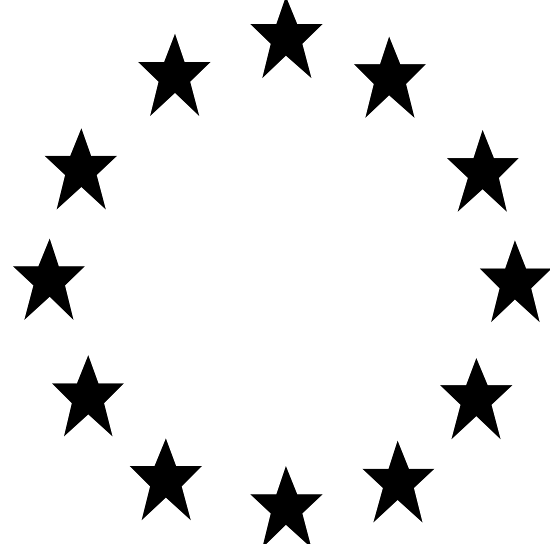

Final addendum to the

Draft Assessment Report (DAR)

- public version -

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

FLUOPICOLIDE

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

January 2008

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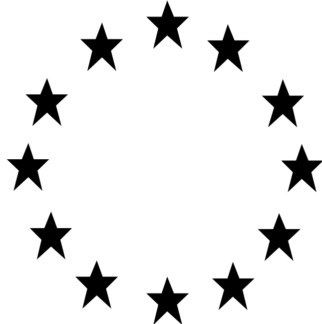
Addendum 3 to Volume 4	February 2009 ..... 507 Confidential

Council Directive 91/414/EEC



Fluopicolide (AE C638206)

ADDENDUM 1 (Chemistry Only)
TO THE DRAFT ASSESSMENT REPORT PREPARED BY THE UNITED KINGDOM

Draft: November 2007

PESTICIDES SAFETY DIRECTORATE
Mallard House, Kings Pool,
3 Peasholme Green,
York YO1 7PX, UK
Website: www.pesticides.gov.uk

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B.2 Physical and Chemical Properties

Open point 1.13
The reference Güldner, 2005, Lab. ID. 02-99 should be added to the list of references relied on. The storage stability correction should be considered in a revised DAR or corrigendum (WG).
See reporting table 1(62).

Corrected text to B.2.2.15 (WG) below:

B.2.2b Physical, chemical and technical properties of the plant protection product – WG formulation

Product name: 'EXP11074B' (Water Dispersible Granule containing 4.44%w/w fluopicolide and 66.7%w/w fosetyl-aluminium)

Table B.2.3 Summary of the physical and chemical properties of the plant protection product – WG formulation

section (Annex point)	study	method	results	comment	reference
B.2.2.15 (IIIA 2.7)	Shelf life	GIFAP No.17	Chemically and physically stable for two years at ambient. Physical properties tested before and after storage– appearance, particle size, pH, dispersibility, suspensibility, wet sieve, wettability, attrition, acidity, dustiness and persistent foam.		Güldner, 2005a

B.2.4 References relied on

Open point 1.13

The reference Güldner, 2005, Lab. ID. 02-99 should be added to the list of references relied on. The storage stability correction should be considered in a revised DAR or corrigendum (WG).
See reporting table 1(62).

Open point 1.14

The studies Zietz, 2004b and Billian and Schöning, 2004 should be deleted from the list of references relied on because they belong to Annex II, 6.0.
See reporting table 1(64).

Open Points 1.13 & 1.14 – amended references relied on below

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., GLP status (where relevant) published or not	Data protection claimed Y/N	Owner
Annex II, 2.1.1/01	Smeykal H.	2003f	Melting point / melting range AE C638206 substance, pure Code: AE C638206 00 1B99 0002 Generated by: Siemens Axiva GmbH & Co. KG, Frankfurt, Germany Bayer CropScience, Document No: C034152, GLP / GEP Yes. unpublished	Yes	BCS
Annex II, 2.1.2/01 2.1.3/01	Smeykal H.	2003g	Boiling point / boiling range Thermal stability AE C638206 substance, pure Code: AE C638206 00 1B99 0002 Generated by: Siemens Axiva GmbH & Co. KG, Frankfurt, Germany Bayer CropScience Document No: C034153 GLP / GEP Yes unpublished	Yes	BCS
Annex II, 2.2/01	Smeykal H.	2003h	Relative density AE C638206 substance, pure Code: AE C638206 00 1B99 0002 Generated by: Siemens Axiva GmbH & Co. KG, Frankfurt, Germany Bayer CropScience Document No: C034154 GLP / GEP Yes unpublished	Yes	BCS

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., GLP status (where relevant) published or not	Data protection claimed Y/N	Owner
Annex II, 2.3.1/01	Bright A.A.S.	2000a	Vapour pressure AE C638206 99.6 % w/w Code: AE C638206 00 1B99 0002 Generated by: Aventis CropScience UK Limited; Chesterford Park, UK Bayer CropScience Document No: C008406 GLP / GEP Yes unpublished	Yes	BCS
Annex II, 2.3.1/02	Riggs A.S.	2000c	Vapour pressure of 2,6-dichlorobenzamide Generated by: Uniroyal Chemical Co., Ontario, Canada Uniroyal Chemical Company, Inc., Connecticut, USA; Document No: C034076 GLP / GEP Yes unpublished	Yes	Crompton
Annex II, 2.3.2/01	Renaud D.	2003	Henry's law constant calculation AE C638206 Generated by: Bayer CropScience, Lyon, France; Document No: C037664 GLP / GEP Unpublished	Yes	BCS
Annex II, 2.4.1/01	Muehlberger B., Eyrich U.	2003b	Physical, characteristics color, appearance and odor substance, technical Code: AE C638206 00 1C96 0001 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C031788 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.4.1/02	Muehlberger B., Eyrich U.	2003c	Physical, characteristics color, appearance and odor substance, pure Code: AE C638206 00 1B99 0002 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C031787 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.4.2/01	Muehlberger B., Eyrich U.	2003b	Physical, characteristics color, appearance and odor substance, technical Code: AE C638206 00 1C96 0001 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C031788 GLP / GEP Yes Unpublished	Yes	BCS

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., GLP status (where relevant) published or not	Data protection claimed Y/N	Owner
Annex II, 2.4.2/02	Muehlberger B., Eyrich U.	2003c	Physical, characteristics color, appearance and odor substance, pure Code: AE C638206 00 1B99 0002 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C031787 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.5.1/01	Muehlberger B.	2003e	Spectral data (UV / VIS, IR, 1H-NMR, 13C-NMR, MS) and molar extinction coefficient Code: AE C638206 00 1B99 0002 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C034149 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.5.2/01	Bowen T.	2003	AE C653711 - Spectral data (UV / VIS, IR, 1H-NMR, 13C-NMR, MS) Generated by: Bayer CropScience, Frankfurt, Germany Document No: C038927 GLP / GEP Unpublished	Yes	BCS
Annex II, 2.5.2/02	Muehlberger B.	2003g	Spectral data (UV / VIS, IR, 1H-NMR, 13C-NMR, MS) and molar extinction coefficient Code: AE C657188 00 1B97 0001 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C034150 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.5.2/03	Muehlberger B.	2003f	Spectral data (UV / VIS, IR, 1H-NMR, 13C-NMR, MS) and molar extinction coefficient Code: AE 060800 00 1C94 0001 Bayer CropScience, Frankfurt, Germany Document No: C034156 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.6/01	Muehlberger B.	2003h	Water solubility of AE C638206 at pH4, pH7 and pH9 (Column-elution method) Code: AE C638206 00 1B99 0002 Bayer CropScience, Frankfurt, Germany Document No: C034161 GLP / GEP Yes Unpublished	Yes	BCS

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., GLP status (where relevant) published or not	Data protection claimed Y/N	Owner
Annex II, 2.6/02	Riggs A.S.	2000a	Solubility of 2,6-dichlorobenzamide in water Generated by: Uniroyal Chemical Co., Ontario, Canada Uniroyal Chemical Company, Inc., Connecticut, USA Document No: C034077 GLP / GEP Yes Unpublished	Yes	Crompton
Annex II, 2.6/03	Muehlberger B., Eyrich U.	2003j	Water solubility of AE C657188 (flask method) Code: AE C657188 00 1B97 0001 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C037026 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.6/04	Muehlberger B.	2003k	Water solubility of AE 0608000 (flask method) Code: AE 0608000 00 1C94 0001 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C037587 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.7/01	Muehlberger B.	2003a	Solubility in organic solvents Code: AE C638206 00 1B99 0002 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C031136 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.8/01	Muehlberger B.	2003d	Partition coefficient 1-octanol/water (HPLC-method) Code: AE C638206 00 1B99 0002 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C032556 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.8/02	Riggs A.S.	2000b	The partition coefficient (n-octanol/water) of 2,6-dichlorobenzamide Generated by: Uniroyal Chemical Co., Ontario, Canada Uniroyal Chemical Company, Inc., Connecticut, USA; Document No: C034074 GLP / GEP Yes Unpublished	Yes	Crompton

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., GLP status (where relevant) published or not	Data protection claimed Y/N	Owner
Annex II, 2.8/03	Muehlberger B., Eyrich U.	2004a	Partition coefficient 1-octanol/ water AE C657188 00 1B97 0001 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C040103 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.8/04	Muehlberger B., Eyrich U.	2003i	Partition coefficient 1-octanol/ water at pH 5, pH 7 and pH 9 (HPLC-method) AE 0608000 00 1C94 0001 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C035847 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.9.1/01	Shepler K., Runes H.	2002	Hydrolysis of [14C]- AE C638206 at pH 4,5,7 and 9 Generated by: PTRL West, Inc., USA; Bayer CropScience; Document No: B004202 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.9.2/01	Runes H., Shepler K	2003	Photolysis and Quantum Yield of [14C]- AE C638206 in Buffered Aqueous Solution Generated by: PTRL West, Inc., USA; Bayer CropSience; Document No: B004201 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.9.3/01	Runes H., Shepler K	2003	Photolysis and Quantum Yield of [14C]- AE C638206 in Buffered Aqueous Solution Generated by: PTRL West, Inc., USA; Bayer CropSience; Document No: B004201 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.9.4/01	Bright A.A.S.	2000b	Dissociation constant AE C638206 99.6 % w/w Code: AE C638206 00 1B99 0002 Generated by: Aventis CropScience UK Limited; Chesterford Park, UK Document No: C008405 GLP / GEP Yes Unpublished	Yes	BCS

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., GLP status (where relevant) published or not	Data protection claimed Y/N	Owner
Annex II, 2.9.4/02	White C.K.	2000	Dissociation constant of 2,6-dichlorobenzamide in water Generated by: Uniroyal Chemical Division , CK Witco Corp., CT, USA; Uniroyal Chemical Company, Inc., USA; Document No: C034075 GLP / GEP Unpublished	Yes	Crompton
Annex II, 2.9.4/03	Muehlberger B.	2004b	Determination of the dissociation constant Code: AE C657188 00 1B97 0001 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C040202 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.9.4/04	Muehlberger B.	2003l	AE 0608000 Determination of the dissociation constant Code: AE 0608000 00 1B97 0001 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C038993 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.10/01	Rupprecht K.	2004	Estimation of the Reaction of AE C638206 with Photochemically Produced Hydroxyl Radicals in the Atmosphere Generated by: Bayer CropScience, RTP, USA Document No: B004573 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.11.1/01	Smeykal H.	2003a	Flammability (solids) AE C638206; substance technical Code: AE C638206 00 1C96 0001 Generated by: Siemens Axiva GmbH & Co. KG, Frankfurt, Germany Bayer CropScience Document No: C033117 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.11.2/01	Smeykal H.	2003b	Auto-flammability (Solids - Determination of relative self-ignition temperature) AE C638206; substance technical Code: AE C638206 00 1C96 0001 Generated by: Siemens Axiva GmbH & Co. KG, Frankfurt, Germany Bayer CropScience Document No: C033119 GLP / GEP Yes Unpublished	Yes	BCS

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., GLP status (where relevant) published or not	Data protection claimed Y/N	Owner
Annex II, 2.13/01	Smeykal H.	2003c	Explosive properties AE C638206; substance technical Code: AE C638206 00 1C96 0001 Generated by: Siemens Axiva GmbH & Co. KG, Frankfurt, Germany Bayer CropScience Document No: C033118 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.14/01	Smeykal H.	2003d	Surface tension AE C638206; substance technical Code: AE C638206 00 1C96 0001 Generated by: Siemens Axiva GmbH & Co. KG, Frankfurt, Germany Bayer CropScience Document No: C033116 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.15/01	Smeykal H.	2003e	Oxidizing properties AE C638206; substance technical Code: AE C638206 00 1C96 0001 Generated by: Siemens Axiva GmbH & Co. KG, Frankfurt, Germany Bayer CropScience Document No: C033120 GLP / GEP Yes Unpublished	Yes	BCS

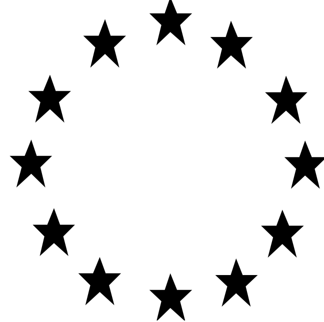
Plant Protection Product - EXP11074B

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., GLP status (where relevant) published or not	Data protection claimed Y/N	Owner
Annex III, 2.1.1/01 2.1.2/01 2.1.3/01 2.4.1/01 2.4.2/01 2.6.2/01 2.7.1/01 2.7.3/01 2.8.1/01 2.8.2/01 2.8.3.1/01 2.8.3.2/01 2.8.5.2/01 2.8.6.1/01 2.8.6.2/01 2.8.6.3/01 2.8.8.1/01	Uceda L., Le Gren I.	2003	Determination of physio-chemical characteristics and storage stability EXP11074B (AE F053616 06 WG71 A1) Generated by: Bayer CropScience, Lyon, France; Document No: C028444 GLP / GEP Yes unpublished	Yes	BCS
Annex III, 2.2.1/01 2.2.2/01 2.3.2/01 2.3.3/01	Allard O.	2002	Determination of the explosion properties, flammability, ability for self heating, relative self-ignition temperature and oxidising properties of EXP11074B (AE F053616 06 WG71 A1) Generated by: Rhoditech, Process Safety Laboratory Decines Charpieu, France; Bayer CropScience; Document No: C024918 GLP / GEP Yes Unpublished	Yes	BCS
Annex III, 4.2.2/01	Friessleben R.	2003	Results to characterise spray tank cleaning behaviour - tank wash recommendations Code: AE F053616 06 WG71 acyl - picolide & fosetyl - Al Generated by: Bayer CropScience, Monheim, Germany; Document No: C036782 GLP / GEP unpublished	Yes	BCS
Annex III, 2.7.3/02	Güldner W.	2005	Storage stability and shelf life of EXP11074B – final report (2 years) Generated by: Bayer CropScience, Monheim, Germany; Document No: ID: 02-99 GLP / GEP : no unpublished	Yes	Bayer CropScience

Plant Protection Product - EXP11120A

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., GLP status (where relevant) published or not	Data protection claimed Y/N	Owner
Annex III, 2.1.1/01 2.1.2/01 2.1.3/01	Uceda L., Le Gren I.	2003	Determination of physico-chemical characteristics and storage stability Code: AE B066752 04 SC61 A1 (EXP11120A) Generated by: Bayer CropScience, Lyon, France; Document No: C030423 GLP / GEP Yes unpublished	Yes	BCS
Annex III, 2.2.1/01 2.3.3/01	Francois J. M.	2003	Determination of the flash point, the auto-flammability and the explosion properties of EXP11120A (AE B066752 04 SC61 A1) Generated by: Rhoditech, Process Safety Laboratory Decines Charpieu, France; Bayer CropScience; Document No: C028144 GLP / GEP Yes unpublished	Yes	BCS
Annex III, 2.4.2/01 2.5.2/01 2.5.3/01 2.6.1/01 2.7.1/01 2.7.2/01 2.7.3/01 2.8.2/01 2.8.3.1/01 2.8.3.2/01 2.8.5.2/01 2.8.8.2/01	Uceda L., Le Gren I.	2003	Determination of physico-chemical characteristics and storage stability Code: AE B066752 04 SC61 A1 (EXP11120A) Generated by: Bayer CropScience, Lyon, France; Document No: C030423 GLP / GEP Yes unpublished	Yes	BCS
Annex III, 2.7.3/02	Güldner W.	2005	Storage stability and shelf life of EXP11120A – final report (2 years) Generated by: Bayer CropScience, Monheim, Germany; Document No: M-253575-02-1 GLP / GEP : no unpublished	Yes	Bayer CropScience
Annex III, 4.2.2/01	Friessleben R.	2003	Results to characterise spray tank cleaning behaviour - tank wash recommendations Code: AE B066752 04 SC61 A1 acyl-picolide & propamocarb-HCl Generated by: Bayer CropScience, Monheim, Germany; Document No: C036783 GLP / GEP unpublished	Yes	BCS

Council Directive 91/414/EEC



Fluopicolide (AE C638206)

ADDENDUM 1
TO THE DRAFT ASSESSMENT REPORT PREPARED BY THE UNITED KINGDOM

Draft: November 2007

PESTICIDES SAFETY DIRECTORATE
Mallard House, Kings Pool,
3 Peasholme Green,
York YO1 7PX, UK
Website: www.pesticides.gov.uk

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B.2 Physical and Chemical Properties

Open point 1.13

The reference Güldner, 2005, Lab. ID. 02-99 should be added to the list of references relied on. The storage stability correction should be considered in a revised DAR or corrigendum (WG).
See reporting table 1(62).

Corrected text to B.2.2.15 (WG) below:

B.2.2b Physical, chemical and technical properties of the plant protection product – WG formulation

Product name: 'EXP11074B' (Water Dispersible Granule containing 4.44%w/w fluopicolide and 66.7%w/w fosetyl-aluminium)

Table B.2.3 Summary of the physical and chemical properties of the plant protection product – WG formulation

section (Annex point)	study	method	results	comment	reference
B.2.2.15 (IIIA 2.7)	Shelf life	GIFAP No.17	Chemically and physically stable for two years at ambient. Physical properties tested before and after storage– appearance, particle size, pH, dispersibility, suspensibility, wet sieve, wettability, attrition, acidity, dustiness and persistent foam.		Güldner, 2005a

B.2.4 References relied on

Open point 1.13

The reference Güldner, 2005, Lab. ID. 02-99 should be added to the list of references relied on. The storage stability correction should be considered in a revised DAR or corrigendum (WG).

See reporting table 1(62).

Open point 1.14

The studies Zietz, 2004b and Billian and Schöning, 2 004 should be deleted from the list of references relied on because they belong to Annex II, 6.0.

See reporting table 1(64).

Open Points 1.13 & 1.14 – amended references relied on from the original DAR below

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., GLP status (where relevant) published or not	Data protection claimed Y/N	Owner
Annex II, 2.1.1/01	Smeykal H.	2003f	Melting point / melting range AE C638206 substance, pure Code: AE C638206 00 1B99 0002 Generated by: Siemens Axiva GmbH & Co. KG, Frankfurt, Germany Bayer CropScience, Document No: C034152, GLP / GEP Yes. unpublished	Yes	BCS
Annex II, 2.1.2/01 2.1.3/01	Smeykal H.	2003g	Boiling point / boiling range Thermal stability AE C638206 substance, pure Code: AE C638206 00 1B99 0002 Generated by: Siemens Axiva GmbH & Co. KG, Frankfurt, Germany Bayer CropScience Document No: C034153 GLP / GEP Yes unpublished	Yes	BCS
Annex II, 2.2/01	Smeykal H.	2003h	Relative density AE C638206 substance, pure Code: AE C638206 00 1B99 0002 Generated by: Siemens Axiva GmbH & Co. KG, Frankfurt, Germany Bayer CropScience Document No: C034154 GLP / GEP Yes unpublished	Yes	BCS

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., GLP status (where relevant) published or not	Data protection claimed Y/N	Owner
Annex II, 2.3.1/01	Bright A.A.S.	2000a	Vapour pressure AE C638206 99.6 % w/w Code: AE C638206 00 1B99 0002 Generated by: Aventis CropScience UK Limited; Chesterford Park, UK Bayer CropScience Document No: C008406 GLP / GEP Yes unpublished	Yes	BCS
Annex II, 2.3.1/02	Riggs A.S.	2000c	Vapour pressure of 2,6-dichlorobenzamide Generated by: Uniroyal Chemical Co., Ontario, Canada Uniroyal Chemical Company, Inc., Connecticut, USA; Document No: C034076 GLP / GEP Yes unpublished	Yes	Crompton
Annex II, 2.3.2/01	Renaud D.	2003	Henry's law constant calculation AE C638206 Generated by: Bayer CropScience, Lyon, France; Document No: C037664 GLP / GEP Unpublished	Yes	BCS
Annex II, 2.4.1/01	Muehlberger B., Eyrich U.	2003b	Physical, characteristics color, appearance and odor substance, technical Code: AE C638206 00 1C96 0001 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C031788 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.4.1/02	Muehlberger B., Eyrich U.	2003c	Physical, characteristics color, appearance and odor substance, pure Code: AE C638206 00 1B99 0002 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C031787 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.4.2/01	Muehlberger B., Eyrich U.	2003b	Physical, characteristics color, appearance and odor substance, technical Code: AE C638206 00 1C96 0001 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C031788 GLP / GEP Yes Unpublished	Yes	BCS

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., GLP status (where relevant) published or not	Data protection claimed Y/N	Owner
Annex II, 2.4.2/02	Muehlberger B., Eyrich U.	2003c	Physical, characteristics color, appearance and odor substance, pure Code: AE C638206 00 1B99 0002 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C031787 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.5.1/01	Muehlberger B.	2003e	Spectral data (UV / VIS, IR, 1H-NMR, 13C-NMR, MS) and molar extinction coefficient Code: AE C638206 00 1B99 0002 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C034149 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.5.2/01	Bowen T.	2003	AE C653711 - Spectral data (UV / VIS, IR, 1H-NMR, 13C-NMR, MS) Generated by: Bayer CropScience, Frankfurt, Germany Document No: C038927 GLP / GEP Unpublished	Yes	BCS
Annex II, 2.5.2/02	Muehlberger B.	2003g	Spectral data (UV / VIS, IR, 1H-NMR, 13C-NMR, MS) and molar extinction coefficient Code: AE C657188 00 1B97 0001 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C034150 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.5.2/03	Muehlberger B.	2003f	Spectral data (UV / VIS, IR, 1H-NMR, 13C-NMR, MS) and molar extinction coefficient Code: AE 060800 00 1C94 0001 Bayer CropScience, Frankfurt, Germany Document No: C034156 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.6/01	Muehlberger B.	2003h	Water solubility of AE C638206 at pH4, pH7 and pH9 (Column-elution method) Code: AE C638206 00 1B99 0002 Bayer CropScience, Frankfurt, Germany Document No: C034161 GLP / GEP Yes Unpublished	Yes	BCS

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., GLP status (where relevant) published or not	Data protection claimed Y/N	Owner
Annex II, 2.6/02	Riggs A.S.	2000a	Solubility of 2,6-dichlorobenzamide in water Generated by: Uniroyal Chemical Co., Ontario, Canada Uniroyal Chemical Company, Inc., Connecticut, USA Document No: C034077 GLP / GEP Yes Unpublished	Yes	Crompton
Annex II, 2.6/03	Muehlberger B., Eyrich U.	2003j	Water solubility of AE C657188 (flask method) Code: AE C657188 00 1B97 0001 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C037026 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.6/04	Muehlberger B.	2003k	Water solubility of AE 0608000 (flask method) Code: AE 0608000 00 1C94 0001 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C037587 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.7/01	Muehlberger B.	2003a	Solubility in organic solvents Code: AE C638206 00 1B99 0002 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C031136 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.8/01	Muehlberger B.	2003d	Partition coefficient 1-octanol/water (HPLC-method) Code: AE C638206 00 1B99 0002 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C032556 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.8/02	Riggs A.S.	2000b	The partition coefficient (n-octanol/water) of 2,6-dichlorobenzamide Generated by: Uniroyal Chemical Co., Ontario, Canada Uniroyal Chemical Company, Inc., Connecticut, USA; Document No: C034074 GLP / GEP Yes Unpublished	Yes	Crompton

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., GLP status (where relevant) published or not	Data protection claimed Y/N	Owner
Annex II, 2.8/03	Muehlberger B., Eyrich U.	2004a	Partition coefficient 1-octanol/ water AE C657188 00 1B97 0001 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C040103 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.8/04	Muehlberger B., Eyrich U.	2003i	Partition coefficient 1-octanol/ water at pH 5, pH 7 and pH 9 (HPLC-method) AE 0608000 00 1C94 0001 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C035847 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.9.1/01	Shepler K., Runes H.	2002	Hydrolysis of [14C]- AE C638206 at pH 4,5,7 and 9 Generated by: PTRL West, Inc., USA; Bayer CropScience; Document No: B004202 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.9.2/01	Runes H., Shepler K	2003	Photolysis and Quantum Yield of [14C]- AE C638206 in Buffered Aqueous Solution Generated by: PTRL West, Inc., USA; Bayer CropSience; Document No: B004201 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.9.3/01	Runes H., Shepler K	2003	Photolysis and Quantum Yield of [14C]- AE C638206 in Buffered Aqueous Solution Generated by: PTRL West, Inc., USA; Bayer CropSience; Document No: B004201 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.9.4/01	Bright A.A.S.	2000b	Dissociation constant AE C638206 99.6 % w/w Code: AE C638206 00 1B99 0002 Generated by: Aventis CropScience UK Limited; Chesterford Park, UK Document No: C008405 GLP / GEP Yes Unpublished	Yes	BCS

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., GLP status (where relevant) published or not	Data protection claimed Y/N	Owner
Annex II, 2.9.4/02	White C.K.	2000	Dissociation constant of 2,6-dichlorobenzamide in water Generated by: Uniroyal Chemical Division , CK Witco Corp., CT, USA; Uniroyal Chemical Company, Inc., USA; Document No: C034075 GLP / GEP Unpublished	Yes	Crompton
Annex II, 2.9.4/03	Muehlberger B.	2004b	Determination of the dissociation constant Code: AE C657188 00 1B97 0001 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C040202 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.9.4/04	Muehlberger B.	2003l	AE 0608000 Determination of the dissociation constant Code: AE 0608000 00 1B97 0001 Generated by: Bayer CropScience, Frankfurt, Germany Document No: C038993 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.10/01	Rupprecht K.	2004	Estimation of the Reaction of AE C638206 with Photochemically Produced Hydroxyl Radicals in the Atmosphere Generated by: Bayer CropScience, RTP, USA Document No: B004573 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.11.1/01	Smeykal H.	2003a	Flammability (solids) AE C638206; substance technical Code: AE C638206 00 1C96 0001 Generated by: Siemens Axiva GmbH & Co. KG, Frankfurt, Germany Bayer CropScience Document No: C033117 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.11.2/01	Smeykal H.	2003b	Auto-flammability (Solids - Determination of relative self-ignition temperature) AE C638206; substance technical Code: AE C638206 00 1C96 0001 Generated by: Siemens Axiva GmbH & Co. KG, Frankfurt, Germany Bayer CropScience Document No: C033119 GLP / GEP Yes Unpublished	Yes	BCS

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., GLP status (where relevant) published or not	Data protection claimed Y/N	Owner
Annex II, 2.13/01	Smeykal H.	2003c	Explosive properties AE C638206; substance technical Code: AE C638206 00 1C96 0001 Generated by: Siemens Axiva GmbH & Co. KG, Frankfurt, Germany Bayer CropScience Document No: C033118 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.14/01	Smeykal H.	2003d	Surface tension AE C638206; substance technical Code: AE C638206 00 1C96 0001 Generated by: Siemens Axiva GmbH & Co. KG, Frankfurt, Germany Bayer CropScience Document No: C033116 GLP / GEP Yes Unpublished	Yes	BCS
Annex II, 2.15/01	Smeykal H.	2003e	Oxidizing properties AE C638206; substance technical Code: AE C638206 00 1C96 0001 Generated by: Siemens Axiva GmbH & Co. KG, Frankfurt, Germany Bayer CropScience Document No: C033120 GLP / GEP Yes Unpublished	Yes	BCS

Plant Protection Product - EXP11074B

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., GLP status (where relevant) published or not	Data protection claimed Y/N	Owner
Annex III, 2.1.1/01 2.1.2/01 2.1.3/01 2.4.1/01 2.4.2/01 2.6.2/01 2.7.1/01 2.7.3/01 2.8.1/01 2.8.2/01 2.8.3.1/01 2.8.3.2/01 2.8.5.2/01 2.8.6.1/01 2.8.6.2/01 2.8.6.3/01 2.8.8.1/01	Uceda L., Le Gren I.	2003	Determination of physio-chemical characteristics and storage stability EXP11074B (AE F053616 06 WG71 A1) Generated by: Bayer CropScience, Lyon, France; Document No: C028444 GLP / GEP Yes unpublished	Yes	BCS
Annex III, 2.2.1/01 2.2.2/01 2.3.2/01 2.3.3/01	Allard O.	2002	Determination of the explosion properties, flammability, ability for self heating, relative self-ignition temperature and oxidising properties of EXP11074B (AE F053616 06 WG71 A1) Generated by: Rhoditech, Process Safety Laboratory Decines Charpieu, France; Bayer CropScience; Document No: C024918 GLP / GEP Yes Unpublished	Yes	BCS
Annex III, 4.2.2/01	Friessleben R.	2003	Results to characterise spray tank cleaning behaviour - tank wash recommendations Code: AE F053616 06 WG71 acyl - picolide & fosetyl - Al Generated by: Bayer CropScience, Monheim, Germany; Document No: C036782 GLP / GEP unpublished	Yes	BCS
Annex III, 2.7.3/02	Güldner W.	2005	Storage stability and shelf life of EXP11074B – final report (2 years) Generated by: Bayer CropScience, Monheim, Germany; Document No: ID: 02-99 GLP / GEP : no unpublished	Yes	Bayer CropScience

Plant Protection Product - EXP11120A

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., GLP status (where relevant) published or not	Data protection claimed Y/N	Owner
Annex III, 2.1.1/01 2.1.2/01 2.1.3/01	Uceda L., Le Gren I.	2003	Determination of physico-chemical characteristics and storage stability Code: AE B066752 04 SC61 A1 (EXP11120A) Generated by: Bayer CropScience, Lyon, France; Document No: C030423 GLP / GEP Yes unpublished	Yes	BCS
Annex III, 2.2.1/01 2.3.3/01	Francois J. M.	2003	Determination of the flash point, the auto-flammability and the explosion properties of EXP11120A (AE B066752 04 SC61 A1) Generated by: Rhoditech, Process Safety Laboratory Decines Charpieu, France; Bayer CropScience; Document No: C028144 GLP / GEP Yes unpublished	Yes	BCS
Annex III, 2.4.2/01 2.5.2/01 2.5.3/01 2.6.1/01 2.7.1/01 2.7.2/01 2.7.3/01 2.8.2/01 2.8.3.1/01 2.8.3.2/01 2.8.5.2/01 2.8.8.2/01	Uceda L., Le Gren I.	2003	Determination of physico-chemical characteristics and storage stability Code: AE B066752 04 SC61 A1 (EXP11120A) Generated by: Bayer CropScience, Lyon, France; Document No: C030423 GLP / GEP Yes unpublished	Yes	BCS
Annex III, 2.7.3/02	Güldner W.	2005	Storage stability and shelf life of EXP11120A – final report (2 years) Generated by: Bayer CropScience, Monheim, Germany; Document No: M-253575-02-1 GLP / GEP : no unpublished	Yes	Bayer CropScien ce
Annex III, 4.2.2/01	Friessleben R.	2003	Results to characterise spray tank cleaning behaviour - tank wash recommendations Code: AE B066752 04 SC61 A1 acyl-picolide & propamocarb-HCl Generated by: Bayer CropScience, Monheim, Germany; Document No: C036783 GLP / GEP unpublished	Yes	BCS

B.2.5 Additional References Relied On:

Annex point/ Location in Dossier	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Data protect. claimed	Owner
Document D1	J. Cousin; H. Schenk-Epp	2007	Summary of Good Agricultural Practices for Intended Pesticide Uses for AE C638206 and the Plant Protection Products AE F053616 06 WG71 A1 and AE B066752 04 SC61 A1 Bayer CropScience AG, Edition No.: M-226985 02-1, Date: 2007-05-07 Non GLP, unpublished	Yes	BCS
Doc K AII 2.13	Smeykal, H.	2006	Fluopicolide (AE C638206), Substance technical, Explosive Properties A. 14. Bayer CropScience AG Edition no.: M-269406-01-1 Date: 2004-01-12 GLP, unpublished	Yes	BCS
Doc K AII 3.8.1	Renaud, D.	2004	Incineration as a safe means of disposal and pyrolytic behaviour under controlled conditions Bayer CropScience AG, Edition No.: M-226555-01-1, Date: 2004-01-12 Non GLP, unpublished	Yes	BCS
Doc K AII 4.1.3	Bowen, T.	2005	Response to the Fluopicolide product chemistry questions raised by the German authorities during the pre-evaluation of Bayer CropScience`s submission of Infinito (Fluopicolide and propamocarb hydrochloride) Bayer CropScience AG Edition no.: M-261425-01-1 Date: 2005-11-28 Non GLP, unpublished	Yes	BCS
Doc K AII 4.1.3	Bowen, T.	2007	Justification for the use of corrected response factors described in the analytical methods AM000203FP1 and AM000303FP1 when determining the fluopicolide impurities AE 1050605 and AE 1423809 Bayer CropScience Edition No. M-287053-01-1 Date: 2007-04-24 no GLP, unpublished	BAY	BCS

Annex point/ Location in Dossier	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Data protect. claimed	Owner
Doc K AII 4.1.3	Bowen, T.	2007	Response to the Fluopicolide Product Chemistry Question Raised by the German Authorities during the Pre-evaluation of Bayer CropScience's Submission of Infinito (Fluopicolide and Propamocarb Hydrochloride) Bayer CropScience AG Edition no.: M-284628-01-1 Date: 2007-02-26 No GLP, unpublished	yes	BCS

Plant Protection Product - EXP11074B

Annex point/ Location in Dossier	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Data protect. claimed	Owner
Doc J IIIa	Cousin, J.	2006	Document JIIIa Confidential data concerning industrial and commercial secrets on the plant protection product AE F053616 06 WG 71 A (correction of composition) Bayer CropScience AG Edition no. M-227000-02-1 Date: 2006-08-30 No GLP, unpublished	Yes	BCS
Doc J IIIa	Cousin, J; Guesnet, J.L; Schenk-Epp, H.	2006	Position paper Correction of the composition statement and document J IIIa for the plant protection product AE F053616 06 WG 71 A Bayer CropScience AG Edition no. M-277097-02-1 Date: 2006-10-24 No GLP, unpublished	Yes	BCS

B.6 TOXICOLOGY AND METABOLISM

B.6.3 Short-term toxicity studies (IIA 5.3)

B.6.3.3 Oral short term toxicity in the dog (IIA 5.3.2)

Open point 2.1
The relevance of the liver weight increase in the 90 day study in dog to be agreed on in an experts' meeting
See reporting table 2(3).

Discussion on NOAEL for liver effects in 90-day dog study

Further detailed information is provided in amendment to Table 6.57 in Volume 3 of DAR. In the study report, no mention is made of apparent treatment-related effects in the clinical chemistry findings for cholesterol and alkaline phosphate by the Investigators both of which have been indicated to be suggestive of relevant treatment-related effects in the liver at the 1000 mg/kg bw/day dose. Whilst the values at 7 weeks and 13 weeks were not statistically significant (with the exception of alkaline phosphatase levels in females at week 13), mean cholesterol and alkaline phosphatase were clearly elevated at 1000 mg/kg bw/day. Considering that only 4 dogs are used in the study, the RMS notes that it is inappropriate to rely on statistical significance alone but to consider the biological relevance and information from the other dog studies.

Table 6.57 [amended]: Summary of relevant clinical chemistry and organ weight changes in the liver in the 90-day oral study in dogs

Parameter	Dose level (mg/kg bw/day)							
	Males				Females			
	0	5	70	1000	0	5	70	1000
Cliinical chemistry								
Cholesterol mmol/L								
Day -15	4.06	3.45	3.94	3.82	3.56	3.40	3.46	3.64
Day -7	3.80	3.12	3.74	3.83	4.06	3.33	3.25	3.53
Week 7	3.98	3.78	3.68	4.63	3.72	3.47	3.70	4.29
Week 13	3.85	3.66	3.82	4.65	3.71	3.73	3.51	4.49
Alkaline phosphatase (U/L)								
Day-15	132	128	119	139	120	135	99	112
Day -7	140	133	124	140	115	130	100	118
Week 7	126	122	117	156	131	119	96	185
Week 13	105	105	99	165	116	97	82	222*
`Terminal Body weight (kg)	12.74	12.88	12.16	11.80	10.64	11.15	11.13	10.19
Organ weight at Week 13 Absolute liver weight (g)	368.1	351.1	350.6	438.8*	300.1	322.3	318.6	396.4
Relative liver weight (%)	2.9	2.7	2.9	3.7*	2.8	2.9	2.9	4.0**

* $p \leq 0.05$; ** $p \leq 0.01$.

Excerpt of evaluation as reported in DAR Vol3 of 90-day oral toxicity study in dogs.

Study	Dog 90-day oral toxicity study Code: fluopicolide 00 1C99 0005
Reference	Mallyon B., 5/12/2000
Date performed	9/11/1999 – 10/3/2000
Test facility	[redacted], UK
Report reference	Lab. Ref: TOX/00/283-25/ Notifier ref: Tox 99292 Dossier ref: C010655.
Guideline(s)	OECD 409 (21/9/1998); EU (=EEC) 88/302/EEC VB 1987; USEPA OPPTS 870.3150 1998; JMAF 4200 1985
Deviations from the guideline	No significant deviation
GLP	Yes and QA
Test material	Batch no., PP/241024/2 and PP/241067/1, purity 97.7%)
Study acceptable	Yes

In a study (2000), groups of 4 male and 4 female outbred beagle dogs were administered by gavage fluopicolide (batch mixture PP/241024/2 and PP/241067/1, purity 97.7 %) at dose levels of 0 (control), 5, 70 or 1000 mg/kg bw/day for at least 13 weeks. The test material was administered in a vehicle of 1 % w/v methyl cellulose in distilled water at a constant volume of 5 ml/kg body weight. Controls received the vehicle alone. Animals were observed daily for clinical signs. Body weight was recorded weekly, whilst food consumption was measured daily. A detailed clinical examination and ophthalmoscopic examination were carried out pre-treatment and prior to necropsy. Haematology and biochemistry investigations were conducted twice prior to treatment, during week 7, and prior to termination. Urinalysis was conducted prior to treatment, during week 6, and at termination. At necropsy, the weights of selected organs were recorded, and subsequently a range of tissues was examined histopathologically.

Statistical analysis

The significance of differences between control and treated groups was analysed by either parametric or non-parametric statistical tests as appropriate. A maximum 2-tailed probability value of 5 % ($p < 0.05$) was considered statistically significant.

The convention employed to indicate statistical significance at the levels of $p < 0.05$, $p < 0.01$ and $p < 0.001$ is indicated below the relevant tables.

Body weight, food consumption and organ weights were analysed for homogeneity of group variances using Bartlett's test. If not significant at the 5% level, the data were then analysed by one way analysis of variance to establish the significance of variability amongst the groups. If significant, pair-wise comparisons of each treated group in turn with the controls were made using Dunnett's test of significance. Where group variances were heterogeneous using Bartlett's test, pair-wise comparisons were made using a modified t test. For pair-wise comparisons, significance was tested at the 5 and 1 % levels ($p < 0.05$, $p < 0.01$). The following convention has been used to indicate statistical significance: * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$.

Haematology and clinical chemistry data were subjected to the following statistical evaluation: Bartlett's Test was used to determine homogeneity of variance between groups; if significant at the 5 % level, a log transformation was applied to the data to attempt to remove the heterogeneity. If homogeneity of variance was demonstrated on either the untransformed or transformed data, parametric tests to detect significant difference between control and test groups at the 5, 1 or 0.1 % level comprised: One-Way Analysis of Variance to establish the significance of variability among all groups and Student's "t" Test, based on a pooled variance estimate, for intergroup comparisons, i.e. control versus each test group. If significant heterogeneity of variance was indicated, even with transformation, then non-parametric analysis was by the Kruskal-Wallis Rank Test to detect any significant group differences at the 5, 1 or 0.1 % level.

The study was certified to be GLP compliant and satisfies the essential requirements of OECD guideline # 408. Test diets were prepared weekly and analysed for test material content. The mean results for the test diet samples analysed were within the range 94.3 - 105.9 % of nominal (laboratory's acceptable range was +10% to -15% of nominal). Homogeneity was shown to be satisfactory at all levels i.e. mean values obtained for top, middle and bottom samples were within the acceptable range 90 - 110% of nominal and these mean % nominal values differed by < 10%. Stability was satisfactory over the time of use of the diet (8 days) i.e.: % nominal levels declined by a maximum of 7 % over 15 days of storage at room temperature. The study is considered acceptable.

The dogs were fed daily over a period of at least 1.5 hours with 400 g of expanded pellet dog diet. On some occasions food bowls were withdrawn earlier if all animals in a pen had consumed all food before 1.5 hours had elapsed. Dogs were normally fed between approximately 1 to 3.5 hours after dosing. A certificate of analysis for each batch of diet was provided by the manufacturer prior to its use.

Prior to the start of treatment, a procedure was developed to reliably prepare homogeneous and suitably stable mixtures of the test material in the vehicle, 1 % w/v methyl cellulose in distilled water, at the required nominal concentrations of 1, 14 and 200 mg/ml (equivalent to dose levels of 5, 70 and 1000 mg/kg bw/day, respectively).

There were no mortalities or clinical signs of toxicity observed during the study. There were no treatment-related effects on the eyes, bodyweight, food intake, haematology, biochemistry, urinalysis, macroscopic pathology or histopathology parameters investigated.

At 1000 mg/kg bw/day, absolute liver weight was increased by 19% in males and by 32% in females, compared with controls. Relative liver weight to bodyweight was increased by 28% and 43% in males and females, respectively, when compared with controls. Organ weights were unaffected at 5 or 70 mg/kg bw/day.

Table 6.57 Summary of the organ weight changes in the liver in the 90-day oral study in dogs

Parameter	Dose level (ppm)							
	Males				Females			
	0	5	70	1000	0	5	70	1000
Organ weight at Week 13 Absolute liver weight (g)	368.1	351.1	350.6	438.8*	300.1	322.3	318.6	396.4
Relative liver weight (%)	2.9	2.7	2.9	3.7*	2.8	2.9	2.9	4.0*

The NOAEL in the 90-day dietary study in dogs was 70 mg/kg bw/day based on increased absolute and relative liver weight at 1000 mg/kg bw/day for both sexes. Dogs are noted to be a non-rodent species and the large increase in liver weight is considered toxicologically relevant.

(Mallyon, 2000d)

Open point 2.2
The carcinogenic potential of fluopicolide to be discussed in an experts' meeting, in particular with regard to the possible mode of action involved and the need for classification
See reporting table 2(6).

The RMS notes that in the chronic toxicity and carcinogenicity study in mice, Fluopicolide caused an increase in hepatocellular adenomas in male and female mice at a dose level of 3200 ppm a dose level at which the MTD had been attained by a mechanism considered to be not relevant to humans. In a mechanistic study, dietary administration of fluopicolide at 3200ppm in the diet induced liver changes such as higher liver weights, hepatocellular hypertrophy as well as a transient and marked hepatocellular proliferation in C57BL/6mice after 7days of treatment, which returned to control levels after 28 days of treatment. Fluopicolide was shown to be an inducer of cytochrome P-450 and BROD and PROD enzyme activities comparable with the liver enzyme induction profile of phenobarbital. Bromodeoxyuridine-labelling in the 28-day mechanistic study showed a transient marked increase in labelling index which is known to be sufficient to induce hepatocellular tumours in mice (Grasso P et al., 1991, Hildebrand B. et al, 1991) and is considered be be of no relevance to humans. Further investigation with Proliferating Cell Nuclear Antigen staining at 90 days did not reveal any PCNA-positive hepatocytes at 90 days and is consistent with the findings with BrDU at 28 days.

The Notifier provided a position paper (Virginie Payraudeau 2/11/2006). The RMS agrees with the conclusion that the hepatocellular adenomas in mice are caused by a mechanism not relevant to humans.

B.6.5.2 Chronic toxicity and carcinogenicity in the mouse

Study	AEC638206 : carcinogenicity study by oral route (dietary admixture) in C57BL/6 mice
Reference	Chevalier, G. 20/11/2003
Date performed	19/4/2001 to 15/11/2002
Test facility	[redacted] France
Report reference	Lab. Ref: 21557TCS Notifier ref: *Tox 20110* Dossier ref: C038732.
Guideline(s)	OECD 451 (04/1981); USEPA : OPPTS 870/4200 (1998); JMAFF 59 Nohsan 4200 (1985); EU {96/54/EEC, B32 (1996)}.
Deviations from the guideline	No significant deviation
GLP	Yes and QA
Test material	Batch no., OP2050046, purity 95.9%
Study acceptable	Yes

In a study (2003), the carcinogenic potential of fluopicolide (batch number: OP2050046, purity: 95.9%) after long-term dietary exposure was investigated in C57BL/6 mice. Groups of 50 male and 50 female C57BL/6 mice were administered in the diet fluopicolide (batch no.: OP2050046, purity: 95.9%) at concentrations of 0, 50, 400 or 3200 ppm (equivalent to 7.9, 64.5 and 551.6 mg/kg/day for the males and 11.5, 91.9 and 772.9 mg/kg/day for the females) for 78 weeks. For the evaluation of chronic toxicity, additional satellite groups of 10 males and 10 females per dose group were treated and killed after one year.

Throughout the study, clinical signs and mortality were checked daily, and careful examination was carried out before the beginning of the treatment period and weekly thereafter to assess possible neurotoxic effects. Palpation for possible masses was carried out every 4 weeks from weeks 4 to 52 and every 2 weeks thereafter. Body weight and food consumption were measured at weekly intervals during the first 13 weeks of the study, every 4 weeks until weeks 31/32 and every 2 weeks thereafter. Achieved dosages were calculated. Before the sacrifice of satellite animals in week 52, blood was taken for the determination of liver enzyme activities (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase). At the end of the appropriate scheduled treatment period (weeks 52 or 78 weeks), animals were sacrificed and were submitted to a macroscopic *post-mortem* examination. A complete range of organs and any masses or macroscopic lesions were sampled. A microscopic examination was performed on all principal animals as well as on the liver and on macroscopic abnormalities from the satellite animals (all groups).

Survival rates were compared using the Chi-squared test. The number of neoplasms (per group and per organ) was compared by Peto's test.

The study was certified to be GLP compliant and satisfied the essential requirements of OECD guideline # 451 (1981). Before the start of the treatment period, the suitability of the dosage form preparation was determined by the analysis of concentration, homogeneity and stability of dietary admixtures. HPLC analysis of the test substance

carried out every 6 months confirmed that the purity remained the same throughout the treatment period. The results of the analyses demonstrated the satisfactory homogeneity of each dietary admixture analyzed during the study. Furthermore, there was a good correspondence between the nominal and the measured concentrations of the test item in the diet. Stability of the formulation was shown to be a minimum of 10 days. On the first day of treatment, the animals were approximately 7 weeks old and had a mean body weight of 23.3 g (21.5 - 26.2 g) for the males and 19.5 g (17.7 - 22.3 g) for the females.

The distribution of mortality, as well as the factors contributing to mortality or premature sacrifice, was similar in the control and treated groups. Survival rate over 78 weeks was in males 82, 88, 90 and 88 % and in females 90, 82, 92 and 82% corresponding to the 0 (control), 50, 400 and 3200 ppm dose groups respectively. The incidence, nature and onset of the clinical signs were similar in the control and treated groups. No signs of neurotoxicity were observed during the study. The frequency, time of onset and size of the few palpable masses recorded were similar in the control and treated groups.

Food consumption and body weight of treated animals at 50 or 400 ppm were similar to that of controls. However, the body weight and the body weight gain of the 3200 ppm dose group was severely affected (-45% in males and –35% in females at 78 weeks, Table 6.46). This effect was associated with a slight reduction of the food consumption (-7% in males and – 8% in females) throughout the study in the top dose group.

Table 6.90 Group mean body weights (g) and group mean body weight changes (g) throughout the treatment period in the 78-week dietary study in mice.

Concentration (ppm)	Males				Females			
	0	50	400	3200	0	50	400	3200
Body weights								
. week 2	23.9	23.9	24.0	23.1**	20.3	20.4	20.3	19.2**
. week 13	29.8	29.9	29.0*	26.9*	24.5	24.5	24.8	22.8**
. week 26	34.9	34.8	33.8	29.1**	28.1	28.8	28.7	24.5**
. week 52	40.8	41.3	39.0	31.9**	33.3	34.5	34.1	26.7**
. week 78	41.4	43.5	42.0	33.3**	34.7	36.1	36.3	29.2**
Body weight change								
. weeks 13 vs. 2	5.9	6.0	5.0**	3.9**	4.2	4.1	4.5	3.7*
. weeks 26 vs. 13	5.1	4.9	4.8	2.2**	3.6	4.2	3.9	1.7**
. weeks 52 vs. 26	5.8	6.4	5.3	2.8**	5.2	5.7	5.4	2.2**
. weeks 78 vs. 52	0.6	1.9	3.0**	1.1	1.3	1.7	2.3	2.7
. weeks 78 vs. 1	18.3	20.5	18.8	10.0**	15.3	16.8	16.9	9.9**
Variation from controls (%)	-	12	3	-45	-	10	10	-35

* $p < 0.05$; ** $p < 0.01$ significantly different to controls

Clinical chemistry parameters investigated in satellite animals for signs of liver toxicity did not generally reveal any differences in liver enzyme activities between treated and control animals. However, there was a notable increase in the mean alkaline phosphatase activity in females at 3200 ppm with further increased but statistically significant ASAT and ALAT. This finding was attributed to very large

changes in 2/10 animals by the Investigators, which in itself is an indication of impairment of liver function albeit in a smaller number of animals from the top dose group (*Table 6.47 correction in DAR should read 6.91*).

Table 6.91 Group mean liver enzyme activities at 53 weeks in the 78-week dietary study in mice (mean ± SD, expressed as IU/L, N = 10)

Parameter	Dose Group (ppm)							
	Males				Females			
	0	50	400	3200	0	50	400	3200
Alkaline phosphatase	129 ± 7	122 ± 22	114 ± 6*	135 ± 12	180 ± 22	170 ± 31	183 ± 30	564 ± 895**
Aspartate aminotransferase	80 ± 40	61 ± 22	62 ± 14	65 ± 35	75 ± 23	101 ± 56	115 ± 78	194 ± 312
Alanine aminotransferase	28 ± 23	24 ± 8	40 ± 25	64 ± 66	45 ± 29	29 ± 17	34 ± 48	145 ± 263

Organ weights at post-mortem examination revealed increased absolute and relative liver weights at dose levels of 400 ppm and 3200 ppm compared with controls at the end of the 52-week and 78-week treatment periods (Correction: Table 6.48 in original DAR should read 6.92). These changes were noted to be associated with hepatocellular hypertrophy in these animals.

Table 6.92 Summary of liver weight changes after 52 weeks and 78 weeks of treatment compared with controls (%)

Sex	Males			Females		
Concentration (ppm)	50	400	3200	50	400	3200
Body weight gain (g)	+5	+1	-19**	+4	+3	-17**
After 52 weeks of treatment (N=10)						
.absolute	+8	+30**	+35**	-5	+4	+50*
. relative	+8	+15**	+63**	0	+10	+99**
After 78 weeks of treatment (N=50)						
. absolute	+14	+18**	+46**	-1	+33**	+56**
. relative	+9	+15**	+79**	-5	+28	+81**

*: p<0.05;**: p<0.01

Macroscopic post-mortem examination revealed after 52 weeks: liver enlargement at 400 or 3200 ppm in males only, and presence of masses and nodules in the liver in females treated at 3200 ppm. After 78 weeks, marked increase of liver enlargement at 3200 ppm, and the number of animals bearing masses and nodules in the liver in treated groups at 400 and 3200 ppm was higher when compared with controls.

Microscopic examination at both 52- and 78-week showed: a dose-related hepatocellular hypertrophy at 400 ppm or 3200 ppm; and higher incidence of altered cell foci at 3200 ppm, and markedly higher incidence of hepatocellular adenoma at 3200 ppm.

After 52-weeks, dose-related centrilobular hepatocellular hypertrophy was observed at 400 ppm (5/10 males and 6/10 females) and 3200 ppm (10/10 males and 9/10 females). Hepatocellular adenoma was found in 1/10 females given 400 ppm and in 3/10 female

mice given 3200 ppm. The higher incidence of hepatocellular adenoma at 3200 ppm (p< 0.036) was considered to be treatment-related.

After 78 weeks, the overall number of animals with neoplasms, the number of animals with more than one primary neoplasm and the number of animals with benign and malignant tumours were comparatively similar in all groups. However, a higher incidence of hepatocellular adenoma was noted in the males and females given 3200 ppm (Table 6.49) and attained a statistically significant level in the females only (p<0.0005). The incidence and time of onset of the hepatocellular neoplastic lesions in the other treated groups (50 ppm and 400 ppm) were comparatively similar to that of the controls.

Hepatocellular neoplasms were diagnosed as "hepatocellular adenoma" and "hepatocellular carcinoma". Adenoma was diagnosed when cells resembling relatively normal hepatocytes formed discrete nodules which significantly compressed the adjacent parenchyma and sometimes bulged above the surface. The diagnosis of hepatocellular carcinoma was made when the liver plates were more than one layer thick, irregular and composed of well- to moderately differentiated hepatocytes. The lesions were either solid or trabecular and showed great variability in cell and nuclear size. Large cells with large hyperchromatic nuclei were commonly present. Many such cells seemed to be undergoing necrosis.

Table 6.93 Summary of the incidence of animals bearing liver neoplasms and non-neoplastic lesions (hepatocellular hypertrophy and altered cell foci) after 78 weeks in the carcinogenicity study in mice

Sex	Males				Females			
Concentration (ppm)	0	50	400	3200	0	50	400	3200
Number of animals examined	50	50	50	50	50	50	50	50
Hepatocellular hypertrophy	0	0	20	49	0	0	41	46
Total Altered cell foci: (percentage)	2 (1) (4)	8 (10)	6 (5) (12)	19 (18) (38)	2 (1) (4)	5 (3) (10)	4 (8)	27 (25) (54)
Hepatocellular adenoma	5	0	5	11***	1	2	0	16**
(percentage)	(10)		(10)	(22)	(2)	(4)		(32)
Hepatocellular carcinoma	3	1	0	2	0	0	2	0
(% to controls)	(6)	(2)		(4)			(4)	
Total	8	1	5	13	1	2	2	16
(percentage)	(16)	(2)	(10)	(a)(26)	(2)	(4)	(4)	(b)(32)

**: p<0.0005; ***: p<0.0314; (a): p<0.0655; (b): p<0.0005 using the Peto’s test

Daily administration by the oral route (dietary admixture) of fluopicolide at concentrations of at 50, 400 or 3200 ppm to C57BL/6 mice for 78 weeks resulted in severe reduction of the body weight gain and food consumption at 3200 ppm, suggesting that the Maximum Tolerated Dose (MTD) was reached. The liver was the principal target organ and higher liver weights, enlarged liver, masses and nodules in the liver, and hepatocellular hypertrophy was observed at dose levels of ≥ 400 ppm and increased incidence of altered cell foci and hepatocellular adenoma at 3200 ppm. The Applicant submitted that this finding in the liver tissue might be attributed, at least in part, to the fact that 3200 ppm reached the MTD.

The NOAEL in the 78-week dietary study in mice was 50 ppm (corresponding to 7.9 mg/kg bw/day in males and 11.5 mg/kg/day in females) based on increased liver weights, enlarged liver, masses and nodules in the liver, and hepatocellular hypertrophy at dose levels of ≥ 400 ppm (corresponding to 64.5 mg/kg bw/day for males and 91.9 mg/kg bw/day for the females). Fluopicolide caused an increase in hepatocellular adenomas in male and female mice at a dose level of 3200 ppm a dose level at which the MTD had been attained by a mechanism considered to be not relevant to humans.

(Chevalier, 2003)

Open point 2.3

The amount of bioavailable fluopicolide after oral administration to be agreed on in an experts' meeting
See reporting table 2(8).

The Applicant has submitted the position paper - Evaluation of the oral bioavailability of Fluopicolide in the rat. - Fisher, P. Dated: 10th April 2007. See Appendix 4.

RMS Comment:

Extent of oral absorption and correction factor for AOEL

The main route of elimination of radiolabel is in faeces. The critical point is the difference in biliary excretion levels between pyridyl and phenyl radiolabel and the biological reasons for such a difference. For the biliary studies, recovery of radiolabel was excellent, approximately 100% so justification for attempting to use another study in which biliary study is unknown is not necessary. "A correction factor of 0.62 was allowed to account for the extent of oral absorption which is based on that determined for the pyridyl radiolabel in the biliary excretion study. The basis for using the lower oral absorption estimate (pyridyl radiolabel - 62% rather than phenyl radiolabel - 80% or an average of the two is because the mechanism or biological reasons for the difference is unclear and hence the more conservative estimate has been relied upon for the derivation of the AOEL."

B.6.10.2 Acute Reference Dose (ARfD)

Open point 2.4
The need for setting an ARfD, and the most relevant study to be considered, to be discussed in an experts' meeting
See reporting table 2(12).

A positon paper has been submitted by the Applicant – See Appendix 5. *AEC63S206 (fluopicolide) - Waiver for an Acute Reference Dose (ARfD) setting. (Payraudeau, V. Dated : 7th March 2006. Report No. M-269338-01-1)*

The RMS has proposed an ARfD in the DAR as follows:

Fluopicolide is of relatively low acute toxicity. Rabbits appeared to be significantly more sensitive compared with other species (rats, mice and dogs) investigated but the clinical findings suggest that toxicity in dams at the LOAEL and indeed at higher dose levels occurred only after repeat administrations. Applying the NOAEL in the 28-day dietary study in rats 200 ppm (17.7 mg/kg bw/day) for systemic toxicity based on impaired growth and histopathological changes in the liver and kidney at 1400 ppm (106 mg/kg bw/day), the ***ARfD is 0.18 mg/kg bw/day*** *and allows for a 100-fold safety margin.*

Further discussions of this can also be found in the reporting table and evaluation table.

B.6.11.2 Acute dermal toxicity (IIIA 7.1.2)

Data requirement (2.1)
Applicant to provide a GLP revision of the acute dermal study (Krotlinger 2003)

The applicant announced in the written procedure that the report M-220872-02-1 (Krotlinger 2003) is available and can be submitted immediately.
See reporting table 2(16).

The dose applied to animals was 2000 mg/kg/bw. The Applicant has submitted a revised GLP compliant revision of the study report.

B.6.12 Dermal absorption studies (IIIA 7.3)

Open point 2.5
RMS to provide further details on the results of the in vivo dermal absorption study (see comment by NL) in an addendum
See reporting table 2(18).

Open point 2.6
Dermal absorption to be discussed in a meeting of experts
See reporting table 2(19).

Dermal absorption is to be discussed at the Expert Meeting. See DAR and Evaluation Table for details. As requested, please see below for further details on the results of the in vivo dermal absorption study:

Discussion of *in vivo* dermal absorption

Further information on recoveries from the stratum corneum and urinary elimination data are been requested. The RMS notes that the relevant information on recoveries are incorporated in summary table provided for the study (reference B 6.12.2). It appears from the table that the row for sacrifice times is not completed and this is corrected in the text in the addendum below.

The RMS notes in the estimate of dermal absorption (reference B6.12.3 DAR Vol 3) that correcting for comparative absorption for rat and human skin on the basis that absorption for rat skin is 11.48x greater for the concentrate and 8.07x greater for the in-use dilution the estimates of dermal absorption for human skin are **0.24 %** for the concentrate and **2.75 %** for the in-use dilution.

The estimates of dermal absorption were based on all the material present in the skin after 144 hours collection of urine as the amount of radiolabel in recovered in the urine continued to increase up to 144 h whilst the amount of radiolabel recovered in the skin including the stratum corneum continued to decrease over this period such that no conclusions can be drawn on which fraction of radiolabel in the stratum corneum is not bioavailable.

The most critical factor in the estimate of the dermal absorption are the findings in the in vitro dermal absorption study in rat and human skin used as a correction for dermal absorption for the in vivo rat study. No questions have been raised on this critical aspect of the assessment. Considering the already very low estimates of dermal absorption obtained, there is unlikely to be any value in a discussion over which number of tape stripping should be considered relevant, noting that the Rapporteur has taken the worst case estimates.

Nonetheless the relevant tables have been provided below for discussion at the expert meeting.

Study as reported in DAR Volume 3

Dermal absorption *in vivo*

Study	*In vivo* dermal absorption in the male rat of [^{14}C]-EXP11120A
Reference	Kemp L. 2/10/2003
Date performed	dates of the in-life phase 29/4/2003 - 18/7/2003
Test facility	[redacted] UK
Report reference	Study no.: BAG 367/033193 /Report no.:C037215
Guideline(s)	OECD 417 adopted 4 April 1984 and draft OECD 427 and draft Guidance document April 2002
Deviations from the guideline	No significant deviations
GLP	Yes and QA.
Test material	Batch no. OP220159, containing fluopicolide 62.5 g/l and propamocarb 625 g/l
Study acceptable	Yes

In a study (2003), the rate and extent of absorption of radioactive material was investigated following topical application of the fungicide formulation EXP 11120A, containing [14C]-fluopicolide as the active ingredient, to male rats. The dose was applied at two concentrations, nominally 62.5 mg/ml (equivalent to the commercially supplied concentrate), and 0.25 mg/ml (equivalent to an in-use dilution of the product). A preliminary study was conducted on one group of 6 male rats, and one group of 5 male rats, with an exposure time of 8 hours (analogous to the normal working day) and sacrifice times at 24, 72 and 144 hours after dose application, to obtain an indication of the proportion of the test substance absorbed through the skin and excreted, retained in the skin or on the skin surface at each dose level. The results obtained from the preliminary study were used to determine the sacrifice times in the main study, the need to investigate the material remaining in the skin, and its localisation, and any requirement to examine the tissue distribution of the radioactivity. On the basis of the results of the preliminary study the same sacrifice times were employed in the main experiments at each dose level, together with a sacrifice time at 8 hours to investigate absorption prior to the dose swabbing procedure. In addition, tape stripping was included in the main studies to determine the distribution of the radioactivity through the skin. There was no reason to investigate tissue distribution of the radioactivity, since the levels of the radiolabelled material absorbed into the body were low and mostly eliminated by 144 hours after dosing. The main study consisted of eight groups of five male rats, four groups at each dose level, which were exposed to the test material for 8 hours. At the end of the exposure period the remaining dose was washed off the skin with a mild detergent solution. One group of animals at each dose level were sacrificed at 8, 24, 72 and 144 hours after application. Urine, faeces and cage wash were collected at 8 hours, 24 hours and daily until termination. At termination the dose site was tape stripped to remove the stratum corneum. The remaining treated skin, a small area of skin surrounding the dose site, untreated skin and residual carcass were retained for analysis.

The radioactive material was absorbed steadily over the 8 hour exposure period, and the absorption rate was shown to be greater at the low dose level. A large proportion of the applied material (>86% at the high dose level and 40% to 56% at the low dose level) was removed by washing at the end of the exposure period. Absorption from the stratum corneum continued subsequent to washing, although some of the material in the stratum corneum was lost by desquamation. For the purposes of risk assessment, however, the stratum corneum fraction has been included in the total percentage absorbed value.

Summary of the findings of the in vivo dermal absorption study

Parameter		Dose Group							
Dose level		High dose				Low dose			
Sacrifice time (h)		8	24	72	144	8	24	72	144
Total Absorbed									
(direct absorption + treated skin + stratum corneum)	%	3.79	7.73	2.69	2.65	30.3	34.3	28.4	22.2
	µg	300	611	213	210	5.20	5.89	4.87	3.81
Non-Absorbed									
(gauze wash, skin swabs + surface dose)	%	95.1	91.7	94.6	93.9	66.6	56.3	68.7	78.8
	µg	7521	7252	7481	7426	11.4	9.66	11.8	13.5
Total Recovery	%	98.8	99.5	97.3	96.6	96.9	90.6	97.1	101

Results are expressed as mean % applied radioactivity.
Mean values were calculated from the individual recoveries and not the sum of the mean components

The investigators concluded that the total amount of radioactive material absorbed was 2.65% at 144 hours for the high dose and 22.2% for the low dose. It is noted that total absorption after 144 hours is the most conservative estimate and includes the recoveries from the stratum corneum. Correcting for comparative absorption for rat and human skin on the basis that absorption for rat skin is 11.48x greater for the concentrate and 8.07x greater for the in-use dilution the estimates of dermal absorption for human skin are **0.24 %** for the concentrate and **2.75%** for the in-use dilution. These derived estimates are still comparable to estimates for human skin obtained in the in vitro study.

Additional tables for the assessment of dermal absorption in the in vivo dermal absorption study

Main study - Summary of the mean distribution of radioactivity following dermal application of [^{14}C]-EXP 11120A to male rats in a concentrated formulation (high level, 658.9 µg AE C638206/cm^2).

Results are expressed as percent applied radiochemical dose.

Group Number		3			4			5			6		
Sacrifice time (h)			8			24			72			144	
Urine		0.00	±	0.00	0.00	±	0.00	0.02	±	0.02	0.10	±	0.03
Faeces		0.00	±	0.00	0.04	±	0.02	0.17	±	0.04	0.62	±	0.16
Cage Wash		ND	±	-	0.00	±	0.01	ND	±	-	0.03	±	0.01
Tissues		0.22	±	0.14	0.05	±	0.03	0.09	±	0.02	0.15	±	0.08
TOTAL DIRECT ABSORPTION		**0.22**	**±**	**0.14**	**0.10**	**±**	**0.05**	**0.29**	**±**	**0.05**	**0.91**	**±**	**0.20**
Dose Site	Treated Skin [1]	0.05	±	0.04	0.04	±	0.02	0.04	±	0.03	0.05	±	0.02
Stratum Corneum	(Tape strips [2])	3.52	±	1.28	7.59	±	1.79	2.36	±	0.86	1.69	±	0.75
Scissor Wash [3]		0.00	±	0.00	0.00	±	0.00	0.01	±	0.01	ND	±	-
TOTAL AT DOSE SITE		3.57	±	1.28	7.63	±	1.81	2.42	±	0.85	1.74	±	0.75
TOTAL AVAILABLE FOR ABSORPTION (Direct absorption + treated skin + stratum corneum)		**3.79**	**±**	**1.26**	**7.73**	**±**	**1.78**	**2.69**	**±**	**0.81**	**2.65**	**±**	**0.64**
Razor Head Wash [4]		NS	±	-	NS	±	-	0.13	±	0.08	0.09	±	0.07
Dose Site Shavings [4]		NS	±	-	NS	±	-	1.23	±	0.38	0.64	±	0.48
Surface Dose	(Tape strips 1 & 2)	0.67	±	0.26	1.25	±	0.36	1.15	±	0.24	0.49	±	0.17
Skin Swabs		90.69	±	2.66	86.82	±	2.93	88.60	±	2.87	86.79	±	4.59
Gauze Wash		3.69	±	3.20	3.67	±	2.57	3.47	±	2.46	5.89	±	1.25
TOTAL NON-ABSORBED		**95.05**	**±**	**3.46**	**91.74**	**±**	**2.19**	**94.58**	**±**	**1.13**	**93.90**	**±**	**5.07**
OVERALL RECOVERY		**98.84**	**±**	**2.47**	**99.47**	**±**	**1.05**	**97.28**	**±**	**1.01**	**96.55**	**±**	**4.75**

ND Results within background range

NS No Sample

[1] Treated skin remaining after tape-stripping

[2] Excluding tape strips 1 & 2 which are considered to be non-absorbed surface dose

[3] Wash of scissors used to dissect dose site

[4] Prior to tape stripping regrowth of hair at the dose site was shaved off using disposable razors

Main study - Summary of the mean distribution of radioactivity following dermal application of [^{14}C]-EXP 11120A to male rats in a diluted formulation (low level, 1.43 µg AE C638206/cm^2).

Results are expressed as percent applied radiochemical dose.

Group Number		7			8			9			10		
Sacrifice time (h)		8			24			72			144		
Urine		0.01	±	0.01	0.27	±	0.13	2.06	±	0.65	3.16	±	1.42
Faeces		0.02	±	0.02	3.03	±	1.15	8.99	±	1.27	12.56	±	5.71
Cage Wash		ND	±	-	0.14	±	0.03	0.36	±	0.21	0.63	±	0.71
Tissues		1.67	±	0.71	3.60	±	1.33	2.89	±	0.74	1.21	±	0.49
TOTAL DIRECT ABSORPTION		**1.70**	**±**	**0.70**	**7.04**	**±**	**2.42**	**14.30**	**±**	**1.92**	**17.55**	**±**	**8.14**
Dose Site	Treated Skin [1]	0.14	±	0.07	0.18	±	0.06	0.24	±	0.24	0.07	±	0.02
Stratum Corneum	(Tape strips [2])	28.41	±	13.26	27.03	±	4.75	13.86	±	5.75	4.55	±	2.88
Scissor Wash [3]		ND	±	-	0.04	±	0.03	0.00	±	0.02	0.00	±	-
TOTAL AT DOSE SITE		**28.55**	**±**	**13.33**	**27.24**	**±**	**4.78**	**14.11**	**±**	**5.84**	**4.62**	**±**	**2.88**
TOTAL AVAILABLE FOR ABSORPTION (Direct absorption + treated skin + stratum corneum)		**30.25**	**±**	**13.85**	**34.25**	**±**	**5.56**	**28.40**	**±**	**6.01**	**22.18**	**±**	**6.51**
Razor Head Wash [4]		NS	±	-	NS	±	-	0.53	±	0.27	0.24	±	0.15
Dose Site Shavings [4]		NS	±	-	NS	±	-	3.19	±	1.75	2.75	±	1.80
Surface Dose	(Tape strips 1 & 2)	5.81	±	2.51	7.48	±	1.86	6.43	±	2.24	2.32	±	1.19
Skin Swabs		56.13	±	13.67	40.89	±	7.73	47.27	±	9.35	55.31	±	8.17
Gauze Wash		4.67	±	5.41	7.96	±	3.37	11.31	±	7.53	18.13	±	5.03
TOTAL NON-ABSORBED		**66.61**	**±**	**13.47**	**56.33**	**±**	**8.35**	**68.74**	**±**	**11.09**	**78.75**	**±**	**9.42**
OVERALL RECOVERY		**96.86**	**±**	**5.33**	**90.61**	**±**	**4.06**	**97.14**	**±**	**7.44**	**100.9**	**±**	**3.73**

ND Results within background range
NS No Sample
[1] Treated skin remaining after tape-stripping
[2] Excluding tape strips 1 & 2 which are considered to be non-absorbed surface dose
[3] Wash of scissors used to dissect dose site
[4] Prior to tape stripping regrowth of hair at the dose site was shaved off using disposable razors

Main study - Summary of the distribution of radioactivity in the stratum corneum at 8, 24, 72 and 144 hours after a single topical application of [^{14}C]-EXP 11120A at 658.9 µg AE C638206/cm^2 to male rats. Results are expressed as percent applied radiochemical dose.

Group number		3			4			5			6		
Sacrifice time (h)		8			24			72			144		
		mean	±	sd	mean	±	sd	mean	±	sd	mean	±	sd
Tape Strip Numbers	3-5	0.80	±	0.33	2.31	±	1.25	1.63	±	0.54	0.60	±	0.21
	6-8	1.27	±	0.51	3.09	±	1.16	0.64	±	0.40	0.70	±	0.25
	9-11	0.88	±	0.24	1.76	±	0.83	0.07	±	0.06	0.34	±	0.35
	12-14	0.40	±	0.27	0.41	±	0.11	0.02	±	0.01	0.14	±	-
	15-17	0.13	±	0.12	0.08	±	-	0.00	±	-	NS	±	-
	18-20	0.20	±	-	NS	±	-	NS	±	-	NS	±	-
	21-23	0.02	±	-	NS	±	-	NS	±	-	NS	±	-
OVERALL RECOVERY IN STRATUM CORNEUM		**3.52**	**±**	**1.28**	**7.59**	**±**	**1.79**	**2.36**	**±**	**0.86**	**1.69**	**±**	**0.75**

Main study - Summary of the distribution of radioactivity in the stratum corneum at 8, 24, 72 and 144 hours after a single topical application of [^{14}C]-EXP 11120A at 1.43 µg AE C638206/cm^2 to male rats. Results are expressed as percent applied radiochemical dose.

Group number Sacrifice time (h)		7 8			8 24			9 72			10 144		
		mean	±	sd	mean	±	sd	mean	±	sd	mean	±	sd
Tape Strip Numbers	3-5	12.45	±	5.73	11.04	±	2.57	6.28	±	2.25	2.41	±	1.20
	6-8	13.71	±	7.34	11.78	±	3.39	4.96	±	2.78	1.30	±	0.95
	9-11	2.19	±	1.39	3.73	±	3.89	2.23	±	2.16	0.61	±	0.85
	12-14	0.15	±	-	0.76	±	1.03	0.66	±	0.55	0.33	±	0.30
	15-17	NS	±	-	0.16	±	-	ND	±	-	0.10	±	-
OVERALL RECOVERY IN STRATUM CORNEUM		**28.41**	±	**13.26**	**27.03**	±	**4.75**	**13.86**	±	**5.75**	**4.55**	±	**2.88**

NS No sample

Summary of the distribution of radioactivity in blood at 8, 24, 72 and 144 hours after a single topical application of [^{14}C]-EXP 11120A at two dose levels to male rats. Results are expressed as ng AE C638206/g of blood and percent applied radiochemical dose.

Group number	Dose level (µg/cm^2)	Sacrifice time (h)	Mean concentration of [^{14}C]-AE C638206 in blood at sacrifice (ng/g)			Mean % applied radiochemical dose in blood *		
1	679	24	ND	±	-	ND	±	-
		72	ND	±	-	ND	±	-
		144	32.70	±	-	0.006	±	-
2	1.76	24	0.71	±	-	0.043	±	-
		72	0.68	±	-	0.047	±	-
		144	0.66	±	-	0.058	±	-
3	659	8	9.05	±	12.92	0.002	±	0.002
4		24	ND	±	-	ND	±	-
5		72	6.72	±	6.27	0.001	±	0.001
6		144	16.39	±	5.66	0.003	±	0.001
7	1.43	8	0.52	±	0.09	0.042	±	0.010
8		24	0.91	±	0.26	0.079	±	0.023
9		72	1.14	±	0.23	0.099	±	0.025
10		144	0.57	±	0.12	0.049	±	0.009

* Calculated assuming total blood weight is 7% of bodyweight.

ND Results within background range.

B.6.12.3 Summary of dermal absorption and calculation of dermal absorption values

(As reported in DAR Volume 3)

In an in vitro dermal absorption study using rat and human skin, the investigators concluded that the data showed the total amounts of applied radioactivity absorbed by 24 hours at the high dose level were 0.022% and 0.172% while at the low dose level the amounts absorbed were 1.454% and 14.26% in human and rat skin, respectively. The total amount of applied radioactivity absorbed by 24 hours was 7.800 times greater for rat skin than human skin following application of the high level formulation, and 9.807 times greater for rat skin than human skin following the low dose application.

However, the reviewer noted that estimates of dermal absorption for risk assessment must include the absorbed and absorbable fraction present and biologically available in particular from in vitro skin samples. Hence the estimates of comparative relative dermal absorption for rat skin compared with human skin were 11.48 times greater for the high dose and 8.07 times greater for the low dose. In vivo data in rats showed that the amount of radiolabel in blood continued to increase up to 144 hours suggesting that bioavailable radiolabel in the skin should not be discounted.

In the in vivo dermal absorption study in rats, the investigators concluded that the total amount of radioactive material absorbed was 2.65% at 144 hours for the high dose and 22.2% for the low dose. It is noted that total absorption after 144 hours is the most conservative estimate and includes the recoveries from the stratum corneum. Correcting for comparative absorption for rat and human skin on the basis that absorption for rat skin is 11.48x greater for the concentrate and 8.07x greater for the in-use dilution the estimates of dermal absorption for human skin are **0.24 %** for the concentrate and **2.75 %** for the in-use dilution. These derived estimates are still comparable to estimates for human skin obtained in the in vitro study.

B.6.1.4.1 Assessment of Relevance of Groundwater metabolites

Open Point 2.10, 3.6, 4.20 & 5.12:
RMS to present the complete assessment for the relevance of ground water metabolites in an addendum. Special attention should be paid to the fact that at this stage for metabolites M-01, M-05 and M-10 the trigger of 0.75 μg/L is also exceeded either in the lysimeter or the FOCUS modelling.

In the environmental fate and behaviour assessments Sections B8.9 and B8.10, of the original DAR, a need for an assessment of the relevance of the metabolites M-01, M-05, M-10, M-11, M-12, M-13 and M-14 was identified. These metabolites were either predicted to occur in groundwater at >0.1 μg/l or were found in lysimeter leachate at an annual average concentration >0.1 μg/l.

New FOCUS groundwater modelling has now been submitted by the applicant and this has been evaluated by the RMS and presented in Section B.8.6.2 of Addendum 1 (November 2007). Following consideration of this new FOCUS groundwater modelling, the following metabolites are predicted to have potential to exceed 0.1 μg/l in groundwater: M-01, M-03 (acidic soils), M-05, M-10, M-11, M-12 and M-13 (NB. M-14 was not predicted >0.1 μg/l in the new modelling, but it was >0.1 μg/l in the lysimeter leachate).

Following these findings, a full revised relevance of metabolites in groundwater assessment following EU Guidance Document - Sanco/221/200-rev 10, 25 February 2003 is presented below for all those metabolites that exceed 0.1 μg/l in either consideration:

The assessment follows the step-wise approaches as outlined in the Guidance Document.

STEP 1: EXCLUSION OF DEGRADATION PRODUCTS OF NO CONCERN

All of the metabolites observed in the soil metabolism and lysimeter studies contain either the pyridine ring or the phenyl ring and therefore are not automatically of no concern. In addition there was insufficient information available on their possible natural occurrence and/or of their toxicological or ecotoxicological properties prior to initiating the testing program (See Appendix 1 for chemical structures).

STEP 2: QUANTIFICATION OF POTENTIAL GROUNDWATER CONTAMINATION

As summarised in the original DAR a comprehensive range of studies have been conducted under laboratory, outdoor and field conditions to quantify the potential concentrations in groundwater. The following metabolites were identified in the original DAR to be < 0.1 μg/l: M-15 (AE 1413903 or P8) and M-02 (AE C657188 or PCA). Based on the new FOCUS groundwater modelling the following metabolites are predicted to have potential to exceed 0.1 μg/l in groundwater: M-01, M-03 (acidic soils), M-05, M-10, M-11, M-12 and M-13. Therefore, a summary of the overall

position comparing both results are presented below (See Section B.8.6.2 for full details):

Comparison of results with original groundwater assessment in DAR, B.8.6.2

Metabolites exceeding 0.1 µg/l

The original groundwater assessment for fluopicolide and its metabolites (reported in the DAR, B.8.6.2) was carried out using FOCUS PELMO with standard degradation and sorption parameters and for use on vines, assumed greater crop interception than considered here. The results indicated that parent and the metabolites, M-01, M-03, M-05, M-10, M-11, M-12 and M-13 had potential to exceed 0.1 µg/l at various scenarios (see Table 8.32, Addendum 1(Nov 2007)).

The new groundwater modelling with PELMO (assuming less crop interception for vines) and PEARL (incorporating sorption kinetics), results in the same metabolites being predicted to have potential to contaminate groundwater above 0.1 µg/l. No additional metabolites are predicted to exceed 0.1 µg/l, following proposed use of fluopicolide to vines.

The original groundwater assessment (DAR, B.8.6.2) with FOCUS PELMO assumed application to potatoes, once every 3 years. It resulted in predicted concentrations of fluopicolide being < 0.1 µg/l, but metabolites M-01, M-5, M-10, M-11, M-12 and M-13 were predicted to have potential to contaminate groundwater > 0.1 µg/l.

The new groundwater modelling with PELMO (assuming application to potatoes also every 2 and every 3 years) and with PEARL (incorporating sorption kinetics), results in the same metabolites being predicted to have potential to contaminate groundwater above 0.1 µg/l. However, for application every year, parent compound and M-03 are also predicted to exceed 0.1 µg/l for certain scenarios.

Predicted concentrations of M-03 exceed 0.1 µg/l in both the PEARL and PELMO models, following application to potatoes every 2 years, and also in PEARL after application every 3 years, (though not in PELMO). Following application to potatoes every 3 years, M-13 did not exceed 0.1 µg/l in PEARL, though it did at one scenario in PELMO.

Number of scenarios where 0.1 µg/l is exceeded

For use of fluopicolide on vines, the number of scenarios where 0.1 µg/l was exceeded by parent or metabolites is almost the same, when comparing the results of new and previous PELMO modelling. Incorporation of sorption kinetics in PEARL modelling, gave slightly fewer scenarios exceeding 0.1 µg/l for parent, M-05, M-10 and M-12, but otherwise was similar.

For use of fluopicolide on potatoes, the results of PELMO modelling for application once every 3 years are essentially the same as previously reported in the DAR. Assuming more frequent application, i.e. every year or every 2 years, modelling with PELMO gave a greater number of scenarios where 0.1 µg/l was exceeded, as shown in Table 8.39.

Incorporation of sorption kinetics in PEARL modelling for use on potatoes generally gave an increased number of scenarios at which concentrations of metabolites exceeded 0.1 µg/l, (increasing with frequency of application). There were some exceptions: for M-13, the number of scenarios with concentrations > 0.1 µg/l were similar to those with PELMO and for application every 3 years were all <0.1 µg/l in PEARL. For M-12, the number of scenarios with concentrations >0.1µg/l were slightly fewer in PEARL, than those with PELMO. For M-11, the number of scenarios with concentrations >0.1µg/l were one less than in PEARL, for application once every 3 years).

Differences in 80th percentile concentrations of parent and metabolites

For use of fluopicolide on vines, the assumption of less crop interception in PELMO modelling resulted in higher 80th percentile annual average concentrations for parent and metabolites, as would be expected. The incorporation of sorption kinetics in PEARL modelling gave lower PECgw values for parent fluopicolide, than in the original PELMO assessment, but in some cases concentrations of metabolites were higher (e.g. M-01, M-03, M-05, M-10, M-11, M-12 and M-13. Compare Tables 8.31 and 8.32).

For use of fluopicolide on potatoes, revised PELMO modelling assuming more frequent application (every year or every 2 years) gave higher PECgw values for parent and metabolites, as would be expected. Incorporating sorption kinetics into PEARL modelling generally gave similar or slightly lower PECgw, compared to the results of PELMO modelling, with application every 3 years. (See Table 8.38 compared with the column for "application 1 in 3 years" of Table 8.39, the results of which are equivalent to those originally reported in the DAR).

For application to potatoes every 2 years, PEARL modelling gave a slightly higher 80th percentile concentration for M-05, but similar or lower concentrations for parent and other metabolites, compared to corresponding results with PELMO. For application every 3 years, PEARL gave higher 80th percentile concentrations for M-03, M-05 and M-14, but similar or lower concentrations for parent and the other metabolites, compared to corresponding results with PELMO.

Conclusion on potential groundwater contamination:

For use on vines, fluopicolide is predicted to contaminate groundwater above the maximum acceptable concentration (0.1 µg/l) at one or two of the 7 scenarios modelled, (Châteaudun and or Piacenza). Concentrations of the metabolites M-01, M-05, M-10, M-11, M-12 and M-13 were predicted to exceed 0.1 µg/l in groundwater. Of these, M-01, M-05, M-10, M-11 and M-12 exceeded 0.1 µg/l in all, or almost all of the scenarios simulated in both PELMO and PEARL. In particular, predicted concentrations of M-01 were significantly higher than this limit (range 1.6-6.3 µg/l). Metabolites M-03 and M-13 only exceeded 0.1 µg/l in some scenarios, (and for M-03 the scenarios were those with acidic soils). Therefore, the relevance of these metabolites needs to be assessed further, in accordance with the EU Guidance Document on the assessment of the relevance of metabolites in groundwater.

In the view of the RMS, application every year to potatoes is considered to be extreme and not representative in the vast majority of cases. For use of fluopicolide as proposed on potatoes, assuming application every 2 or 3 years, fluopicolide was not predicted to contaminate groundwater above 0.1 µg/l. However, M-01 exceeded 0.1 µg/l in all or almost all of the modelled scenarios (up to 2 µg/l for application every 2 years and 3.2 µg/l for application every 3 years). Metabolites M-03, M-05, M-10, M-11, M-12 and M-13 also exceeded the 0.1 µg/l limit for various scenarios. Therefore, as above for vines, the relevance of these metabolites need to be assessed further, in accordance with the EU Guidance Document.

Overall, it can be seen that the revised modelling has not resulted in any additional metabolites being predicted to occur at >0.1 µg/l on an annual average basis. The highest concentrations of fluopicolide metabolites from either modelling or lysimeter study seen in the original DAR compared to the highest results from modelling in this addendum are presented below. These have been tabulated simply on the basis of concentration and ignore the GAP used to produce the PEC values and the model used. However, it should be noted that some of the highest concentrations from modelling in this addendum are from use every year on potatoes which the RMS considers to be extreme worst-case and inappropriate as a regulatory scenario.

Comparison of highest metabolite groundwater PEC values from original DAR and this addendum for regulatory decision-making (µg/l)

	Highest concentrations in original DAR	**Highest concentrations in addendum**
M-01	4.614 (H)	**6.733** (H)
M-02	0.033 (P)	0.038 (P)
M-03	0.381 (H)	**0.525** (H)
M-05	0.90 (L)	0.715 (H)
M-10	0.83 (L)	0.586 (H)
M-11	0.55 (L)	**0.813** (J)
M-12	0.36 (L)	**0.542** (J)
M-13	0.160 (H)	**0.369** (J)
M-14	0.19 (L)	0.033 (H)

Values in **bold** are increases from the original DAR values
P = Piacenza; H = Hamburg; L = lysimeter; J = Jokioinen

Thus it can be seen that the highest concentrations of regulatory significance for most metabolites have increased as a result of this new assessment. It should be noted that for M-11, the revised concentration is >0.75 µg/l, whereas in the original DAR the concentration was <0.75 µg/l. This has implications for the relevance assessment. However, it must be realised that the highest concentration occurred on potatoes assuming that the crop was grown every year. In the opinion of the RMS, this is an extreme and unrepresentative GAP for potato, and in GAP assuming a rotation of 1 in 2 years or longer, 0.75 µg/l was not exceeded. In vines, the concentration of M-11 was <0.75 µg/l.

STEP 3: HAZARD ASSESSMENT -- IDENTIFICATION OF RELEVANT METABOLITES

Progressing to step 3 requires the assessment to be conducted in three stages:

- Stage 1: screening for biological activity
- Stage 2: screening for genotoxicity
- Stage 3: screening for toxicity

STEP 3, Stage 1: screening for biological activity

Based on the quantification of potential groundwater contamination the following metabolites are identified as metabolites with the potential to exceed the 0.1 µg/l and need to be considered for biological activity: M-01, M-03, M-05, M-10, M-11, M-12, M-13 and M-14.

One of the key stages in the assessment of potential relevance of a metabolite is the determination of biological activity. Many small molecules with molecular weights below 200 can be found to occur naturally in soil as a result of organic matter decomposition. The key distinguishing feature of the metabolites formed from plant protection products is the potential to have biological activity and therefore retain the properties of the xenobiotic.

As stated in the Guidance Document Sanco/221/2000, rev. 10, 25 Feb 2003, the goal is to identify metabolites which have comparable target activity as the parent active ingredient. It also states that efficacy testing should be focused on the question of comparing the activity against the biological target. Included in this assessment is the structure-activity relationship and the necessary functional groups to give the fungicidal activity that is present in the parent fluopicolide (AE C638206) molecule.

The metabolites M-01 (AE C653711), M-02 (AE C657188), M-05 (AE 1344122), M-10 (AE 1344123), M-14 (AE 1388273) and M-15 (AE 1413903) were therefore tested for their fungicidal activity in comparison with the parent AE C638206 (Latorse, M.P., Flahout, J. 2004, C038369) (see Appendix 3 (B.10.7.5). The six metabolites did not show any biological activity in comparative tests with the parent fluopicolide, which showed biological effects in the range of 80 -100%.

It is known from the biological screens that both the pyridine and phenyl ring parts of the molecule are required for fungicidal activity therefore the metabolites without both these rings would be predicted to have no fungicidal activity. It is also known that adding functional groups, especially polar ones, to the phenyl ring causes loss of fungicidal activity. Therefore the addition of SO_3H in the case of M-15 or SO_3H and OH in the case of M-16 would result in the loss of fungicidal activity. Of the remaining metabolites that triggered a consideration of biological activity only M-03, M-11, M-12 (mixture of 2 isomers) and M-13 were not tested for fungicidal activity. Three (M-11, M-12 and M-13) are all single pyridine ring structures and are unlikely to have any significant fungicidal activity. M-03 is a structurally-related transient hydroxylated-derivative of fluopicolide and is an unstable intermediate prior to cleavage of fluopicolide to M-01 and M-02. It is very unstable in water and at

environmental pH will rapidly degrade to M-01 and M-02 and the RMS considers it inconceivable that significant exposure to M-03 will occur via groundwater.

The RMS concludes that all metabolites theoretically occurring in groundwater >0.1µg/L will not retain or express biological activity of the parent, fluopicolide.

STEP 3, Stage 2: screening for genotoxicity

In the guidance document Sanco/221/2000 rev.l0, there is a requirement that metabolites that have shown some potential to be mobile and are not biologically active should be screened for their genotoxic activity in a series of three in vitro genotoxicity studies. These three study types are the Ames test, gene mutation test with mammalian cells and the chromosome aberration test. The guidance document also states that equivocal results in *in-vitro* studies should be substantiated by *in vivo* experiments.

Metabolite M-01
The genotoxicity profile of M-01 was assessed in three in vitro and one in vivo assays and no evidence of genotoxicity was observed in any assays. The *in vitro* studies were the bacterial gene mutation assay in bacterial cells, V79/HPRT gene locus assay, and unscheduled DNA synthesis (UDS) assay and the mouse micronucleus assay *in vivo*. Overall, M-01 is not considered a genotoxic compound. Although no *in vitro* chromosmal aberration study has been performed, the liver UDS assay is considered an acceptable equivalent given the hepatotoxicity of M-01.

Metabolite M-02
The new FOCUS modelling has confirmed the findings of the original DAR and M-02 not to be a significant groundwater metabolite (See Section B.8.6.2, Addendum 1, November 2007). However, genotoxicity data are presented here as supporting information to metabolites with similar structures. The genotoxicity profile of M-02 was assessed in three in vitro assays which included the bacterial gene mutation assay in *S. typhimurium* strain and *E. coli* strains, chromosomal aberration assay in cultured human peripheral blood lymphocytes and the V79/HPRT gene locus assay. There was no evidence of genotoxicity in any of the assays. Therefore, as the M-02 is not a significant metabolite or genotoxic it can be considered as non-relevant.

Metabolite M-05
The genotoxicity profile of M-05 was assessed in three in vitro assays which included the bacterial gene mutation assay, chromosomal aberration assay and the V79/HPRT gene locus assay for forward mutations. There was no evidence of genotoxicity in any of the assays.

Metabolite M-10

The genotoxicity profile of M-10 was assessed in three in vitro and two in vivo assays. M-10 was devoid of any mutagenicity potential in the bacterial reverse mutation assay as well as in the chromosome aberration assay performed in human lymphocytes. A positive response was observed in the *in vitro* mammalian HPRT gene locus assay in Chinese hamster V79 cells. A mouse micronucleus assay and a rat UDS assay were run to assess the genotoxic potential of M-10 in the whole animal. No evidence of genotoxicity was observed in these two in vivo assays at concentrations showing cytotoxicity of the target organs. Therefore, these findings clearly show that the metabolite M-10 is not a genotoxic compound.

Metabolite M-14

The genotoxicity profile of M-14 (AE 1388273 or P7) was assessed in three *in vitro* and two *in vivo* assays. M-14 was devoid of any mutagenicity potential in the bacterial reverse mutation assay as well as in the mammalian HPRT assay in cultured Chinese hamster V79 cells. A positive response was observed in the *in vitro* chromosomal aberration assay performed in human lymphocytes. A mouse micronucleus assay and a rat UDS assay were run to assess the genotoxic potential of M-14 in the whole animal. There was no evidence of genotoxicity in the two *in vivo* assays at concentrations showing cytotoxicity of the target organs. Overall, M-14 has no genotoxic potential. In the new groundwater modelling assessment (see See Section B.8.6.2, Addendum 1, November 2007), M-14 has been shown not to be a significant groundwater metabolite. It is therefore concluded that M-14 is a non-relevant metabolite.

Metabolites M-03, M-11, M-12 and M-13

Specific genotoxicity data has not been provided for M-03, M-11, M-12 and M-13.

M-03 (P3) is a proposed transient intermediate metabolite of fluopicolide in rats, formed by hydroxylation of the methyl group adjoining the benzamide group before further metabolism by cleavage to provide M-02, M-01 and their derivative metabolites and conjugates (Figure 6.6). Approximately 10% of a dose of fluopicolide appears to be metabolised via M-03 (based on Table 6.25 of the original DAR). The genotoxic potential of M-03 is considered to be addressed by the specific information on the genotoxic potential of fluopicolide, and the metabolites M-01 and M-02 (see above). M-03 is considered unlikely to be genotoxic.

M-11, M-12 (mixtures of isomers P2a & P2b), M-13 and indeed M-14 have been shown in the threshold of concern assessment for exposure by the Applicant to be not relevant. Nonetheless, comparison of the molecular structures of the metabolites M-11, M-12 and M-13 with that of M-10 and M-14 for which genotoxicity studies have been conducted suggests that M-11, M-12 and M-13 are quite similar and unlikely to possess a significantly different biological and genotoxic properties than that assessed for M-10 and M-14. M-11, M-12 & M-13 do not have any structural alerts for genotoxicity and are considered unlikely to be genotoxic.

STEP 3, Stage 3: screening for toxicity

Stage 3 of Step 3 is aimed at the question of whether a metabolite has certain toxicological properties, which - from a regulatory perspective - qualify for considering it “relevant”. A metabolite is considered “relevant” if its toxicological properties lead to a classification as toxic of very toxic (T or T+) according to Directive 67/548/EEC. Therefore, in addition to genotoxicity testing, further toxicity testing has been conducted to determine whether the metabolite has certain toxicological properties which from a regulatory perspective would qualify it to be classified as relevant. These studies include metabolism studies to understand the adsorption, distribution, metabolism and elimination from the body.

Metabolite M-01

Following single oral administration of [14C]-M-01 to the male and female rat at the rates of 10 and 150 mg/kg most of the administered radioactivity was eliminated in the urine (ca 82 %dose) although the rate of elimination was relatively slow. Lower levels (ca 13 %dose) were eliminated via the faeces. The highest concentrations in tissues were seen in the kidney (ca 0.57 µg equiv./g) and liver (ca 0.44 µg equiv./g) for the 10mg/kg dose group and in the skin & fur (3.8 to 5.0 µg equiv./g), kidneys (2.8 to 3.0 µg equiv./g) and liver (2.1 to 2.3 µg equiv./g) for the 150 mg/kg dose group. Tissue concentrations therefore increased by approximately five-fold for a fifteen-fold increase in dose rate. Overall, multiple dosing (14 daily doses at 10 mg/kg) did not have any significant impact in the absorption, distribution, metabolism and elimination compared to results after single oral dosing. Thus, the results in this study showed that the routes and the rates of excretion were maintained despite the multiple dosing, which meant that most of the radioactivity was eliminated via the urinary route. The distribution pattern in the tissues was also similar between single and multiple dosing with the highest mean concentrations observed in the skin & fur (3.0 µg equiv./g), kidney (1.9 µg equiv./g) and liver (1.3 µg equiv./g). Bioretention or accumulation was therefore not indicated. The routes of biotransformation was similar between dose levels and sexes with hydrolysis of the amide group to form AE C416656, hydroxylation to form hydroxy-BAM (M-04) and subsequent conjugation with either glucuronic acid or sulphate, and the loss of a chlorine atom following glutathione conjugation. Further metabolism of the glutathione group to the mercapturic acid or S-methyl metabolites was observed.

M-01 was shown to be of relatively low acute oral toxicity, however the available data indicate that it is of greater acute oral toxicity than fluopicolide (LD50 >5000 mg/kg bw). The LD50 of M-01 was found to be >2000 mg/kg bw in males and >500 mg/kg bw in females in a modern study using the acute toxic class method (OECD 423). However, in an older non-GLP study (performed to OECD 401) LD50 values of 1470 (951–2270) and 2330 (1430–3780) mg/kg bw were calculated for male and female rats respectively. The findings of the two studies are therefore inconsistent, but taken together do indicate that M-01 is of slightly greater acute oral toxicity than fluopicolide.

In a 13-week toxicity study performed in CD rats with M-01 at doses up to 2300 ppm, reduced body weight gains and food consumption was observed at dose levels of ≥ 600 ppm but no target organ toxicity was observed. The NOAEL of M-01 was 180 ppm (equivalent to 14 mg/kg bw/day) in both males and females.

In comparison, the NOAEL for the fluopicolide 90-day rat study was 100 ppm (equivalent to 7.4 or 8.4 mg/kg bw/d in males and females respectively), based on treatment-related haematological (reduced haemoglobin and haematocrit in male rats), clinical chemistry (increased cholesterol) and urinalysis findings (increased urine volume and specific gravity in females); organ weight changes (increased relative liver and kidney weights in males and relative spleen weight in females) and histopathological changes in the liver and kidneys at the LOAEL of 1400 ppm (equivalent to 109 or 119 mg/kg bw/d in males and females respectively). Findings show that the short-term toxicity of fluopicolide and M-01 is comparable, and therefore that further (long-term) studies with M-01 are not required.

Notifier has, however, provided a 2-year rat chronic toxicity study performed with M-01. The results of this study indicate that the liver is the target organ of toxicity; a NOAEL of 180 ppm (equivalent to 5.7 and 8.6 mg/kg bw/d in males and females respectively) can be determined. The NOAEL in this study is therefore comparable to the NOAEL of 200 ppm from the rat chronic toxicity/carcinogenicity study performed with fluopicolide, indicating similar long-term toxicity. In the M-01 study, a slight (but not statistically significant) increase in the incidence of hepatocellular adenoma was seen in males at the top dose level of 500 ppm; a dose level considered to exceed the MTD. No evidence of carcinogenicity was seen in this study. A detailed assessment of the carcinogenic potential of M-01 is presented after the conclusion section below.

In conclusion, these data showed that the toxicological profile of the metabolite M-01 is similar to that of fluopicolide.

Metabolite M-02

Metabolism studies in rats dosed with [pyridyl-2,6-14C]-M-02 at a nominal dose level of 10 mg/kg bw showed that the rate of elimination was rapid for both male and female rats with at least 90% of the total administered radioactivity eliminated within the first 48 hours post dose. The total recovery in urine accounted for a mean of 78.5 % dose. Lower levels were found in the faeces with mean values that accounted for cumulative mean values of 6.6 %dose. No pulmonary excretion was detected. The estimated minimum mean level of absorption was calculated to be 87%. Thus this metabolite of fluopicolide demonstrated high oral bioavailability and low potential for bioaccumulation. No sex difference was observed in terms of the routes and rates of elimination of [14C]-M-02. There was little remaining radioactivity in the tissues with 0.23% and 0.30% of the administered dose for males and females respectively. The metabolism investigations showed that M-02 was the major fraction excreted in urine and faeces.

M-02 was shown to be of very low acute toxicity with an oral LD50 of > 2000 mg/kg bw in rats. Therefore, no classification is required for acute oral toxicity.

In a 28-day toxicity study in CD rats with M-02 at doses up to 20000 ppm, there was no evidence any effects up to and including the top dose ; no target organ toxicity was identified. The NOAEL of M-02 was 20000 ppm, equivalent to 1500 mg/kg bw/day in both males and females.

In conclusion, these data showed that the metabolite M-02 is less toxic than parent fluopicolide.

Metabolite M-05
M-05 was shown to be of very low acute toxicity with an oral LD50 >5000 mg/kg bw in rats. Therefore, no classification is required.

In a 28-day oral toxicity study in rats with M-05 at doses up to 20000 ppm, slight reduction in body weight gains and degeneration/regeneration in the kidneys was observed in animals at 20000 ppm, the highest test dose. The NOAEL was 2000 ppm (equivalent to 152 mg/kg/day and 167 mg/kg/day in males and females, respectively).

In conclusion, the data showed that the metabolite AE1344122 is less toxic than parent fluopicolide.

Metabolite M-10
M-10 was shown to be of very low acute toxicity with an oral LD50 of >5000 mg/kg bw in rats. No classification is required for acute oral toxicity of M-10.

In a 28-day oral toxicity study in rats with M-10 at doses of up to 20000 ppm, there was no evidence of any systemic effects at dose levels of up to 20000 ppm. Diarrhoea was increased at 20000 ppm. The clear NOAEL of M-10 was 2000ppm (equivalent to 164 & 240 mg/kg bw/day, in males and females, respectively).

The data showed that the metabolite M-10 is less toxic than parent fluopicolide.

Summary of toxicological Studies with Metabolites

	Genotoxicity						Toxicity		
	In-vitro				*In-vivo*				
Metab-olite	Ames test	C'some aberration	UDS	HPRT	M'nucleus	UDS	LD50 mg/kg	28 / 90 day rat NOAEL (mg/kg/day)	2 year rat NOAEL (mg/kg/day)
M-01 (BAM)	negative	N/R	negative	negative	negative	N/R	1000 - 2000	14 (90 day)	5.7 males / 8.6 females
M-02	negative	negative	N/R	negative	N/R	N/R	>2000	1574 males / 1581 females	N/A
M-05	negative	negative	N/R	negative	N/R	N/R	>5000	152 males/ 167 females	N/A
M-10	negative	negative	N/R	positive	negative	negative	>5000	164 males/ 240 females	N/A
M-14	negative	positive	N/R	negative	negative	negative	N/R	N/R	N/A
Fluopi-colide	negative	equivocal	N/R	negative	negative	negative	>5000	17 (28 d) 7 m/8 f (90 d)	8.4 males / 10.8 females

N/R – not required according to EC guidance documents

Therefore, it can be concluded that none of the metabolites M-01 (BAM), M-02, M-05, M-10 and M-14 lead to a classification as toxic (T) or very toxic (T+). In addition since the parent is not classified as a reproductive toxicant and is not carcinogenic the metabolites also have no reason to qualify for reproductive or carcinogenic testing. It has been confirmed that none of the metabolites are genotoxic. Therefore from a toxicological perspective all the metabolites pass the assessment of Stages 2 and 3 and are considered non-relevant.

STEP 4: EXPOSURE ASSESSMENT - THRESHOLD OF CONCERN APPROACH

For those metabolites for which the exposure assessment shows they are below the threshold of concern which is given in the Guidance Document as 0.75 µg/L they can be determined to be non relevant at Step 4. Therefore, based on the data presented in the original DAR and following the new modelling, the following metabolites can be deemed non relevant at this Step: M-03, M-12, M-13 and M-14. However, the following metabolites are identified as requiring a refined risk assessment as the highest concentration in the DAR or addendum evaluation is predicted to be above 0.75 µg/l: M-01, M-05, M-10 and M-11.

Based on the proposed ADI for fluopicolide, 0.08 mg/kg bw, a health based drinking water limit of 240µg/L can be proposed. This is based on a 60kg person consuming 2L of water per day and allocating 10% of the ADI to drinking water. Predicted levels of the fluopicolide metabolites are all <5% of the health based drinking water limit for fluopicolide. Therefore the metabolites are not considered to present a concern to human health.

STEP 5: REFINED RISK ASSESSMENTS FOR THE REMAINING METABOLITES

The metabolites: M-01, M-05, M-10 and M-11 have been found to lie in the concentration range between 0.75 µg/L and 10 µg/L. Therefore, a refined risk assessment is presented below:

M-05 was negative in three *in vitro* genotoxicity assays and is of low acute oral toxicity and of lower repeat dose toxicity than fluopicolide.

M-10 exhibited evidence of genotoxicity in an *in vitro* assay for gene mutation in mammalian cells, but was negative in *in vivo* assays for micronucleus formation and UDS. M-10 is of low acute toxicity and significantly lower repeat dose toxicity than fluopicolide.

M-11 is structurally very similar to M-10. The only difference is the presence of a hydroxy- group on the pyridinyl ring, which is not a structural alert for genotoxicity. This minor change is considered unlikely to have a significant effect on the observed toxicity of M-11 relative to M-10. The hydroxy- group is likely to enhance the

excretion of M-11 relative to M-10. Metabolite M-14 is also of a similar structure to M-11, having a hydroxy- group on the ring in place of the carboxy acid moiety and a methylated sulphonic acid group. It is considered that the toxicity of M-11 will be similar to that of M-10 and M-14 and thus lower than that of fluopicolide.

M-01 was negative in three *in vitro* genotoxicity assays and in an *in vivo* assay for micronuclei induction. M-01 is more acutely toxic than fluopicolide, but in 90 day and 2-year studies the toxicity of both compounds is considered equivalent, taking account of dose spacing and relative molecular weights. M-01 had no biological (fungicidal) activity. Predicted exposures to M-01 from fluopicolide use are <5% of the health based Maximum Acceptable Concentration (MAC) of fluopicolide in drinking water.

Predicted Dietary exposure to M-01, M-05, M-10 and M-11 is also expected to be low and would not add significantly to consumer exposure via sources other than drinking water. Therefore the metabolites are not considered to present a concern to human health.

CONCLUSION

To conclude, all the metabolites meet the criteria of the guidance document and are found to be toxicologically non-relevant in groundwater. In addition, the ecotoxocological assessment (see Section B.9.2) also concludes that the metabolites can be considered environmentally 'non-relevant'.

A FURTHER DETAILED DISCUSSION ON THE CARCINOGENIC POTENTIAL OF M-01 (BAM/ 2.6-DICHLOROBENZAMIDE)

Genotoxicity

BAM has no genotoxic potential and is not considered toxicologically relevant for this criterion. It was negative in a complete range of studies required for the assessment of genotoxic potential.

i) BAM was not mutagenic without and with S9 mix in the plate incorporation as well as in the pre-incubation modification of the Salmonella/microsome test.
ii) BAM was not mutagenic in the V79/HPRT Forward Mutation Assay both with and without metabolic activation under the conditions of the assay.
iii) BAM was negative in the unscheduled DNA synthesis or DNA repair assay using primary cell cultures of rat hepatocytes.
iv) BAM was not genotoxic in the in vivo mouse micronucleus assay in bone marrow erythrocytes.

Carcinogenicity

The RMS has considered the incidence of adenomas in top dose females in the 2-year dietary study in rats with BAM together with other the indications of liver toxicity, evidence of systemic toxicity and the strengths and weaknesses in the two year study. These are covered in more detail in the text and tables below.

Evidence of significant toxicity in both sexes was characterised by significant reduction in body weight gain in both sexes at 500 ppm, slightly greater in females than in males (Table 1 and significant reduction in erythrocyte parameters (haemoglobin concentration, erythrocyte counts and haematocrit) occasionally mainly in males and to a lesser extent in females at 500 ppm (Table 2) although statistical significance was not attained on every occasion. These effects on body weight and red blood cell parameters would suggest that at a dose of 500 ppm, the maximum tolerated dose was exceeded.

The re-evaluation of the pathological findings in slides produced from liver sections taken in a rat study forms the basis for the assessment of the histopathological findings in the toxicity study. The complete summary of liver findings is provided in Tables 3, 4 and 5a, b & c.

i. The incidence of benign hepatocellular adenomas in female rats at the top dose level was stated to be marginally statistically significant (P=0.049) according to the report of the reviewing pathologist. However the investigating laboratory have subsequently stated that the statistical methods used in this report were not appropriate, and that the tumour incidence in this group is not in fact significant. A statistical re-evaluation by the Notifier identified a P-value of 0.14. However, it should be noted that the statistical evaluation comparing control and top-dose animals is complicated by the small population size for this kind of study and the absence of adenomas in all dose groups except for top dose females.

ii. There was no indication of progression from adenomas to carcinomas.

iii. Non-neoplastic indications of hepatotoxicity (e.g. eosinophilic foci) were similar in both sexes indicating that if M-01 were carcinogenic, a similar tumour response might be expected in both sexes. A combined assessment of liver tumours for both sexes does not suggest a treatment-relationship for the increased number of adenomas in top dose females. Comparatively in males, hepatocellular carcinomas were observed at dose levels of ≤ 180 ppm but no carcinomas were observed at the 500 ppm in males, the dose responsible for the slight increase in adenomas in females, and only a single incidence of adenoma was observed in top dose males.

iv. Changes routinely seen with compounds producing liver tumours were not reported in the study with BAM. Clinical chemistry parameters did not show any changes suggestive of liver toxicity. Organ weights of the liver also did not reveal any changes normally associated with a liver carcinogen.

Conclusion

The RMS concludes that there was no evidence of substance related carcinogenicity and the weight of evidence as discussed above suggests that BAM is unlikely to pose a carcinogenic risk to humans and does not meet the EC criteria for classification for carcinogenicity.

Summary tables of relevant findings in the assessment of carcinogenicity study are provided.

Table 1: Summary of mean body weights

	Dose level (ppm)									
	Males					Females				
	0	**60**	**100**	**180**	**500**	**0**	**60**	**100**	**180**	**500**
Mortality	20	19	25	17	20	18	19	19	22	15
Body weights	137									
Wk 13[a]	521	504	508	502	476	303	299	303	286	273
Wk 26	629	609	618	610	580**	346	349	354	336	311**
Wk 52	720	701	704	698	664*	429	414	425	399	368**
Wk 78	808	773	777	759	732*	520	494	512	495	*
WK106	792	759	740	766	686*	616	580	572	533	454* 489**

* P<0.05; ** P<0.01; *** P<0.001

[a] not statistically assessed.

Table 2: Group mean haematological changes at week 106

Week	Dose level (ppm)									
	Males					Females				
	0	60	100	180	500	0	60	100	180	500
Hematocrit (%)	46	-	-	45	**41***	42	-	-	43	43
Haemoglobin (g%)	14.9	-	-	14.8	**13.5***	14.2	-	-	13.9	**13.5**
RBC ($x10^6$/cmm)	7.80	-	-	7.69	**7.34**	7.12	-	-	6.78	7.29

* p< 0.05 ; significantly different to controls using Student's t test

Table 3: Liver neoplastic findings

	Dose level (ppm)									
	Males					Females				
	0	60	100	180	500	0	60	100	180	500
N° livers examined	26	28	32	25	34	25	28	28	32	35
Hepatocellular adenoma (benign)	1	0	1	0	1	0	1	0	0	5*
Hepatocellular carcinoma (malignant)	2	1	2	1	0	0	0	0	0	0

* Note that this originally identified statistical significance is considered inaccurate in subsequent statistical reassessments

Table 4: Liver non-neoplastic findings

Parameter	Dose level (ppm)									
	Males					Females				
	0	60	100	180	500	0	60	100	180	500
N° livers examined	26	28	32	25	34	25	28	28	32	35
Eosinophilic hepatocytes-foci	6	12	17**	11	21**	5	4	7	16*	23**
Eosinophilic hepatocytes-area	1	3	0	2	4	2	2	1	5	18**
Basophilic hepatocytes-foci	7	11	5	6	9	9	10	6	14	23*
Basophilic hepatocytes-area	1	0	1	0	1	3	3	0	2	5
Centrilobular hepatocyte vacuolation area	5	7	10	5	16*	5	7	5	8	11

*p < 0.05 ; ** p< 0.01 –significantly different from controls using Fisher's Exact test

Table 5a: Summary of the complete findings in the assessment of liver pathology

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 1	Group 2	Group 3	Group 4	Group 5
	Males					Females				
Animals on study	35	35	35	35	35	35	35	35	35	35
Animals completed	26	28	32	25	34	25	28	28	32	35
Liver										
Examined	26	28	32	25	34	24	28	27	32	35
Missing	0	0	0	0	0	1	0	1	0	0
No abnormalities detected	3	0	2	1	1	4	2	3	2	0
Hepatocellular adenoma (Benign)	1	0	1	0	1	0	1	0	0	5
Hepatocellular carcinoma (Malignant)	2	1	2	1	0	0	0	0	0	0
Basophilic hepatocytes - focal										
(Total)	7	11	5	6	9	9	10	6	14	23
Minimal	7	11	5	6	9	8	10	6	14	19
Moderate	0	0	0	0	0	1	0	0	0	4
Basophilic hepatocytes - area (s)										
(Total)	1	0	1	0	1	3	3	0	2	5
Minimal	1	0	1	0	1	3	3	0	1	3
Moderate	0	0	0	0	0	0	0	0	1	1
Marked	0	0	0	0	0	0	0	0	0	1
Eosinophilic hepatocytes - focal										
(Total)	5	12	17	11	21	5	4	7	16	23
Minimal	5	9	16	10	11	5	4	6	13	10
Moderate	0	3	1	1	9	0	0	1	3	12
Marked	0	0	0	0	1	0	0	0	0	1
Eosinophilic hepatocytes - area (s)										
(Total)	1	3	0	2	4	2	2	1	5	18
Minimal	1	3	0	2	3	2	2	1	5	10
Moderate	0	0	0	0	1	0	0	0	0	7
Marked	0	0	0	0	0	0	0	0	0	1
Vacuolated hepatocytes - focal										
(Total)	0	1	1	3	1	0	1	1	0	2
Minimal	0	1	1	3	1	0	1	1	0	0
Moderate	0	0	0	0	0	0	0	0	0	2
Vacuolated hepatocytes - area (s)										
(Total)	0	0	0	0	0	1	2	0	3	1
Minimal	0	0	0	0	0	1	2	0	3	1

Table 5b: Summary of the complete findings in the assessment of liver pathology.

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 1	Group 2	Group 3	Group 4	Group 5
	Males					Females				
Animals on study	35	35	35	35	35	35	35	35	35	35
Animals completed	26	28	32	25	34	25	28	28	32	35
Liver	(Continued)									
Peliosis hepatis (Total)	0	0	0	1	1	2	0	1	1	0
Minimal	0	0	0	1	1	0	0	0	0	0
Hepatocyte vacuolation - periportal (Total)	6	4	7	5	1	12	15	11	19	17
Minimal	5	3	2	3	0	5	10	6	8	10
Moderate	1	0	5	2	1	5	3	2	8	5
Marked	0	1	0	0	0	2	2	3	3	2
Hepatocyte vacuolation - centrilobular (Total)	5	7	10	5	16	5	7	5	8	11
Minimal	4	6	9	2	12	4	2	4	8	6
Moderate	0	1	1	2	4	1	5	1	0	4
Marked	1	0	0	1	0	0	0	0	0	1
Hepatocyte necrosis - focal (Total)	0	2	0	0	0	0	0	0	0	1
Minimal	0	1	0	0	0	0	0	0	0	1
Moderate	0	1	0	0	0	0	0	0	0	0
Hepatocyte necrosis - centrilobular (Total)	2	2	0	1	5	1	0	3	4	1
Slight	0	0	0	0	2	0	0	0	0	0
Minimal	1	1	0	1	2	0	0	2	4	0
Moderate	1	1	0	0	1	1	0	1	0	1
Bile duct proliferation (Total)	10	15	15	10	8	8	5	9	8	6
Minimal	8	13	15	8	7	7	5	8	6	6
Moderate	2	2	0	2	1	1	0	1	1	0
Pericholangitis (Total)	2	3	4	1	3	0	2	1	0	1
Minimal	1	3	4	1	3	0	2	1	0	1
Moderate	1	0	0	0	0	0	0	0	0	0
Parenchymal inflammatory cells (Total)	2	3	5	2	1	0	0	1	0	1
Minimal	2	3	4	2	1	0	0	1	0	1
Moderate	0	0	1	0	0	0	0	0	0	0
Cystic degeneration (Total)	3	10	10	7	7	3	1	1	1	2
Minimal	3	10	10	7	7	3	1	1	1	2

Table 5c Summary of the complete findings in the assessment of liver pathology

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 1	Group 2	Group 3	Group 4	Group 5
	Males					Females				
Animals on study	35	35	35	35	35	35	35	35	35	35
Animals completed	26	28	32	25	34	25	28	28	32	35
Liver	(Continued)									
Portal - Portal fibrous bridging (Total)	1	0	0	1	0	0	0	0	0	0
Minimal	0	0	0	1	0	0	0	0	0	0
Marked	1	0	0	0	0	0	0	0	0	0
Myeloid leukaemia (Malignant)	1	0	0	0	0	0	0	0	0	0
Thrombus	1	0	1	0	0	0	0	0	1	0
Pigmented histiocytes	1	0	2	0	0	0	0	0	0	0
Centrilobular congestion (Total)	1	1	0	0	0	0	0	0	0	0
Minimal	1	1	0	0	0	0	0	0	0	0
Subcapsular sinusoidal dilatation and congestion (Total)	1	4	6	1	1	2	2	1	2	0
Minimal	1	3	6	1	1	2	2	0	2	0
Moderate	0	1	0	0	0	0	0	0	0	0
Marked	0	0	0	0	0	0	0	1	0	0
Portal fibrosis (Total)	0	1	0	0	0	0	0	0	0	0
Minimal	0	1	0	0	0	0	0	0	0	0
Histiocytic sarcoma (Malignant)	0	1	1	1	2	0	1	0	1	0
Hepatocyte necrosis - lobar	0	1	0	0	0	0	0	0	0	0
Hepatocyte - periportal (Total)	0	1	0	0	0	0	0	0	0	0
Minimal	0	1	0	0	0	0	0	0	0	0
Granulomatous inflammation (Total)	0	0	1	0	0	0	0	0	0	0
Minimal	0	0	1	0	0	0	0	0	0	0
Lymphoma (Malignant)	0	0	1	0	0	0	0	0	0	0
Hepatocyte degeneration - centrolobular (Total)	0	0	1	0	0	0	0	0	0	0
Minimal	0	0	1	0	0	0	0	0	0	0
Haemangiosarcoma (Malignant)	0	0	0	1	0	0	0	1	0	0
Periarteritis	0	0	0	0	1	0	0	0	0	0
Cystic bile duct	0	0	0	0	1	0	0	0	0	0
Peribiliary oedema (Total)	0	0	0	0	1	0	0	0	0	0
Moderate	0	0	0	0	1	0	0	0	0	0

B.6.8.1 Toxicity studies on metabolites

Open point 2.11

Some metabolites are found in rotational crops. Their toxicity should be discussed compared to the toxicological properties of the parent.

See reporting table 2(26).

Assessment of toxicological significance of metabolites found in rotational crops but not identified in rat metabolism studies

The metabolites M-04, M-05, M-08 and M-09 found in rotational crops were not found in the rat metabolism studies and therefore and assessment of toxicological relevance is provided. The metabolites are not considered to present any specific significant hazard as they are considered to share the same intermediate metabolic pathways as those found in the rat and share substantial structural similarities with identified or tested rat metabolites.

Metabolite M-04

The genotoxicity profile of **M-04** was assessed in three in vitro and two in vivo assays. M-04 did not show any mutagenicity potential in the bacterial reverse mutation assay as well as in the mammalian HPRT gene locus in Chinese hamster V79 cells. A positive response was observed after 20 h but not after 3 hours in the test without metabolic activation in the in vitro chromosome aberration assay in human lymphocytes. However, the mouse micronucleus assay and a rat UDS assay run to assess the genotoxic potential of M-04 in the whole animal were negative at concentrations showing cytotoxicity of the target organs. Overall M-04 is not considered to present a genotoxic potential to humans.

In a 28-day toxicity study in rats with M-04 at doses up to 20000 ppm, slight reduction in body weight was observed at the top dose. The target organs identified were the liver and the kidneys. The NOAEL of M-04 was 2000 ppm (equivalent to 159.2 and 230.6 mg/kg bw/day in males and females respectively).

In conclusion, the data showed that the metabolite M-04 is less toxic than parent fluopicolide.

Metabolite M-05

The genotoxicity profile of **M-05** was assessed in three in vitro assays which included the bacterial gene mutation assay, chromosomal aberration assay and the V79/HPRT gene locus assay for forward mutations. There was no evidence of genotoxicity in any of the assays. M-05 is noted to be structurally similar to M-14. In a 28-day oral toxicity study in rats with M-05 at doses up to 20000 ppm, slight reduction in body weight gains and degeneration/regeneration in the kidneys was observed in animals at 20000 ppm, the highest test dose. The NOAEL was 2000 ppm (equivalent to 152 mg/kg/day and 167 mg/kg/day in males and females, respectively).

In conclusion, the data showed that the metabolite M-05 (AE1344122) is less toxic than parent fluopicolide.

Metabolite M-08

M-08 has no specific toxicity data, but it is considered to share the same intermediate metabolic pathways as metabolite M-02, which is also a rat metabolites and with which it shows substantial structural similarities. The genotoxicity profile of M-02 was assessed in three in vitro assays which included the bacterial gene mutation assay in S. typhimurium strain and E. coli strains, chromosomal aberration assay in cultured human peripheral blood lymphocytes and the V79/HPRT gene locus assay. There was no evidence of genotoxicity in any of the assays. M-02 was shown to be of very low acute toxicity with an oral LD50 of > 5000 mg/kg bw in rats. Therefore, no classification is required for acute oral toxicity. In a 28-day toxicity study in CD rats with M-02 at doses up to 20000 ppm, there was no evidence any effects up to and including the top dose ; no target organ toxicity was identified. The NOAEL of M-02 was 20000 ppm, equivalent to 1500 mg/kg bw/day in both males and females. In conclusion, these data showed that the metabolite M-02 is less toxic than parent fluopicolide.

One way in which M-08 differs from M-02 is in the presence of a benzamide grouping. Metabolite M-01, which is produced in rats, posesses a benzamide group but is based on a phenyl ring rather than a pyridyl ring. M-01 was negative in three *in vitro* genotoxicity assays and in an *in vivo* assay for micronuclei induction. M-01 is more acutely toxic than flupicolide, but in 90 day and 2-year studies the toxicity of both compounds is considered equivalent, taking account of dose spacing and relative molecular weights.

The toxicity profile of M-08 is predicted to be similar to that of M-02 and no worse than that of M-01. Taking account of the relative levels of M-08 (<25% of the levels of fluopicolide plus M-01), the fact that absolute levels of M-08 are very low (<0.05 mg/kg) and did not increase over time, the RMS considers that residues of M-08 in rotational crops are not of toxicological relevance.

Metabolite M-09

M-09 has no specific toxicity data, but it is considered to share the same intermediate metabolic pathways as metabolite M-02, which is also a rat metabolite and with which it shows substantial structural similarities. M-09 differs from M-02 only in the presence of a hydroxy group in place of a carboxy group on M-02. The toxicity profile of M-09 is unlikely to differ significantly from that of M-02.

The genotoxicity profile of M-02 was assessed in three in vitro assays which included the bacterial gene mutation assay in S. typhimurium strain and E. coli strains, chromosomal aberration assay in cultured human peripheral blood lymphocytes and the V79/HPRT gene locus assay. There was no evidence of genotoxicity in any of the assays. M-02 was shown to be of very low acute toxicity with an oral LD50 of > 5000 mg/kg bw in rats. Therefore, no classification is required for acute oral toxicity. In a 28-day toxicity study in CD rats with M-02 at doses up to 20000 ppm, there was no evidence any effects up to and including the top dose ; no target organ toxicity was identified. The NOAEL of M-02 was 20000 ppm, equivalent to 1500 mg/kg bw/day in both males and females.

In conclusion, these data showed that the metabolite M-02 is less toxic than parent fluopicolide and the same is predicted to apply to M-09

B.6.16 Additional References Relied On:

Location in Dossier	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Data protect. claimed	Owner
Doc K AII 5.5.3	Payraudeau, V.	2006	AE C638206 (fluopicolide): Assessment of hepatocellular proliferation and lack of carcinogenicity potential Bayer CropScience AG, Edition No.: M-275342-01-1, Date: 2006-08-02 Non GLP, unpublished	Yes	BCS
Doc K AII 5.8.1.1	Payraudeau, V; Freeman, E.	2006	2,6-dichlorobenzamide (BAM): Toxicity profile and lack of carcinogenicity potential Bayer CropScience AG, Edition No.: M-274220-02-1, Date: 2006-07-13 Non GLP, unpublished	Yes	BCS / Chem-tura
Doc K AII 5.8.1.1	Pallen, C.	2006	Re-assessment of liver lesions/tumors from study PDR/49 BAM: Dietary administration to rats for 2 years, complementary statistical analysis of hepatocellular tumors in female rats Bayer CropScience AG, Edition No.: M-273467-01-1, Date: 2006-06-13 Non GLP, unpublished	Yes	BCS
Doc K AII 5.8.1.1	Gopinath, C.	2007	Expert opinion on the carcinogenic potential of BAM (2,6-dichlorobenzamide) Huntingtdon Life Science Bayer CropScience Report no: Edition No. M-287543-01-1 Date: 2007-04-26 no GLP, unpublished	BAY	BCS / Chemtura
Doc Kb Position Papers	Payraudeau, V.	2006	AE C638206 (fluopicolide) Waiver for an Acute Reference Dose (ARfD) setting Bayer CropScience AG, Edition No.: M-269338-01-1, Date: 2006-03-07 Non GLP, unpublished	Yes	BCS
Doc Kb Position Papers	Payraudeau, V.	2007	Toxicological relevance of the solvent toluene present as an impurity in the technical grade active substance Bayer CropScience AG Edition no.: M-284199-01-1 Date: 08.02.2007 Non GLP, unpublished	Yes	BCS

Location in Dossier	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Data protect. claimed	Owner
Doc Kb Position Papers	Fisher, P.	2007	Fluopicolide: Evaluation of the oral bioavailability of fluopicolide in the rat Bayer CropScience Edition No. M-287367-01-1 Date: 2007-04-10 no GLP, unpublished	BAY	BCS
Doc K AIIIa 7.1.2	Krötlinger, F.	2003	AE F053616 06 WG71 A1 – EXP11074B: Study for acute dermal toxicity in rats 1st amendment to report no. AT00219 of January 20, 2003 Bayer CropScience AG Edition no. M-220872-02-1 Date: 2003-04-14 GLP, unpublished	Yes	BCS

B.8 ENVIRONMENTAL FATE AND BEHAVIOUR

B.8.1 Route and rate of degradation in soil

Open point 4.1

"Half lives for metabolites derived in the studies where they are dosed as starting material are seen by the RMS as more reliable, specially with respect to M14 (see DAR p 661). Therefore, only these DT50 should be reported in the list of end points. RMS to amend the list of end points accordingly.

MS experts to discuss if the half lives derived from the study dosed with M02 may however still be used for modelling.

See reporting table 4(6)"

Open point 4.8

"MS experts to discuss in an experts meeting the kinetic evaluation of field dissipation studies.

See reporting table 4(36)."

See Addendum 2 (Nov 2007) – For further details on the above Open points.

Open point 4.2

"RMS to clarify normalized laboratory DT50's values for fluopicolide and metabolites, i.e., for fluopicolide in LoEP the range is 194 – 333 d when for example in Allan 2003 c study degradation in one soil results in a normalized DT_{50} = 373 d (or for another example 664 d for Lamberton soil in Allan 2003e). Please do it in an addendum or in an updated list of end points following the updated template where the origin of the different end points and normalization procedures may be easily tracked.

See reporting table 4(10)"

To clarify the values given in the list of endpoints, reference is made to DAR Volume 3, Section B.8.1.8, p. 715, Table B.8.142.

The values given in the LoEP for laboratory degradation rate of fluopicolide are values from the Applicants calculations, rather than from the RMS calculations. This the reason why the range given in the endpoints differs from the specific values stated in the Open Point 4.2 of 373 and 664 days which are from the RMS calculations. The differences between the outcome of the Applicant and RMS calculations can in part be explained by the fact that the Applicant used all data points, whereas the RMS only used data points from within the first 120 days of the study, given that this is the length of study specified by SETAC guidelines and that it is known that microbial viability of soils can become compromised over longer study duration periods. In addition, the DT50 of 664 days is extrapolated well beyond study duration and is associated with an r^2 value of only 0.583, i.e. below the 0.85 value specified in the 'Persistence Guidance Document' for acceptability for use in comparison with Directive persistence triggers,

and below the 0.7 value specified for used in exposure modelling. If the 664 day DT50 is excluded due to low r^2 and results from 2 different radiolabels for the same soil in individual studies are geometrically meaned, the subsequent geometric mean of RMS calculated DT50 values is 260.5 days, comparable with the overall geometric mean of 271 days for the applicant's calculations.

The values quoted for the metabolites are also from the Applicant calculations.

The LoEP has been updated according to the latest template.

In relation to the point made above relating to the 'Persistence Guidance Document', the RMS recognises that whilst the kinetics and associated statistics for this evaluation were not derived in strict accordance with the FOCUS Degradation Kinetics guidance, the evaluation was conducted significantly before agreement/adoption of the guidance document. We are not convinced that applying the principles of the guidance document would significantly influence the endpoints selected for exposure assessment.

Route and rate of degradation in soil

Open point 4.5

"MS experts to discuss potential influence of the different extraction method employed on the respective results of the laboratory and field studies.

Applicant provided an explanatory note in the "Comments to the reporting table". To be considered by MSs experts in their discussion.

See reporting table 4(26)."

As a reminder to MS experts, lab studies used 3-4 extractions with acetonitrile/water at ambient temperature followed by an acetonitrile Soxhlet extraction. Field studies used 2 extractions of acetonitrile/water/formic acid under ambient conditions.

RMS notes the Applicants statement, however, the RMS has further investigated extraction in the lab studies. Considering the representative chromatograms presented in studies, (in the Allen, 2003c study), Soxhlet extractions at 369 DAT accounted for 14.2 – 23.3% AR, with fluopicolide accounting for 9.7 – 17.6% AR in the Soxhlet extracts. In the Allen, 2003b study, at 98 DAT Soxhlet extractions accounted for a further 5.4 – 6.1% AR as fluopicolide. Information relating to the amount of fluopicolide extracted with each successive ambient extraction in lab studies is not available.

RMS considers that in light of this information, there is still some uncertainty over the suitability of the extraction methods for the field dissipation studies and that this should discussed by MS experts with a view to obtaining an appropriate resolution.

B.8.1.3. Route and rate of degradation in soil - photolysis

Data Requirement 4.1

"Notifier to provide an estimation of soil photolysis half lives at other latitudes (i.e. 40 ºN and 45 ºN). Applicant indicated to submit a position paper (Report MEF-06/495) by April 2007.

See reporting table 4(14)."

Background:
Soil photolysis was performed by simulating irradiation in Scotland, (latitude 55°N). As fluopicolide is also intended for use in Southern EU Member States, further estimates were requested of the contribution of photolysis to soil degradation at latitudes around 40°N-45°N.

In the field dissipation studies (DAR B.8.1.8), fluopicolide was sprayed to bare soil surface. This also prompted discussion over the influence of photolysis on the results of field dissipation studies, compared to under normal conditions of use in the field and

the possible relevance of photolysis to the biphasic degradation observed, with faster degradation occurring in the initial period. (See reporting table point 4(42)).

RMS evaluation of new data:
Two photolysis studies were conducted using thin soil layers (sandy loam, *ca*. 3 mm depth, treated with [pyridyl-2,6-^{14}C]-labelled fluopicolide (Keirs and Lowrie, 2001) and [phenyl ring-U-^{14}C]-labelled fluopicolide (Mackie, 1999) and exposed to artificial sunlight for up to 15 days, at 20°C. These studies were assessed in the DAR, B.8.1.8.

Irradiated samples were exposed to continuous illumination (24 hours per day) under artificial sunlight. The level of irradiance was intended to be equivalent to the total radiation received in one summers day (5470 W*h/m^2*d) at East Lothian, Scotland (55°N). Assuming 12 hours of light per day (5470 W*h/m^2*d / 12 d), this gives an intended hourly irradiation value of 456 W/m^2 (or W*h/m^2*h).

Actual irradiance in the studies was measured using a Radialux meter, fitted with a global sensor to measure light intensity in the wavelength region 290-800 nm, at the start and end of the study.

Table 8.1 Study irradiance measurements (290-800 nm) (Keirs & Lowrie, 2001, Mackie, 1999).

Time (days)	[Pyridyl-2,6-^{14}C] labelled experiment (Keirs and Lowrie, 2001)		[Phenyl ring-U-^{14}C] labelled experiment (Mackie, 1999)	
	Irradiance (W/m^2)		Irradiance (W/m^2)	
	Pre-incubation	Termination	Pre-incubation	Termination
3 days	461	467	418	418
5 days	459	464	420	426
7 days	452	457	460	480
10 days	406	412	480	480
15 days	419	420	460	480
Median	455		460	

Hourly levels of irradiance during the study have been described by the applicant using the median values of 455 and 460 W/m^2 from Table 8.1 (pyridyl and phenyl labelled experiment, respectively). This irradiance or light intensity (W/m^2) measured between 290 - 800 nm only represents part of total light intensity (280 - 3000 nm). The applicant provided global radiation data (from CIE publication no. 20, 1972)[1] which gave a breakdown of percentage of total radiation for each wavelength range. For the wavelength bands 200-400 nm and 400-800 nm, the percentage of total radiation was 6.1% and 51.8%, respectively. Based on this the applicant assumed that (6.1+51.8%=) 57.9% of total light intensity falls in the wavelength range 200-800nm and that the

[1] CIE (1972): Empfehlung fur die Gesamtbestrahlungsstarke und die spektrale Verteilung kunstlicher Sonnenstrahlung fur Prufzwecke. Publication CIE, No. 20 (TC-2.2),

filtered light intensity measured in the study represents 57.9% of total light intensity, recalculated as below[2].

Total irradiance (W/m^2, 280-3000 nm) = measured irradiance (W/m^2, 290-800 nm)/ 0.579
Total irradiance = 455 or 460 W/m^2 / 0.579
Total irradiance = 785 or 794 W/m^2 (pyridyl or phenyl label, respectively)

The hourly or instantaneous solar radiation (W/m^2) was then converted into an energy yield of the solar radiation per day (kJ/m^2*d) by:

instantaneous total irradiance (W/m^2, 280 - 3000 nm) * 86400 (seconds/day) /1000
(note: 1 hour = 3600 seconds x 24 hour = 86400 seconds/day)

Assuming total irradiance of 785 W/m^2 or 794 W/m^2, in the above equation gives an energy yield of solar radiation of 67.82 and 68.64 MJ/m^2 per day, respectively for the [pyridyl-2,6-^{14}C] and [phenyl ring-U-^{14}C]-labelled experiments.

The applicant has recalculated the photodegradation rate of fluopicolide, based on these studies, assuming single exponential first-order kinetics using Excel Solver to obtain the best 'least squares fit'. Due to the variability of the recovery data, the soil residue data (%AR) were normalised for total recovery at each time point, with no correction for dark control residues, (since no significant decline was observed in the dark).

[2] using Chemtec, (1995): Solar radiation data - Handbook of Material Weathering, 2nd edition. Chemtec Publishing, Ontario, Canada.

Table 8.2 Decline of [pyridyl-2-6-^{14}C]-fluopicolide (normalised for total recovery) in soil photolysis.

Irradiated Samples

Time (days)	% of applied radioactivity		
	Total Recovery	Measured Fluopicolide	Normalised Fluopicolide
0	100.56	98.44	97.89
3	98.14	90.87	92.59
5	95.76	87.97	91.87
7	96.09	87.33	90.88
10	95.83	85.98	89.72
15	96.32	86.68	89.99

Non-Irradiated Samples

Time (days)	% of applied radioactivity		
	Total Recovery	Measured Fluopicolide	Normalised Fluopicolide
0	100.56	98.44	97.89
3	99.90	96.65	96.75
5	95.59	92.99	97.28
7	94.97	91.65	96.50
10	96.58	94.35	97.69
15	95.86	92.96	96.97

Table 8.3 Decline of [phenyl-2-6-^{14}C]-fluopicolide (normalised for total recovery) in soil photolysis.

Irradiated Samples

Time (days)	% of applied radioactivity		
	Total Recovery	Measured Fluopicolide	Normalised Fluopicolide
0	109.20	102.10	93.50
3	94.14	81.35	86.41
5	91.73	79.51	86.68
7	98.99	82.84	83.69
10	100.00	80.92	80.92
15	90.81	71.60	78.85

Non-Irradiated Samples

Time (days)	% of applied radioactivity		
	Total Recovery	Measured Fluopicolide	Normalised Fluopicolide
0	109.20	102.10	93.50
3	96.07	91.61	95.36
5	96.54	89.61	92.82
7	100.1	94.61	94.52
10	102.1	96.96	94.97
15	100.2	95.13	94.94

Table 8.4 Laboratory photolysis DT50 values and conditions for fluopicolide.

Radiolabel	Rate constant	DT_{50}	Fitting criteria			Instant. irradiance (290 - 800 nm)	Instant. total irradiance (280 - 3000 nm)	Solar energy yield
	(d^{-1})	(d)	(error of χ^2 in %)	(r^2)	(*B* value)	(W/m^2)	(W/m^2)	($kJ/m^2 * d$)
Pyridyl	0.0052	134.13	1.3	0.688	0.9997	454.5	785	67822
Phenyl	0.0111	**62.55**	3.1	0.909	0.9997	460	794	68643

The photodegradation DT50 values above have been independently verified by the RMS, with non-linear regression analysis in MS Excel Solver (SFO, no reps with fit). The DT50 of 62.55 days (i.e. the faster of the two photodegradation rates calculated, representing most photodegradation) was used in further modelling, this is worst case in the context of the applicant trying to demonstrate the impact of photolysis. Note that the calculated DT50 values are extrapolated well beyond study duration which may account, at least in part, for the apparently large difference in DT50 between the two radiolabelling positions.

To assess the influence of photodegradation in the overall degradation of fluopicolide under field conditions, the applicant ran simulations for fluopicolide in the FOCUS PEARL model with and without taking into account photodegradation. As FOCUS PEARL does not take into account photodegradation, a soil surface layer of 2 mm in which photochemical transformations may occur was implemented in the model. A comparison of the residues with depth and time was made for 2 FOCUS groundwater scenarios, Kremsmünster (48.03°N) and Sevilla (37.22°N) and one field dissipation trial, Philippsburg (49.14°N), (latter evaluated at DAR, B.8.1.5. (g) and B.8.1.7.(c)). No justification was provided for this particular selection of scenarios/ sites. However, the RMS presumes that the reason was that the 2 groundwater scenarios were relevant to the intended crops and that the Philippsburg site was chosen as it was a 5 year trial, with soil hydrology data being available.

A 2 mm soil surface layer was implemented in the FOCUS PEARL model to simulate photodegradation by increasing the biodegradation factor (f_r), which is usually set to 1 at the soil surface.

Biodegradation factor $f_r = (k_{soil} + k_{photo}) / k_{soil}$.

where k_{soil} is microbial degradation and k_{photo} is photodegradation, combined to represent total degradation in the top soil.

Therefore, photodegradation is considered as part of the total degradation in the top soil layer and is also connected to the moisture and temperature dependency used for the total degradation rate. The RMS is not convinced that photodegradation processes are influenced by soil temperature and moisture to the same extent as microbial degradation. The RMS considers that in this case, selection of a 2mm soil layer in which photolytic processes occur will have a relatively small influence to overall degradation. This is likely to be case for soil photolysis in practice. As photolytic rate could not be corrected for daily solar radiation values in the PEARL model, site

specific photolytic DT50 values were recalculated, taking into account mean solar radiation for approximately 4 months after application of fluopicolide.

The photodegradation DT50 of 62.55 days from the phenyl-label study, (laboratory solar energy yield 68643 kJ/m^2*d) was recalculated for site specific radiation using the formula below, (verified by the RMS):

DT50 actual = DT50 laboratory * solar energy yield per day of laboratory study / solar energy yield per day of specific site (e.g. in season of interest, in kJ/m^2*d)

e.g. for Kremsmünster
DT50 = 62.55d x 68643 kJ/m^2*d/ 16285 kJ/m^2*d = 263.65 solar days

Table 8.5 Site and season specific photodegradation DT50 values for fluopicolide based on laboratory photodegradation DT50 of 62.55 days at 68643 kJ/m^2*d.

Site	Period	Mean solar energy yield	Site specific photodegradation DT_{50}
		kJ/m^2*d	d
Kremsmünster	June – Sept.	16285 ^{F}	263.7
Sevilla	May – August	24907 ^{F}	172.4
Philippsburg	June – Sept.	13979 ^{K}	307.2

^{F} daily mean values from standard FOCUS gw scenarios (Focus, 2000)

^{K} daily mean values as used for kinetic field evaluation (Kley, 2003a)

The RMS notes that the time periods selected would represent greatest exposure to sunlight. The solar energy yield values given are referenced as from FOCUS 2000, presumably the MARS database and have been accepted as quoted. They appear comparable with values provided by the applicant for a range of 9 other locations (in the UK, EU, USA at latitudes from 36.80°N to 56.26°N) of 16000-27000 kJ/m^2*d, derived from the Solar Radiation Handbook of Material Weathering 2nd edition, Chemtec Publishing 1995.

Table 8.6 Irradiation in the laboratory* in relation to summer days.

Location	Latitude (°N)	Mean solar energy yield (KJ/m^2*d)	DT50 (solar days) Phenyl-label	DT50 (solar days) Pyridyl- label
Philippsburg	49.14	13979	307.15	650.76
Kremsmünster	48.03	16285	263.65	558.61
Sevilla	37.22	24907	172.38	365.24
Dundee (UK)	56.26	17,000	252.56	535.11
London (UK)	51.31	16,000	268.35	568.56
Vienna (Austria)	48.14	19,000	225.98	478.79
Zurich (Switzerland)	47.23	18,000	238.53	505.39
Portland (USA)	43.39	19,000	225.98	478.79
Boston (USA)	42.22	21,000	204.46	433.19
Philadelphia (USA)	39.53	21,000	204.46	433.19
Athens (Greece)	38.03	20,000	214.68	454.85
Tunis (Tunisia)	36.80	27,000	159.02	336.92

*(For phenyl-label suntest irradiation 1501.2 h equated to lab DT50 of 62.55 d. For pyridyl label suntest irradiation 3219.12 h equated to lab DT50 of 134.13 d).

The same standard soil degradation rates, equilibrium sorption coefficients and application schemes were assumed for running the FOCUS scenarios Kremsmünster and Sevilla, as were used in the groundwater assessment for fluopicolide (DAR, B.8.6.2). For the Philippsburg field dissipation site, the soil degradation rates (inverse evaluated (Kley, 2003a)), equilibrium sorption coefficients and application schemes specific for this soil and site were taken from the original kinetic evaluation of field dissipation studies (Kley, 2003a DAR, B.8.1.5.1). The applicant acknowledged that the $DT50_{field}$ values used could potentially have included an element of photodegradation as well as microbial degradation. However, they considered that any effect of the slow photodegradation on the $DT50_{field}$ rates of fluopicolide, occurring at the soil surface only, would be apparent in this evaluation, if significant.

Table 8.7 Parameters input for FOCUS PEARL simulations.

	Kremsmünster	Sevilla	Philippsburg
Crop	Vines	Vines	Bare soil
Application rate (g/ha)	3 x 133	3 x 133	400
Application dates	5 + 15+ 25 June	5 + 16+ 26 May	20 June 2000
Crop interception (%)	70, 70, 85	70, 70, 85	-
Soil $DT50_{field}$ (d)	138.8*	138.8*	108.56#
Site specific photolysis DT50 (solar days)[a]	263.7	172.4	307.2
Biodegradation factor (F_r) for upper 2 mm	1.526	1.805	1.353
Koc (Kom), mean	321.1 l/kg (186.2 l/kg)	321.1 l/kg (186.2 l/kg)	248.3 l/kg [b](soil specific)
1/n, mean	0.9028	0.9028	0.841 [b] (soil specific)

* (mean $DT50_{field}$, bare field). # (site specific $DT50_{field}$, from inverse DT50 evaluation, Kley, 2003a).
[a] worst case (faster $DT50_{lab}$) derived from data from study using phenyl-labelled fluopicolide.
[b] reported in DAR, Table B.8.152.

Model parameters:
Dispersion length (λ) was 5 cm and photolysis layer 2mm. FOCUS-PEARL 3.3.3 was run for Kremsmünster and Sevilla, and FOCUS-PEARL 1.1.1 was run for the field site, Philippsburg (soil hydrology manually calibrated).

The applicant presented depth profiles for individual time points (days 14, 60, 180, 240, 450 and 720) over 2 years for each of the FOCUS GW scenarios, with and without the 2 mm soil layer for photodegradation for comparison. Concentrations of fluopicolide in 50 cm soil depth over approximately 10-12 years were also reported. Dissipation curves for fluopicolide over 30 cm soil depth were also presented with and without photodegradation, for the field dissipation site, Philippsburg. The applicant concluded that there were no significant differences, with or without the additional photodegradation rate, observed for any of the scenarios over time.

Figure 8.1 Depth profiles of fluopicolide residues for Kremsmünster, (applicant calculated).

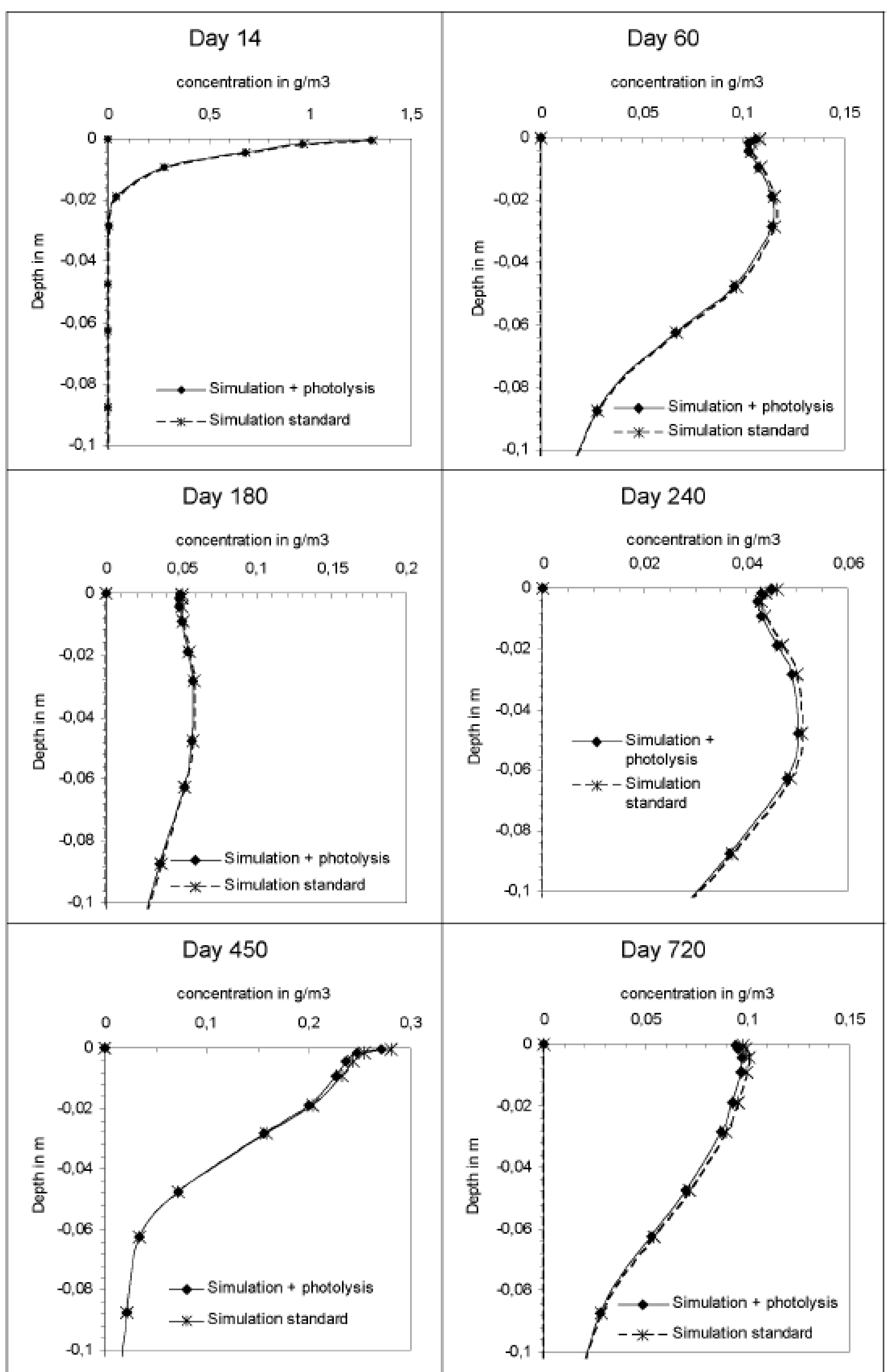


Figure 8.2 Depth profiles of fluopicolide residues for Sevilla (applicant calculated).

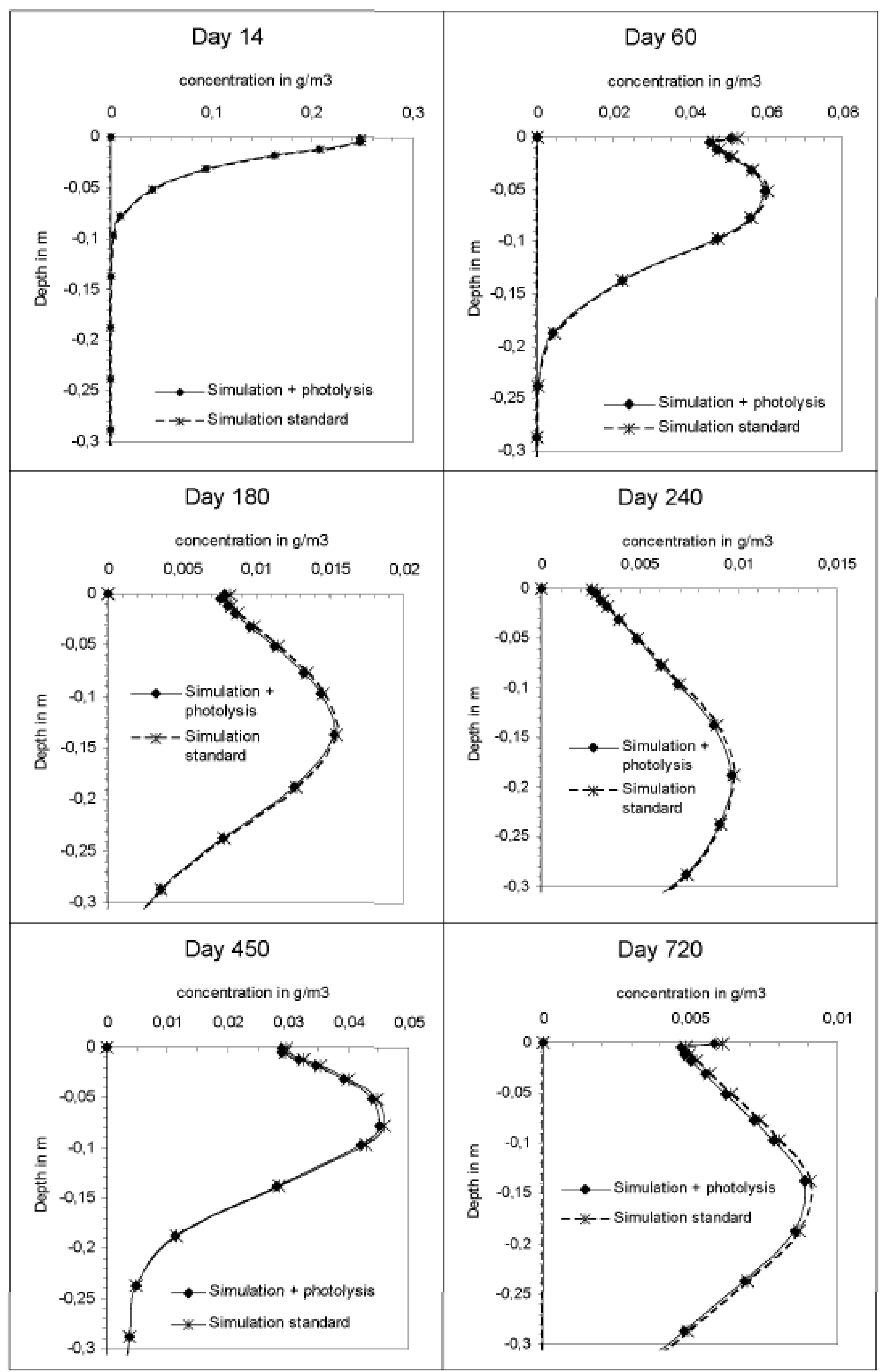


Figure 8.3 Concentration of fluopicolide residues in upper soil layers (50cm) for Kremsmünster scenario.

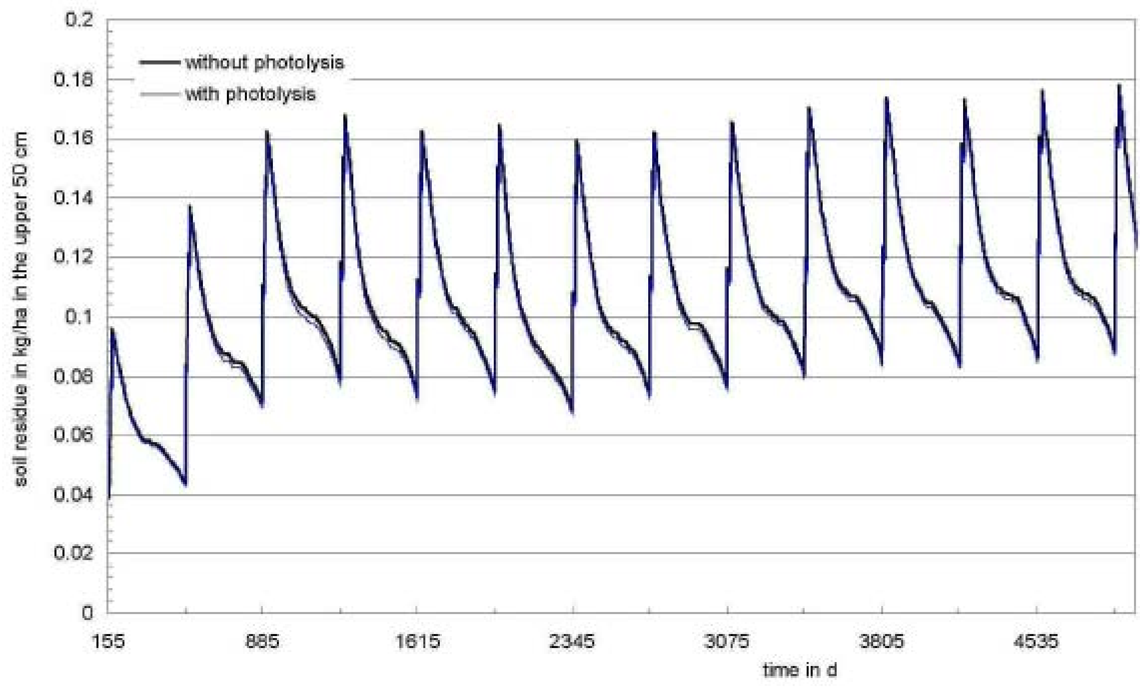


Figure 8.4 Concentration of fluopicolide residues in upper soil layers (50cm) for Sevilla scenario.

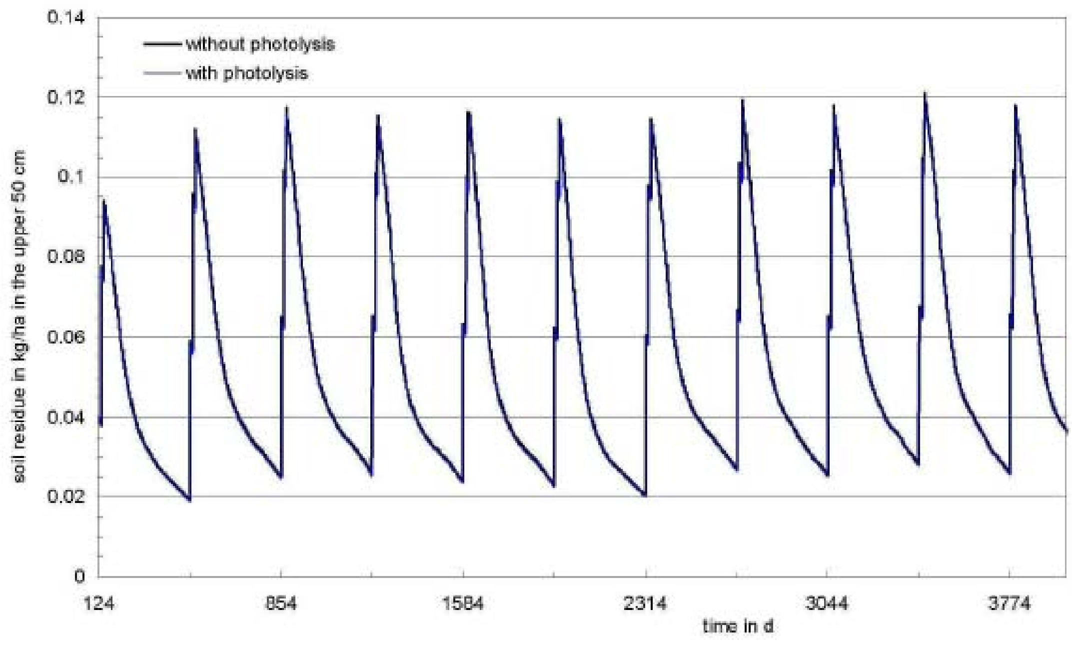
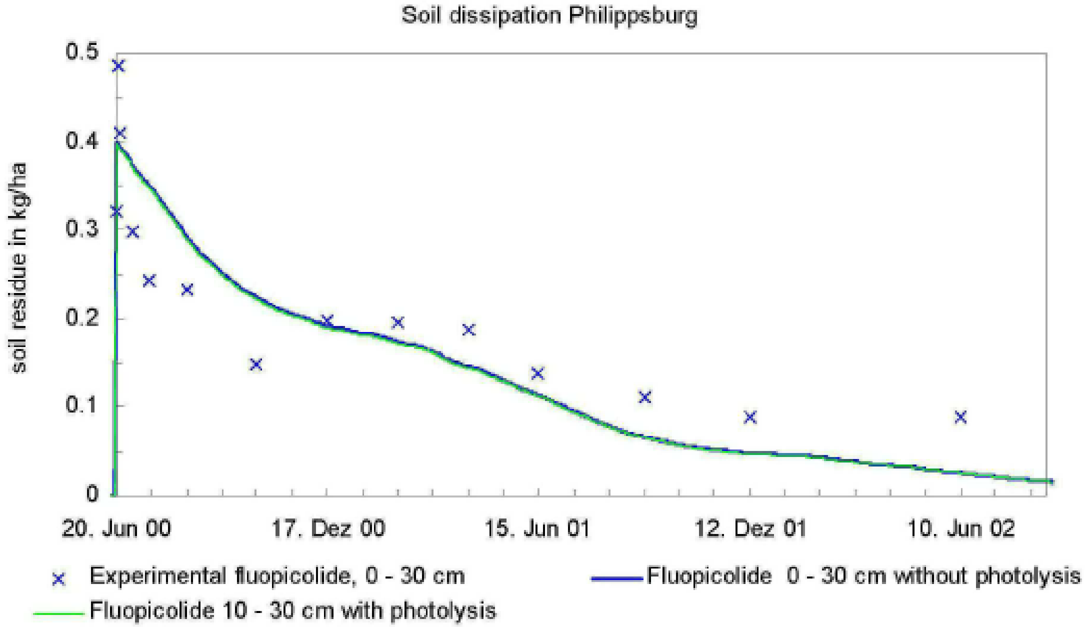


Figure 8.5 Concentration of fluopicolide residues in upper soil layers (30cm) for Philippsburg field dissipation trial.



The applicant concluded that photodegradation did not appear to contribute to the biphasic dissipation pattern seen in the field. Possible alternative explanations for the biphasic dissipation behaviour of fluopicolide in the field were proposed by the applicant, such as experimental artefacts, seasonal climatic changes, or the effect of time dependent sorption.

Experimental artefacts may be due to insufficient soil homogenisation or compression of the soil due to cultivation methods, which could cause artificially high residues at the start of the study. However, the applicant considered that if this explanation was likely it would be expected that biphasic degradation would be seen in only some trials, with other trials appearing to follow SFO kinetics.

The applicant considered that climatic changes were a possible explanation, as the degradation of fluopicolide in the field was moisture and temperature dependent. Degradation occurred more rapidly initially in spring/summer, then slowed over colder winter months.

The effect of time dependent sorption contributing to biphasic dissipation was also investigated by the applicant. The full assessment of this investigation is described later in this Addendum, at B.8.6.2 in the context of the groundwater assessment.

To summarise, sorption of pesticides on soil is described with a Freundlich-type equation. It is assumed in the FOCUS PEARL model that degradation and sorption in soil may be described by both instantaneous (equilibrium sorption) and long-term or gradual sorption processes (non-equilibrium sorption). Transformation of active substance is assumed to only occur in the equilibrium domain, with slow release of compound from the non-equilibrium domain.

The applicant provided additional reports (Kley 2004, MEF-04/346 and MEF - 04/347) describing their approach to calculating a degradation rate constant specific to the equilibrium domain (k_t) and kinetic sorption parameters for use in the PEARL model, (kinetic-sorption rate constant, k_d and the ratio between the Freundlich coefficients in the non-equilibrium and in the equilibrium domain, f_{ne}).

In these reports the applicant fitted the data from previously assessed studies to a kinetic-sorption model implemented in FOCUS-PEARL, using ACSL Optimise 1.2, (Kley 2004, MEF 04/346, Addendum B.8.X). The data used for fitting, were derived from two studies on the effect of ageing on sorption, which were reported in the DAR at B.8.2.1(c/d), (though they were not considered essential to the risk assessment in the original DAR, Allan, 2003b; Fitzmaurice, 2003). This approach was then also applied to the field dissipation data from 6 trials (reported at DAR B.8.1.5.) to derive a more realistic field degradation rate constant specific to the equilibrium domain, for use in the PEARL model, (Kley 2004, MEF 04/347, Addendum B.8.6.2)

The optimised parameters for the kinetic-sorption rate constant (k_d) and the ratio between the Freundlich coefficients in the non-equilibrium and in the equilibrium domain (f_{ne}) are shown in Table 8.8.

Table 8.8 Evaluated parameters of the kinetic-sorption model of all soils in the laboratory time dependent sorption study (Kley, 2004, MEF-04/346)

Soil	k_d (d^{-1})	f_{ne}
Philippsburg	0.0589	0.4692
Rödelsee	0.0835	0.3701
Huntlosen	0.1950	0.3657
Senas	0.1075	0.3230
Abington	0.0362	0.4485
Arithmetic mean	-	0.3953
Geometric mean	0.08211	-

A biphasic dissipation pattern may result from kinetically controlled sorption, due to the combination of degradation rate in the equilibrium domain (k_t) and the rate of transfer from the non-equilibrium to the equilibrium domain (k_d). The mean ratio between the Freundlich coefficients in the non-equilibrium and equilibrium domain (f_{ne}) calculated for fluopicolide was 0.395. The applicant claimed that this indicated that fluopicolide underwent a moderate, but measurable kinetic sorption with time, with a kinetically controlled "sorption capacity" of about 40% of the instantaneous "sorption capacity". (i.e. 60% of applied residue is available for degradation in the equilibrium domain, compared with 40% of applied residue in the non-equilibrium domain, where no degradation is assumed).

RMS Risk Assessment and Conclusions:

The RMS concludes that soil photolysis at more southerly latitudes is unlikely to significantly influence the degradation of fluopicolide. Kinetic adsorption aspects, if implemented into FOCUS modelling of environmental exposure, would be likely to result in lower peak and annual average concentrations.

Implications for Ecotoxicogical Assessment:

No change from the relevant endpoints reported in the DAR.

(Kley, C; Mackenzie, E; MEF-06/495, 2007)

B.8.1.7 Route and rate of degradation in soil – field soil accumulation

Data Requirement 4.2

"Applicant to present the position paper with their evaluation of the accumulation studies. Applicant indicated to submit a position paper assessing the field accumulation studies (Kley, C; Mackenzie, E.; Report no. M-267721-01-1) by April 2007.

See reporting table 4(41)."

Background:
The applicant has submitted a position paper presenting further evaluation of the field accumulation studies, (originally assessed at DAR B.8.1.7. and B.8.1.8), in response to the conclusion of the RMS that residues of fluopicolide and M-01 had not reached a plateau at study termination in the trial at Appilly and that results at Senas were inconclusive. The applicant has also submitted the time-points at which maximum concentrations were estimated to be reached, not previously given. (See reporting table, points 4(41), 4(51) and 4(73)).

RMS Evaluation of new data:
Field dissipation/ accumulation trials with fluopicolide were conducted over a 4 year period at sites in Philippsburg (Southern Germany), Appilly (Northern France) and at Senas (Southern France). Concentrations of fluopicolide and its metabolites, M-01, M-03 and M-02, in soil were measured following repeated annual applications of 400 or 500 g/ha p.a. to bare soil. See the assessment in the DAR at B.8.1.7. and B.8.1.8 for further details.

These data have since been evaluated further by the applicant to assess whether the plateau concentrations of fluopicolide measured in the field were reached after 4 years, or if further increases would be expected in successive years. The accumulation potential of fluopicolide and its metabolite M-01 have been evaluated at each site, using SFO kinetics. Metabolite M-01 was shown to have potential to be mobile and persistent. The metabolite M-02 was only detected at a few time points at low levels and metabolite M-03 was only detected in acidic soils, being degraded rapidly in soils with pH>7. Throughout this evaluation the applicant converted concentrations in mg/kg to g/ha for the total soil depth assuming a soil density of 1.5 g/cm^3.

PHILIPPSBURG
(S. Germany, loamy sand, pH 6.4 and 0.27% oc content)

Fluopicolide was applied annually as detailed below. The applicant measured the unused formulation remaining in the spray tank to confirm the actual amount applied ('calibrated application rate'). Three plots, each 3 m x 26 m, were treated with fluopicolide and a fourth plot left untreated as a control. The treated plots were subdivided into separate areas for the dissipation phase treated once in the first year and for the accumulation phase treated annually for up to 5 years. Details of the treatment and sampling areas were provided. Samples for the dissipation phase were taken for up to 2 years after the first application. Samples for the accumulation phase were taken

immediately after application and at 4 and 12 months after each application, with the final sample taken immediately after the 5th application.

Table 8.9 Application schedule at Philippsburg

Application Date	Days after treatment	Nominal application rate (g/ha)	Calibrated application rate (g/ha)
20 June 2000	0	400	411
24 July 2001	399	400	422
26 June 2002	736	400	398
05 June 2003	1080	400	423
06 July 2004	1478	400	418

The applicant converted the concentrations from the field (mg/kg, individual replicate values for total soil depth, including below 20 cm) into g/ha over 10 cm depth then derived the average g/ha value. The same approach was taken for metabolite M-01, but also assuming that parent compound was 100% transformed to M-01 and residues were converted to a.s. equivalents (by correction for molecular weight differences).

Figure 8.6 Philippsburg Dataset

Soil Accumulation, SFO parent + SFO metabolite
Field Philippsburg

	Fluopicolide	AE C653711	
DT50:	217.52	95.77	d
k:	0.003186661	0.00723731	1/d
C0:	397.48		g/ha
Molar Mass:	383.59	190.03	g/mol
Formation fraction:		1	

soil residues assuming BD = 1.5

	Measured field data			Simulated data		Square of differences	
Application	Time d	Fluopicolide g/ha	AE C653711 g/ha ai eq.	Fluopicolide g/ha	AE C653711 g/ha ai eq.	Fluopicolide	AE C653711
20/06/2000	0	318	0	397.48	0.00	6317	0
400 g/ha nominal	1	486	33	396.21	1.26	7972	1007
	3	400	39	393.70	3.74	40	1243
	14	294	38	380.14	16.49		
	36	235	62	354.40	37.83		
	62	225	72	326.22	57.00		
	120	147	64	271.17	82.12		
	181	191	63	223.26	91.27		
	244	190	16	182.65	90.21		
	308	186	0	148.96	83.53		
	367	135	18	123.43	75.14	123	3265
24/07/2001	399	629	99	508.94	70.27	14415	826
	525	213	141	340.63	135.35		
	762	206	75	160.06	130.83	2064	
26/06/2002	736	657	64	571.37	108.00	7333	1936
	857	300	171	388.56	163.42		
	1078	226	86	192.13	122.42	1113	1326
05/06/2003	1080	485	100	586.39	121.87	10690	478
	1203	298	274	397.60	172.77		
	1448	198	188	182.13	119.51	236	4692
07/07/2004	1478	435	152	563.00	111.08	16384	1674

$\sum(P-O)^2$ 83134

Where;
P=predicted value
O=observed value

The concentrations of fluopicolide and M-01 in soil after annual applications of fluopicolide are shown in Figures 8.7-8.9. Results for both mean and individual plots were presented for fluopicolide. The mean results of the 3 plots were also provided for M-01, though not the individual plots. The final 3 data points (461, 546, 735 DAT) from the second year of the dissipation phase of the study (September 2001-June 2002) were excluded, as they overlapped with the start of the accumulation phase (July 2001) and were not needed to assess the accumulation plateau.

The maximum concentration of fluopicolide was detected immediately after the second application (2001) and was a similar level after the third application (2002). The individual plots are presented separately. The applicant stated that the measured $C_{high\ max}$ and $C_{low\ max}$ values for fluopicolide appeared to reach a plateau and that there appeared to be a tendency for accumulation of the metabolite M-01 over the course of the study, with a plateau not being reached. The RMS notes that while the $C_{high\ max}$ value for fluopicolide seems to have reached a plateau in individual plots, the $C_{low\ max}$ decreased only at last point and slightly increased at the end for plot, T2.

Figure 8.7 Concentration of fluopicolide at Philippsburg (g/ha for total soil depth) (Mean of 3 individual treated plots T1, T2 and T3)

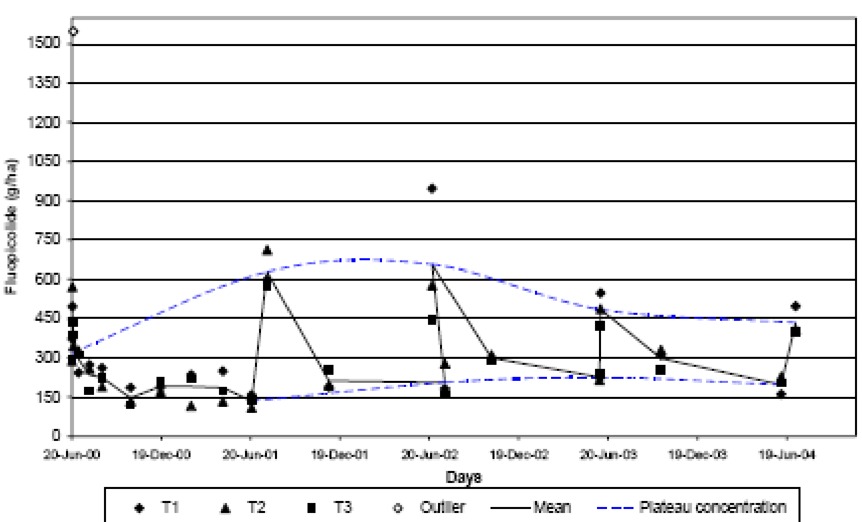


Figure 8.8 Concentration of M-01 at Philippsburg (g M-01 /ha for total soil depth) (Mean of 3 individual treated plots T1, T2 and T3)

NB Scale different.

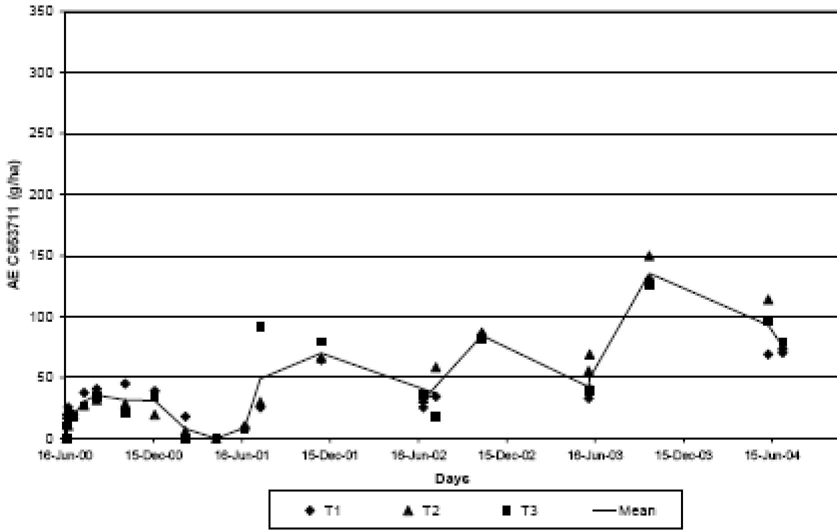
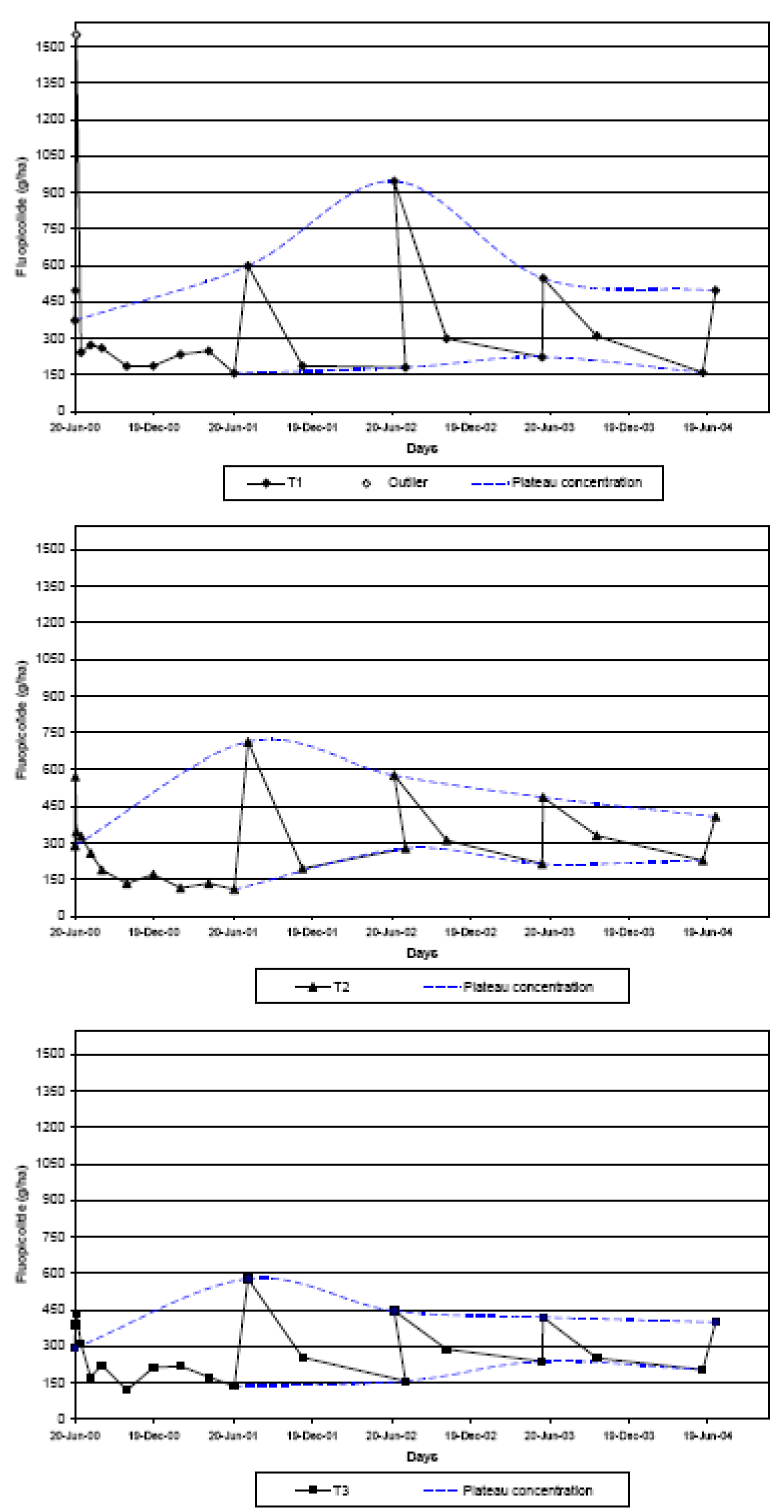


Figure 8.9 Concentration of fluopicolide at Philippsburg in 3 individual plots (T1, T2 and T3) (g/ha for total soil depth).



The plateau concentrations after 4 years were calculated by the applicant based on residues found in the 0-10 cm soil depth only, or by summing and then averaging the levels of residues found in the 0-10 cm and 10-20 cm layer.

Table 8.10 Plateau concentrations of fluopicolide at Philippsburg.

Plateau concentration	Time-point	Measured in soil increments (mg/kg)	
		0-10 cm	0-20 cm
High[1]	Day 0 2nd Application	0.341	0.191
Low[2]	Day 368 after 4th Application	0.094	0.064

[1] maximum of the high values of the "saw teeth" curve
[2] maximum of the low values of the "saw teeth" curve

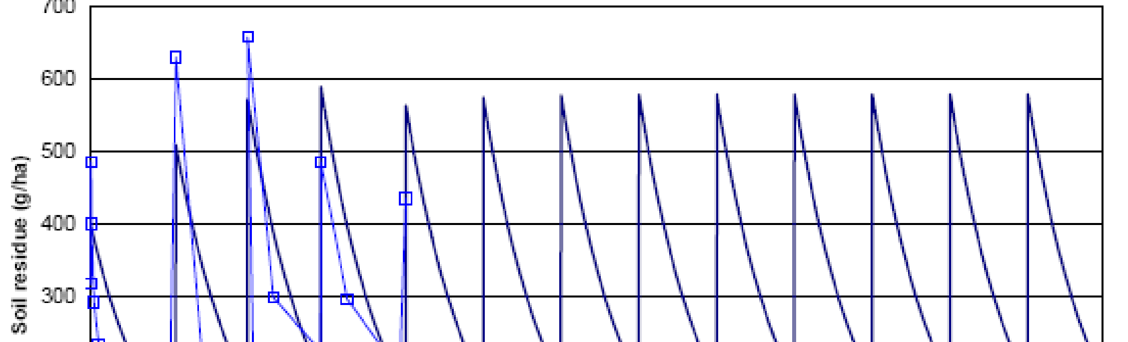
These plateau concentrations are as reported previously in the DAR. The degradation rate of fluopicolide in soil under field conditions was claimed to be moisture and temperature dependent, with faster degradation in spring and summer, followed by slower degradation in winter months. The applicant used simple first order (SFO) evaluation to describe the upper and lower concentration of the 'saw teeth' curve during the accumulation period and to calculate daily concentrations of fluopicolide in soil for each of the sites. However, it was noted by the applicant (and accepted by the RMS in the DAR) that SFO kinetics were not always the best fit for decline of fluopicolide between applications. In the DAR assessment of the field trials, biphasic (Hockey Stick) kinetics were reported as the best fit of decline at the Philippsburg and Apilly sites and SFO kinetics at the Senas site.

To simulate potential accumulation in further successive years, SFO degradation rate constants for fluopicolide (k_1) and M-01(k_2) plus the initial soil residue of fluopicolide applied annually (C_0) were optimised by the applicant using an Excel spreadsheet. The parameters derived for each dataset, k_1, k_2 and C_0, represented overall values for the 4 years. Best overall fit was reported to be derived with Excel Solver using least squares optimisation of the fluopicolide and M-01 soil concentrations measured immediately after each application ($C_{high\ max}$) and the residue remaining each year prior to application ($C_{low\ max}$). There was no detailed statistical assessment of the fit presented clearly in the study report. The optimised SFO degradation rates and annual application rate (C_0) were used in a predicted simulation of fluopicolide and M-01. Actual application dates at each site were used, with following applications at 365 day intervals; C_0 was added to the predicted soil concentration remaining immediately prior to the application date.

The predicted plateau values, $C_{high\ max}$ and $C_{low\ max}$, at each site were compared with the experimental data. At Philippsburg, the applicant reported that concentrations of fluopicolide in soil reached a plateau during the accumulation trial. SFO kinetics was claimed to give a good fit to the measured $C_{low\ max}$ values. The predicted initial concentration of 397 g/ha was close to the nominal/ calibrated rate of ca. 400 g/ha p.a. The predicted $C_{high\ max}$ values differed from the soil concentrations measured immediately after application. The RMS notes that the $C_{high\ max}$ values for year 2 and 3 appear to be under predicted, but then for year 4 and 5 are over predicted. The applicant attributed this to variations resulting from sampling and homogenisation

processes. Concentrations measured 1 and 3 days after the 1st application (2000) as well as the initial measured soil residue were included in the optimisation procedure.

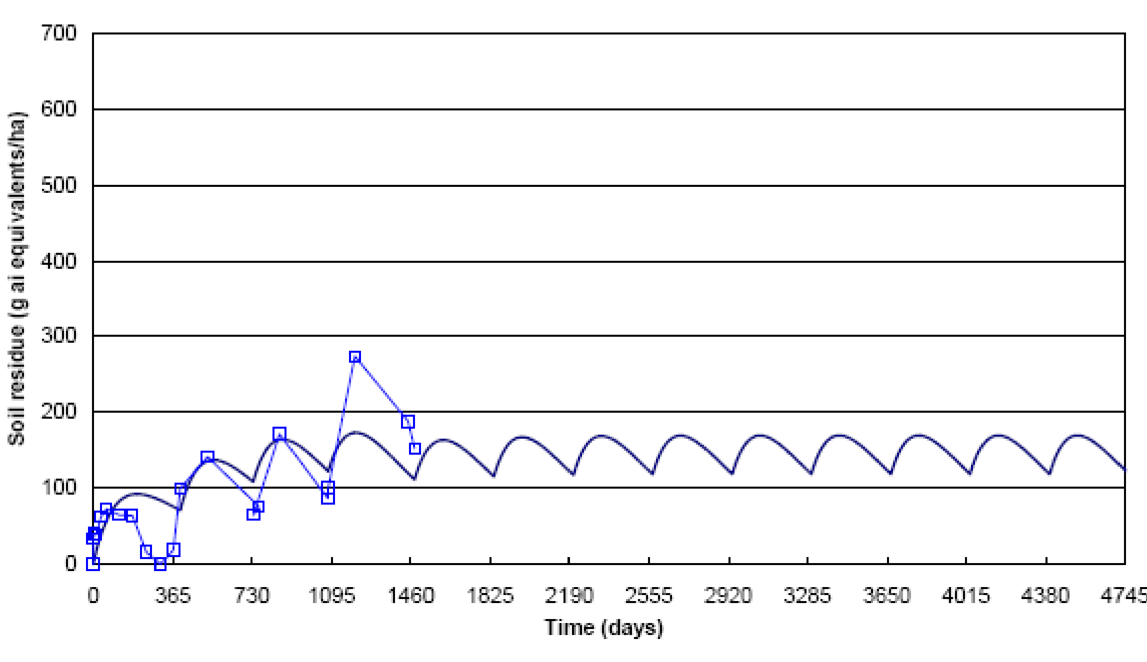
Figure 8.10 Fluopicolide residues at Philippsburg



Predicted $C_{low\ max}$ values were calculated on dates immediately prior to the application dates, which did not always occur in practice, as some measured $C_{low\ max}$ samples were taken earlier than the next application date. At the end of Year 2 (22 July 2002, 762 days) $C_{low\ max}$ samples were taken after the 3rd application (26 June 2002, 736 days), this was made to a different area of the replicate plots, so did not affect the Year 2 sampling.

The applicant concluded that fluopicolide concentrations in soil increased slightly, then reached a plateau during the accumulation study, but that repeated applications were not predicted to result in further increases in soil concentration beyond the duration of the trial. The RMS agrees that accumulation of fluopicolide in soil is not predicted beyond the duration of the study trial at Philippsburg. The predicted plateau concentration was reached by the 5th year (predicted peak plateau concentration 578 g/ha and steady state concentration of 181 g/ha, equivalent to 0.385 mg/kg and 0.121 mg/kg over 10 cm, respectively).

Figure 8.11 M-01 residues at Philippsburg



The fit to the measured concentrations of metabolite M-01 was considered reasonable by the applicant for the 2nd and 3rd years, but in the 1st and 4th years concentrations were over and under predicted, respectively. The RMS considers that the results are not sufficient to conclude that the metabolite M-01 will not accumulate in soil following repeated use of fluopicolide at this site.

Table 8.11 Results of SFO evaluation at Philippsburg

Fluopicolide	
Initial concentration (C_0)	397 g/ha
SFO rate constant (k)	0.00319 d^{-1}
DT50	217.5 days
$C_{high\ max}$	578 g/ha *(0.385 mg/kg over 10 cm)*
$C_{low\ max}$	181 g/ha *(0.121 mg/kg over 10 cm)*
M-01	
DT50	95.8 days
SFO rate constant (k)	0.00724 d^{-1}
$C_{high\ max}$	169 g as equivalents (84 g M-01/ha)
$C_{low\ max}$	118 g as equivalents (58 g M-01/ha)

APPILLY

(S. France, sandy silt, pH 7.1 and 1.51%oc content)

Fluopicolide was applied annually as detailed below.

Table 8.12 Application schedule at Apilly

Application Date	Days after treatment	Nominal application rate (g/ha)	Calibrated application rate (g/ha)
16 June 2000	384	400	397
27 Aug 2001	437	400	413
17 July 2002	761	400	410
18 June 2003	1097	400	382
30 June 2004	1475	400	400

Figure 8.12 Apilly dataset

Soil Accumulation, SFO parent + SFO metabolite
Field Appilly

	Fluopicolide	AE C653711	
DT50:	312.86	150.35	d
k:	0.002215526	0.004610365	1/d
C0:	306.20		g/ha
Molar Mass:	383.59	190.03	g/mol
Formation fraction:		1	

soil residues assuming BD = 1.5

	Measured field data			Simulated data		Square of differences	
	Time	Fluopicolide	AE C653711	Fluopicolide	AE C653711	Fluopicolide	AE C653711
Application	d	g/ha	g/ha ai eq.	g/ha	g/ha ai eq.		
16/06/2000	0	384	0	306.20	0.00	5975	0
400 g/ha	1	319	0	305.52	0.68		0
	3	317	24	304.17	2.01		
	14	338	43	296.85	9.05		
	31	397	50	285.88	18.92		
	62	230	38	266.90	34.07		
	136	147	26	226.54	58.26		
	187	129	20	202.34	67.57		
	245	140	25	177.94	73.07		
	309	141	19	154.41	74.70		
	370	108	29	134.89	73.35	734	1967
27/08/2001	437	361	38	422.49	69.80	3842	1011
	584	317	56	305.05	119.19		
	798	165	88	189.87	137.01	619	
17/07/2002	761	493	93	512.29	118.58	372	654
	889	304	150	385.80	159.95		
	1097	252	188	243.35	149.63	75	1472
18/06/2003	1097	541	107	549.55	149.63	73	1817
	1217	295	177	421.25	183.39		
	1475	240	185	237.85	157.24	5	771
30/06/2004	1475	590	174	544.05	157.24	2112	281

$\sum(P-O)^2$ 21781

Where;
P=predicted value
O=observed value

Figure 8.13 Concentration of fluopicolide at Apilly (g/ha for total soil depth) (Mean of 3 individual treated plots T1, T2 and T3)

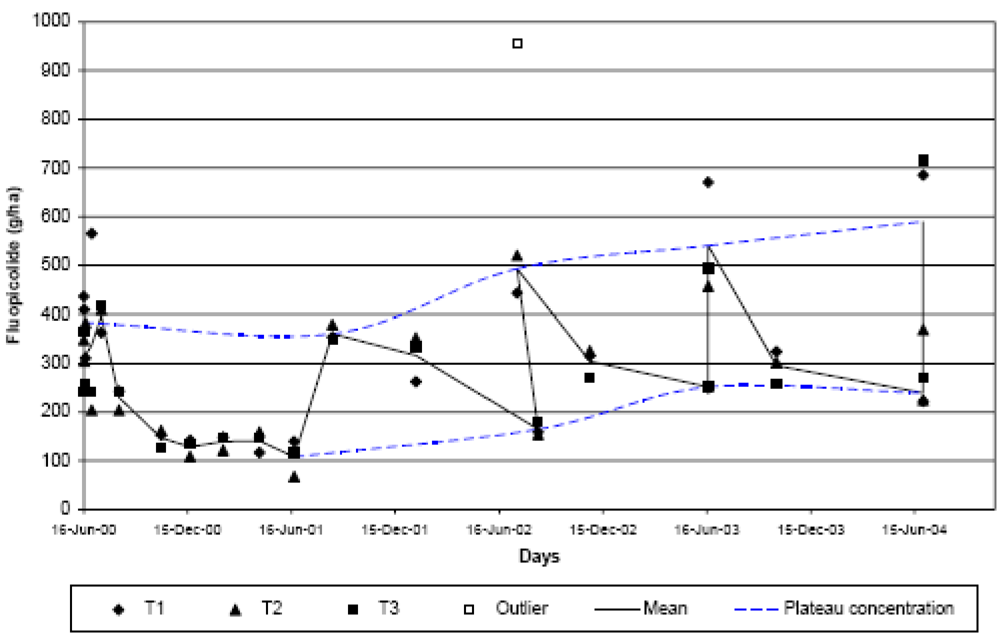
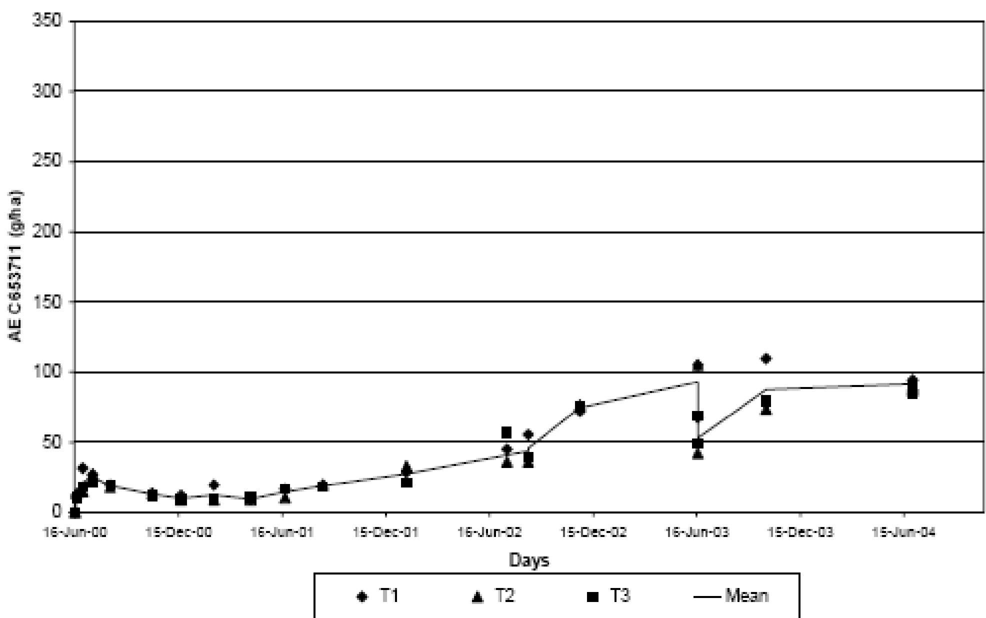


Figure 8.14 Concentration of M-01 at Apilly (g/ha for total soil depth) (Mean of 3 individual treated plots T1, T2 and T3)

NB Scale different.



The maximum mean concentration of fluopicolide detected in soil was immediately after the 5th application (2004). The applicant claimed that although the upper limit of the 'saw teeth' curve still appeared to increase, the plateau concentration at the lower limit had been reached. The applicant stated that the results for individual plots

showed good replication between the plots immediately prior to each application and that the $C_{low\ max}$ values reached a plateau by the study end. There was some variation in concentrations immediately after application ($C_{high\ max}$) between replicate plots, $C_{high\ max}$ appeared level at last point in plot T1, slightly declined in plot T2 and increased in plot T3, (in which one of the applications was excluded as an outlier). The applicant noted that this was compared to good replication at later time points and attributed the variation to the uncertainties of sampling and homogenising soil samples after application when residues were only present in the top ≤1 cm layer of soil core. The applicant concluded that measured $C_{high\ max}$ values reached a plateau in two of the three experimental plots. The RMS notes that for individual plot T3, the $C_{low\ max}$ showed a very slight increase and although for plots T1 and T2, $C_{low\ max}$ decreased by the study end, this was only at the last sample point. The RMS does not consider that there is sufficient evidence to show that a plateau concentration was reached at this site at two of the three plots.

The RMS considers there is insufficient evidence to show that the metabolite M-01 had reached a plateau concentration at this site during the study.

Figure 8.15 Concentration of fluopicolide at Apilly in 3 individual plots (g/ha for total soil depth) (T1, T2 and T3) (g/ha for total soil depth).

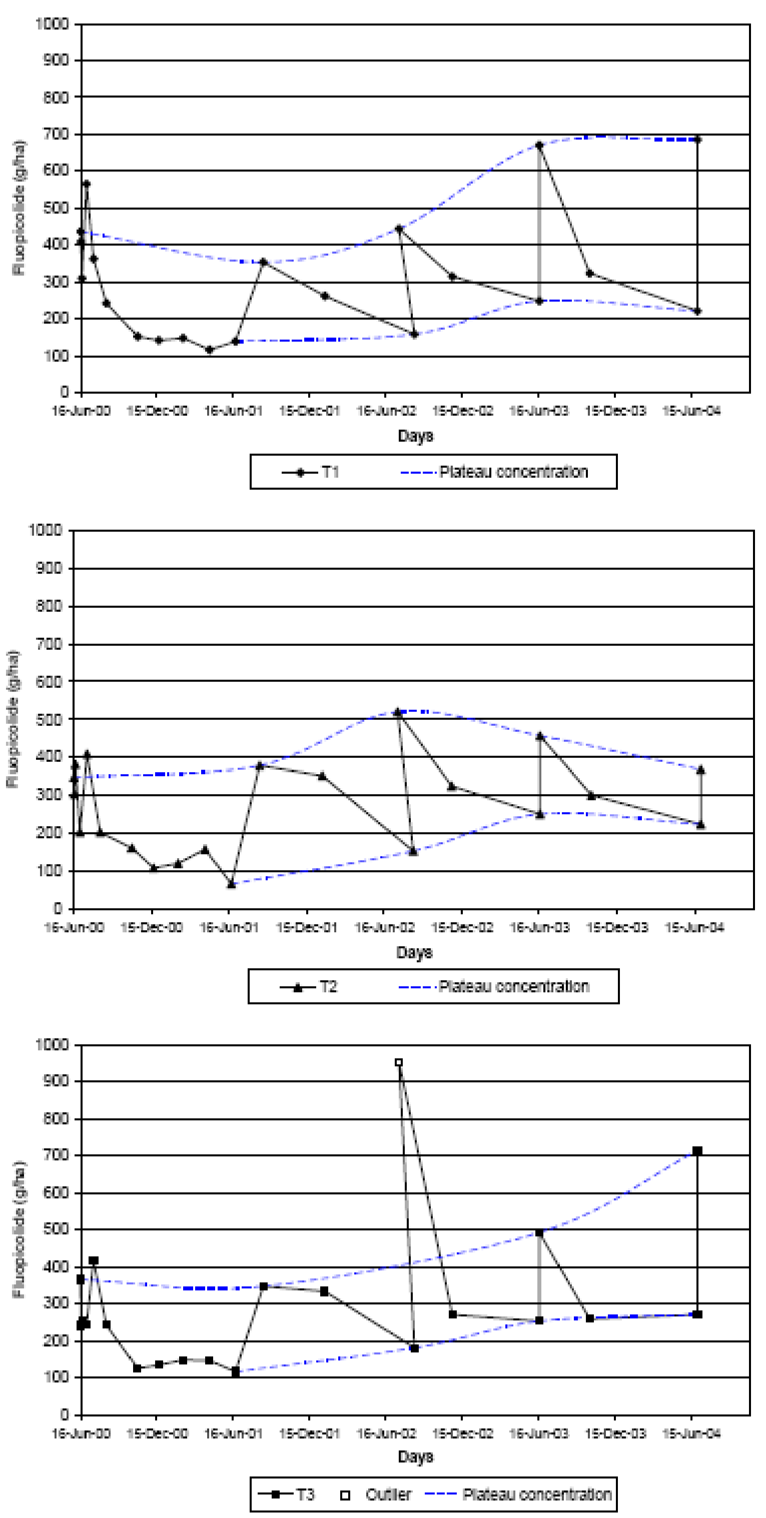


Table 8.13 Plateau concentrations of fluopicolide at Apilly

Plateau concentration	Time-point	Measured in soil increments (mg/kg)	
		0-10 cm	0-20 cm
High[1]	Day 0 5th Application	0.387	0.199
Low[2]	Day 378 after 4th Application	0.144	0.080

[1] maximum of the high values of the "saw teeth" curve
[2] maximum of the low values of the "saw teeth" curve

These plateau concentrations are the same as reported in the DAR, except for 0-20 cm (High = 0.196 mg/kg in DAR).

The applicant concluded from comparison of modelling predictions with measured values that concentrations of fluopicolide in soil reached a plateau during the accumulation trial. The predicted initial concentration at 306 g/ha was lower than the nominal and calibrated rates of *ca*. 400 g/ha p.a. SFO kinetics were reported by the applicant to give a good fit to the measured $C_{low\ max}$ values and a reasonable fit to the measured $C_{high\ max}$ values. There was no detailed statistical assessment of the fit presented clearly in the study report. The RMS notes that the last measured $C_{high\ max}$ value was under predicted.

Figure 8.16 Fluopicolide residues at Apilly

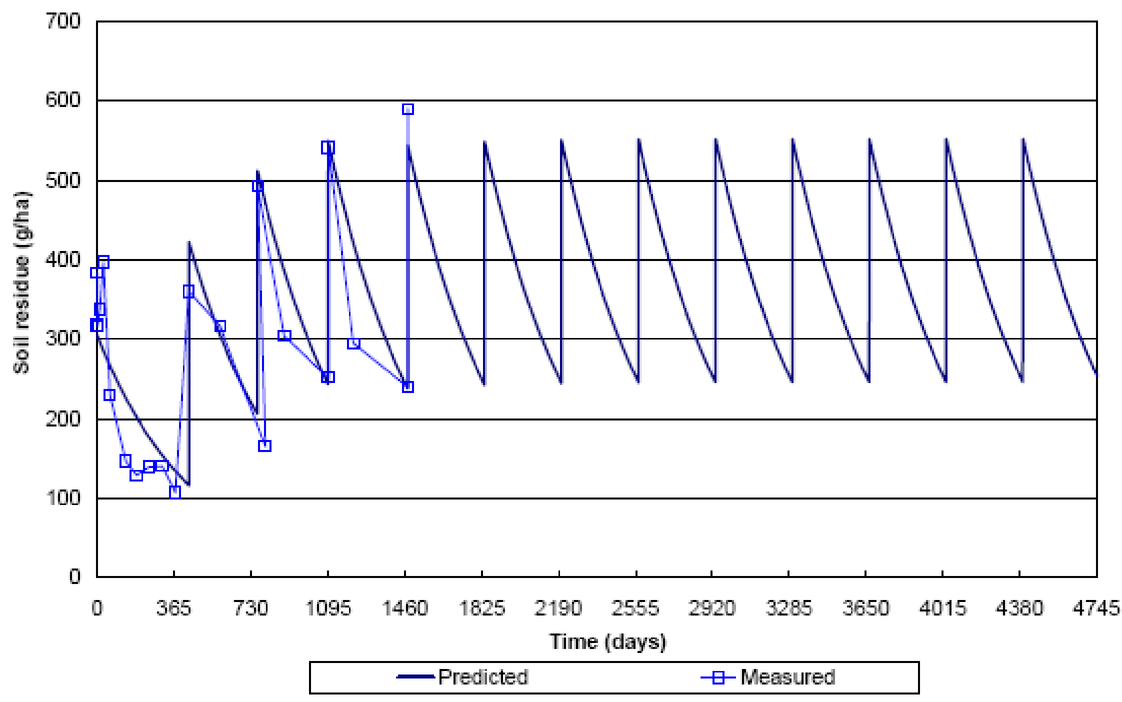
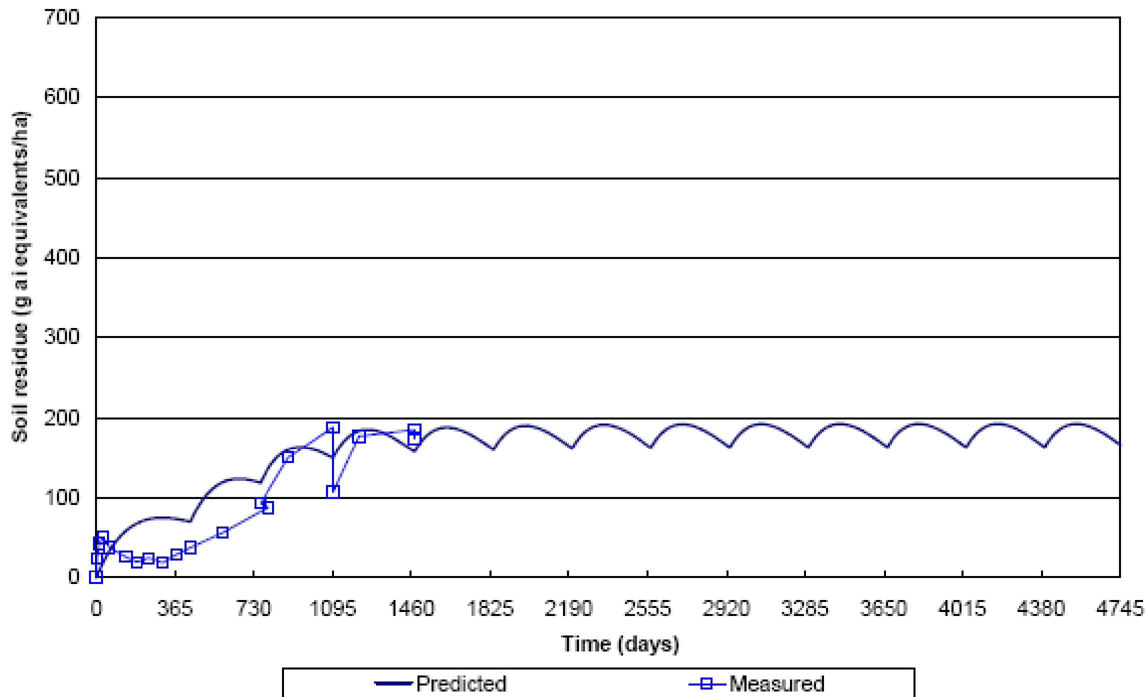


Figure 8.17 M-01 residues at Apilly



Sampling dates for the measured and predicted $C_{low\ max}$ differed, with measured values in practice being taken earlier or later than the application date. The final Year 1 sample was taken on 21 June 2001 (Day 370), before the 2nd application on 27 August 2001 (Day 437) and the final sample in Year 2 was taken on 23 August 2002 (Day 798) after the 3rd application on 17 July 2002 (Day 761). However, the plot layout allowed these samples to be unaffected by the subsequent applications.

Although accumulation of fluopicolide residues was seen, the applicant concluded that a comparison of predicted and measured concentrations confirmed that a plateau was reached during the study, and modelling did not predict further increases in successive years. The applicant's conclusion relied particularly on the $C_{low\ max}$ values. Based on the measured $C_{high\ max}$ concentrations, especially at the last time point, which was under-predicted, the RMS does not agree there is sufficient evidence that fluopicolide will not accumulate beyond the study duration at this site.

Concentrations of M-01 appeared to be over predicted by the modelling compared to the measured concentrations observed in the first 2 years, but fit the data better from day 730 – 1460. The RMS considers that the SFO evaluation is inconclusive with regards to a plateau concentration being reached for metabolite M-01 at this site.

Table 8.14 Results of SFO evaluation at Apilly

	Fluopicolide	
Initial concentration (C_0)	306 g/ha	
SFO rate constant (k)	0.00222 d^{-1}	
DT50	312.9 days	
$C_{high\ max}$	552 g/ha	*(0.368 mg/kg over 10 cm)*
$C_{low\ max}$	246 g/ha	*(0.164 mg/kg over 10 cm)*
	M-01	
DT50	150.4 days	
SFO rate constant (k)	0.00461 d^{-1}	
$C_{high\ max}$	192 g as equivalents (95 g M-01/ha)	
$C_{low\ max}$	163 g as equivalents (81 g M-01/ha)	

SENAS
(S. France. Eyre, 2003a Report: sandy silt loam, pH 7.6 and 1.6% oc content. Pollmann, 2004 Report: loamy silt, pH 7.3 and 1.65% oc content).

The study design at the Senas site differed from at Philippsburg and Appilly. The field dissipation study was started with the first application in June 1999 and ran for 2 years (Eyre, 2003a). Additional applications were continued at the same site/ treated area from 2000-2002 (Pollmann, 2004). The RMS noted in the DAR that the application in Year 3 was made before the final sample was taken in Year 2. The applicant has since provided details of the plot and sampling layout which confirms that the final Year 2 sample would have been unaffected by the Year 3 application.

At the start of the accumulation study, the original control plot in the dissipation study, Plot C, was treated in error on 20 June 2000. Consequently Plot T2n (previously Plot 1 in Eyre, 2003) was treated later on 4 August 2000 and a new control plot, Plot Cn set up.

Fluopicolide was applied as shown below.

Table 8.15 Application schedule at Senas

Application Date	Days after treatment	Nominal application rate (g/ha)	Calibrated application rate (g/ha)
24 June 1999	0	500	500
20 June 2000 (Plots T1, T3)	362	500	524
4 Aug 2000 (Plot T2n)	407	500	500
19 June 2001	726	500	519
27 June 2002	1099	500	513

Figure 8.18 Senas Dataset

Soil Accumulation, SFO parent + SFO metabolite
Field Senas, plot T1 + T2 + T3

	Fluopicolide	AE C653711	
DT50:	**166.70**	**105.48**	d
k:	0.004158018	0.006571505	1/d
C0:	**494.41**		g/ha
Molar Mass:	383.59	190.03	g/mol
Formation fraction:		1	

soil residues assuming BD = 1.5

	Measured field data			Simulated data		Square of differences	
Application	Time d	Fluopicolide g/ha	AE C653711 g/ha ai eq.	Fluopicolide g/ha	AE C653711 g/ha ai eq.	Fluopicolide	AE C653711
24/06/1999	0	327	0	494.41	0.00		0
500 g/ha nominal	1	302	36	492.36	2.04		1176
	3	264	38	488.28	6.07		
	14	223	49	466.45	26.70		
	28	231	63	440.07	49.54		
	60	182	78	385.24	89.48		
	130	113	48	287.96	133.60		
	161	146	50	232.93	142.03		
	231	122	78	189.21	139.31		
	300	99	63	142.02	126.06		4030
	368	69	63	107.04	120.49	1447	
20/06/2000	362	575	65	604.15	110.15	850	2075
	363	670	107	601.64	111.93		
	365	759	145	596.66	115.42		
	376	477	197	569.99	133.09		
	390	459	218	537.75	152.18		
	421	353	221	472.72	182.84		
	483	228	196	365.30	209.12		
	543	241	197	284.64	207.09		
	606	232	145	219.04	190.12		
	664	134	132	172.10	168.60		
	725	125	164	133.55	144.41	69	364
19/06/2001	726	670	159	627.40	144.02	1772	239
	845	414	199	382.52	230.41		
	1098	131	189	133.59	148.87	7	1589
27/06/2002	1099	629	167	627.45	148.45	1	327
	1182	349	167	444.32	225.00		
	1454	138	132	143.39	156.56	29	593
						$\sum(P-O)^2$	14568

Where;
P=predicted value
O=observed value

Figure 8.19 Concentration of fluopicolide at Senas (g/ha for total soil depth) (Mean of 3 individual treated plots T1, T2 and T3)

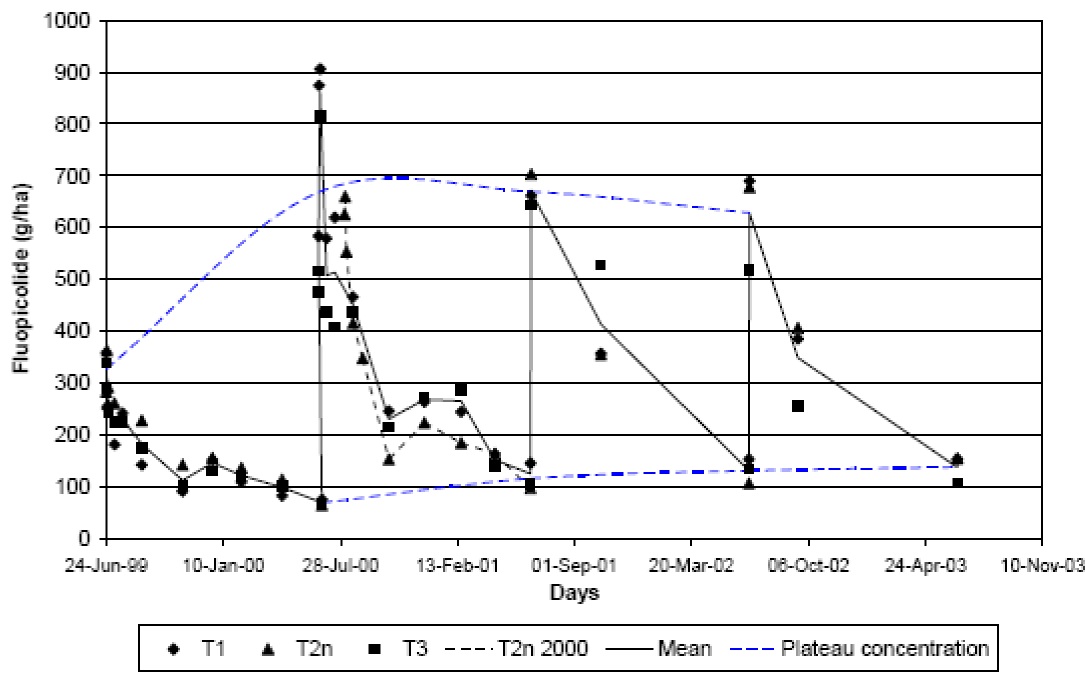
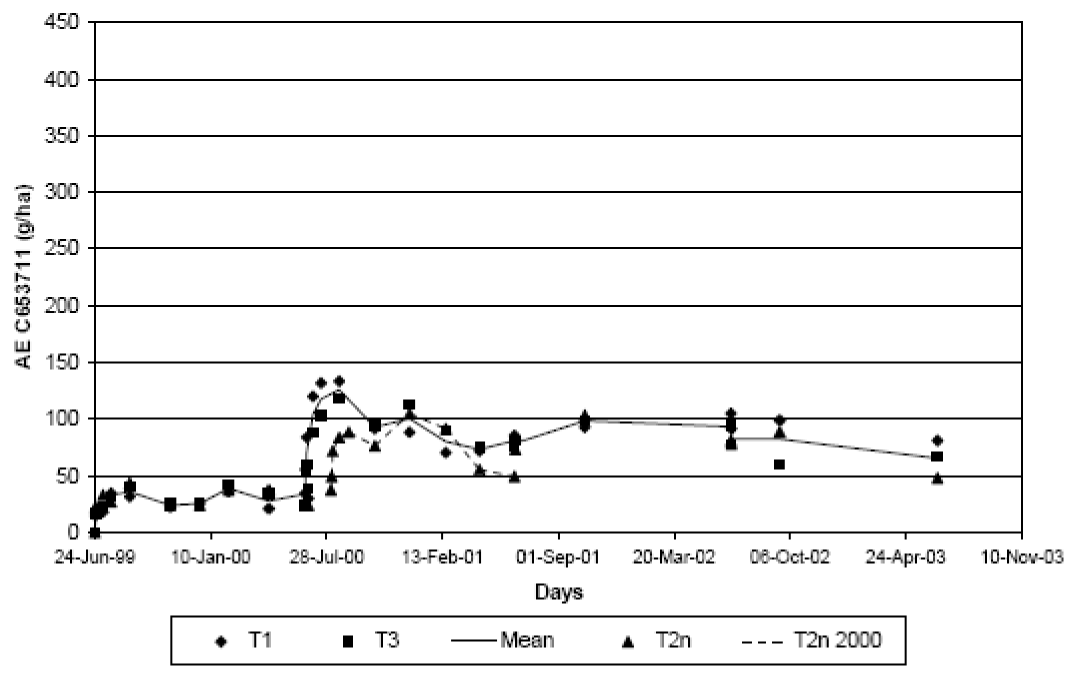


Figure 8.20 Concentration of M-01 at Senas (g/ha for total soil depth) (Mean of 3 individual treated plots T1, T2 and T3)

NB Scale different.



The maximum concentration of fluopicolide was detected immediately after the 2nd application (2000). It was stated that the rate applied to plots T1 and T3 was apparently higher than the nominal application rate of 500 g/ha. The applicant claimed no further accumulation in the upper limit of the 'saw teeth' curve was detected in subsequent applications. It was reported that the lower limit of the 'saw teeth' curve reached a plateau concentration after the 2nd application (2000) and remained relatively constant to the study end. However, the RMS observes that for plot T1 the $C_{high\ max}$ appeared to slightly increase at the last time point, while the $C_{low\ max}$ levelled off. For plot T2, the $C_{high\ max}$ values appeared to reach a plateau, though the $C_{low\ max}$ slightly increased at the end. For Plot T3, both $C_{high\ max}$ and $C_{low\ max}$ appeared to have reached a plateau.

The applicant attributed the slight increase observed in the mean $C_{low\ max}$ values from 2002 (131 g/ha) to 2003 (138 g/ha) as due to experimental variation and to not be significant, (difference between the 2 measurements equated to 0.005 mg/kg, the limit of detection). The applicant concluded that measured $C_{high\ max}$ values in the 3 experimental plots and $C_{low\ max}$ values in 2 of the 3 plots had reached a plateau at Senas.

Measured soil concentrations after the 1st and 2nd applications did not match the nominal and calibrated application rates. In the report Eyre, 2003 the apparent application rate in 1999 (at 327 g/ha) was lower than intended (500 g/ha). In the report (Pollmann, 2004) residue levels after application in 2000 were not considered appropriate as the soil concentrations of fluopicolide measured indicated the rate applied had significantly exceeded the nominal and calibrated application rate (of 500 g/ha). Therefore, only the subsequent years following application in 2001 and 2002 were considered for the evaluation of the plateau concentrations.

Figure 8.21 Concentration of fluopicolide at Senas in 3 individual plots (g/ha for total soil depth).

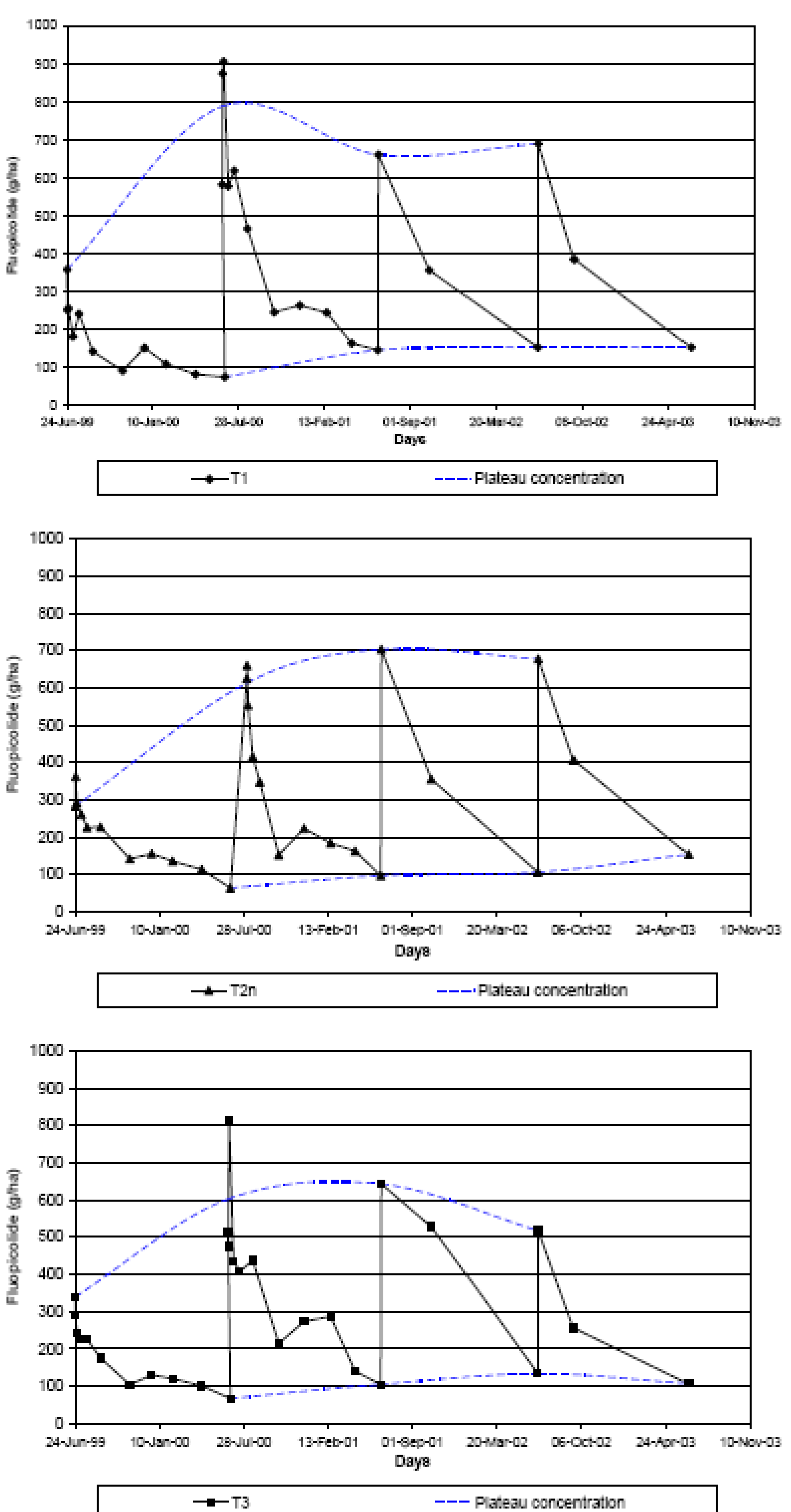


Table 8.16 Plateau concentrations of fluopicolide at Senas

Plateau concentration	Time-point	Measured in soil increments (mg/kg)	
		0-10 cm	0-20 cm
High[1]	Day 0 (4th application overall)	0.354	0.186
Low[2]	Day 372 (3rd application overall)[3]	0.082	0.044

[1] maximum of the high values of the "saw teeth" curve
[2] maximum of the low values of the "saw teeth" curve
[3] $C_{low\ max}$ (0.046 mg/kg) was measured in 0-20 cm in 2003 (Day 355 after 4th application overall)

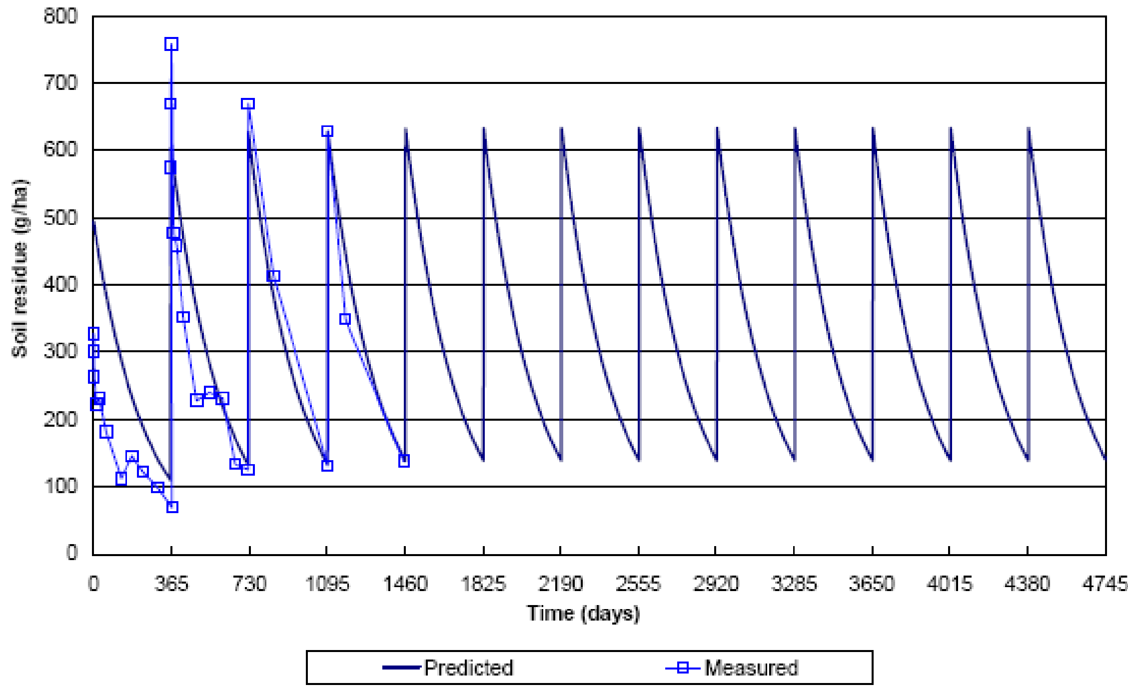
These plateau concentrations are as reported in the DAR except Low (0-10 cm/0-20 cm) was previously 0.061/0.046 mg/kg (day 355 after application 3).

The applicant concluded that based on comparison of measured and predicted concentrations of fluopicolide in soil, a plateau concentration was reached at Senas, with SFO kinetics providing a good fit to the measured $C_{low\ max}$ values. (There was no detailed statistical assessment of fit presented clearly in the study report). The predicted initial concentration (494 g/ha) was close to the nominal and calibrated application rates (*ca*. 500 g/ha). However, the predicted and measured soil concentrations immediately after the 1st and 2nd applications differed from nominal and calibrated application rates.

In the original assessment, the residue levels after application in 2000 were excluded for the assessment of the plateau concentrations, as there were indications that the rate applied had significantly exceeded 500 g/ha. Only the later years (application in 2001 and 2002) were considered. In this evaluation the initial soil residue measured in the first year (1999) was omitted from the optimisation, as the apparent application rate (327 g/ha) was lower than that achieved in later years, but all other years were considered.

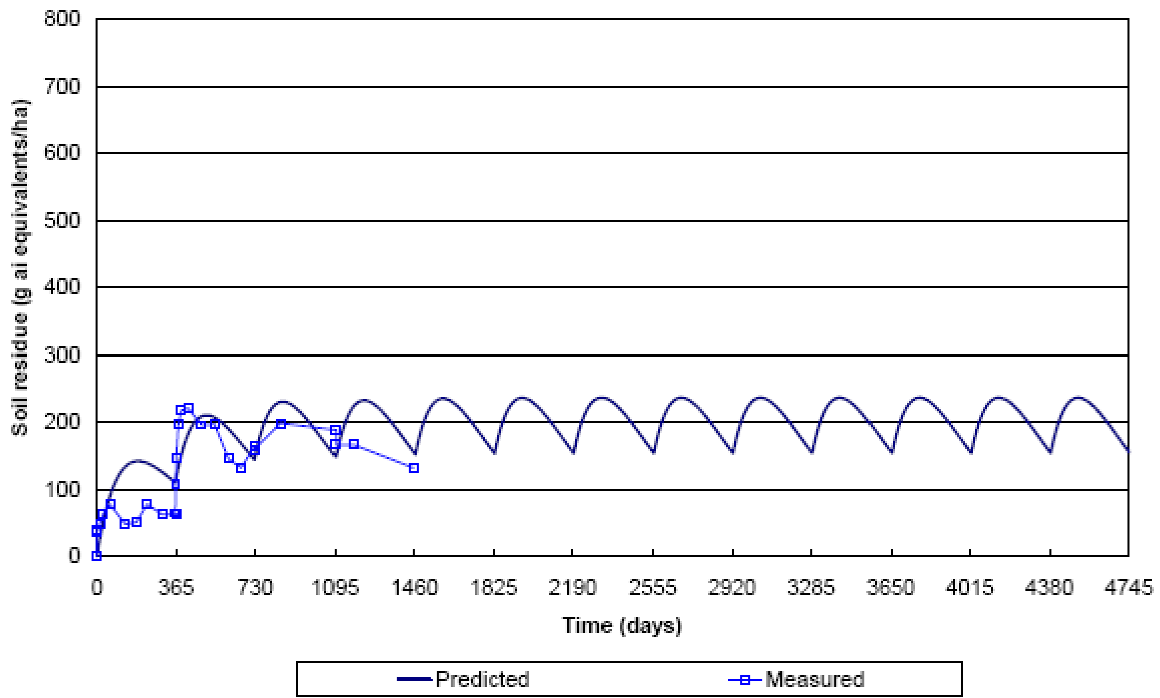
The final measured $C_{low\ max}$ value of Year 1 (26 June 2000, 368 days) was taken after the 2nd application date (20 June 2000, 362 days, Plots T1 and T3), but details of the plot layout confirmed that this sample was not affected by the Year 2 application.

Figure 8.22 Fluopicolide residues at Senas.



The applicant concluded that at the Senas trial, concentrations of fluopicolide in soil increased slightly, but reached a plateau during the study. No further increases were predicted by modelling simulations of additional applications in successive years.

Figure 8.23 Residues of M-01 at Senas.



The applicant considered that the fit to the measured concentrations of M-01 over predicted the concentration observed in the 1st year, but described the remaining years data better. There was no detailed statistical assessment of fit presented clearly in the study report. The RMS agrees that a plateau appeared to be reached for both fluopicolide and metabolite M-01 within the trial duration (4th application, with predicted peak plateau and steady state concentrations of 633 and 139 g/ha for fluopicolide, respectively).

Table 8.17 Results of the SFO evaluation at Senas.

	Fluopicolide	
Initial concentration (C_0)	494ha	
SFO rate constant (k)	0.00416 d^{-1}	
DT50	166.7 days	
$C_{high\ max}$	633 g/ha	*(0.422 mg/kg over 10 cm)*
$C_{low\ max}$	139 g/ha	*(0.026 mg/kg over 10 cm)*
	M-01	
DT50	105.5 days	
SFO rate constant (k)	0.00657 d^{-1}	
$C_{high\ max}$	237 g as equivalents (117 g M-01/ha)	
$C_{low\ max}$	154 g as equivalents (76 g M-01/ha)	

RMS Risk Assessment and Conclusions:

Fluopicolide

To summarise the results from the three sites: At the Philippsburg site, the measured concentrations of fluopicolide from two of three trial plots (T1 and T3) indicated that a plateau concentration was likely to have been reached during the trial. For the third plot T2, the $C_{high\ max}$ values appeared to have plateaued by the study end, though $C_{low\ max}$ slightly increased at the last sampling point. However, based on mean values a plateau concentration appeared to be reached by the study end. Modelling, with SFO kinetics predicted no further increase in residues from repeated applications in successive years after the trial. This modelling underestimated measured $C_{high\ max}$ residues at the 2nd and 3rd applications, but overestimated them for the 4th and 5th applications. The plateau concentration was predicted to be reached by the 5th year with $C_{high\ max}$ and $C_{low\ max}$ values of 578 and 181 g/ha, respectively. The RMS considers that the overall data indicate that fluopicolide appeared to have reached a plateau concentration within the study duration.

At the Apilly site, the measured concentrations of fluopicolide from one of three trial plots (T3) indicated that a plateau was not reached, both $C_{high\ max}$ and $C_{low\ max}$ values were still increasing at the study end. In plot T1, $C_{high\ max}$ and $C_{low\ max}$ appeared to plateau, but only at the last sampling point and at plot T2, $C_{high\ max}$ clearly declined, though $C_{low\ max}$ values were again only level at the last point. Based on mean values the $C_{low\ max}$ values appeared to plateau but the $C_{high\ max}$ values did not. No further increase in residues beyond the trial was predicted by modelling, with SFO kinetics after repeated applications in successive years. The plateau concentration was predicted by the applicant to be reached by the 5th year with $C_{high\ max}$ and $C_{low\ max}$ values of 552and 246 g/ha, respectively. The RMS considers that this modelling underestimated measured $C_{high\ max}$ residues at last (5th) application and that the data are inconclusive as to whether a plateau was reached during the trial.

At the Senas site, the measured concentrations of fluopicolide from one of three trial plots (T3) indicated that a plateau was reached. At the T1 plot, $C_{high\ max}$ slightly increased at the last point though $C_{low\ max}$ had reached a plateau. For plot T2, $C_{high\ max}$ values had plateaued, though the $C_{low\ max}$ value was still increasing at the study end. The RMS considers that overall, based on mean $C_{high\ max}$ values, concentrations had reached a plateau and the $C_{low\ max}$ values, though close to levelling off, were very slightly increasing at the study end. Modelling, assuming SFO kinetics predicted no further increases based on repeated applications in successive years after the trial. This modelling underestimated measured $C_{high\ max}$ residues at 2nd application, but the fit to later years was reasonable. The plateau concentration was predicted by the applicant to be reached by the last (4th) year with $C_{high\ max}$ and $C_{low\ max}$ values of 633and 139 g/ha, respectively. Therefore, the RMS accepts that a plateau concentration appeared to have been reached for fluopicolide at the Senas site within the study duration.

The applicant compared the maximum residue level observed for fluopicolide at each site after 4 years ($C_{high\ max}$ 0.341-0.387 mg/kg over 10 cm) as equivalent to 1.1 -1.5 times the residue in soil after a single application. (In support of this, the RMS estimates an initial PEC_{soil} after a single application of 400-500 g a.s/ha of 0.267-0.333 mg/kg over 10 cm soil depth, based on a simple first tier calculation with no interception assumed).

M-01

Concentrations of the metabolite M-01 (AE C653711) were not predicted by the applicant to significantly increase in soil, in successive years after the study duration at each site. However, the agreement between the concentrations predicted by SFO modelling and the measured concentrations was less robust.

The RMS considered that based on the measured data there was insufficient evidence of a plateau concentration being reached for M-01 at the Philippsburg and Apilly sites during the trials, although a plateau concentration for M-01 did appear to be reached at the Senas site. The RMS considered that for Philippsburg site the modelling clearly underestimated the concentrations of M-01 at the last time point. For the Apilly site, the RMS considered that the predicted concentrations for M-01 were closer to the measured data (except for under-estimations in the first year) and that at the Senas site, the modelling appeared to generally over predict concentrations of M-01.

The RMS proposes that further discussion is needed at the expert meeting over the general acceptability of this type of higher tier approach, versus a simple first tier calculation of PEC_{soil} accumulation. Further discussion may also be warranted over how best to interpret measured versus predicted concentrations in soil and the results of individual plots compared to mean results at each site, in reaching an overall conclusion on the potential for accumulation of an active substance.

Implications for Ecotoxicogical Assessment:

No implications for the ecotoxicolgical assessment at present. However, the PEC_{soil} may need to be reassessed on the basis of the PRAPeR expert meeting discussion.

(Kley, C; Mackenzie, E; M-267721-01-1, 2007)

B.8.6.2 Predicted environmental concentrations in groundwater.

Data Requirement 4.3

"Applicant to provide results with a second FOCUS model following the recommendations given in the PPR Opinion: Opinion of the Scientific Panel on Plant Health, Plant Protection Products and their Residues on a request of EFSA related to FOCUS groundwater models. The EFSA Journal (2004) 93, 1-20.

For some of the metabolites it may not be confirmed that the triggers of 0.75 µg/L and 10 µg/L are not exceeded in some scenarios. A second model is necessary to reduce the uncertainty and confirm the non relevance of the metabolites.

Applicant indicated to submit new PEC GW calculations with a second model and lower interception rate for vines by May 2007.

See reporting table 4(79)."

Data Requirement 4.4

"Applicant to repeat the FOCUS GW calculations following the GAP as reported in the Representative uses table. Applicant indicated to submit repeated PEC GW calculations with a lower interception rate for vines by May 2007.

See reporting table 4(80)."

Background:

Potential contamination of groundwater by fluopicolide was assessed with only one FOCUS model, (DAR, B.8.6.2), as the submission was made prior to the PPR Opinion[3] recommending the results of two models are needed to complete the risk assessment. The applicant was requested to provide results for FOCUS GW modelling with a second FOCUS model to reduce uncertainty and confirm the non-relevance of metabolites, following recommendations given in the PPR Opinion (EFSA Journal (2004) 93, 1-20).

For the PECgw calculation (DAR, B.8.6.2) it was assumed a one in three year crop rotation was representative of good agricultural practice in potatoes. However, as crop rotation is not mandatory and the 'representative' use concept implies the assessment is also applicable to other crops represented by the specific crop listed, the applicant was requested to repeat the FOCUS GW calculations following the GAP as reported in the Representative uses table. Similarly, the applicant was requested to repeat the FOCUS

[3] Opinion of the Scientific Panel on Plant Health, Plant Protection Products and their Residues on a request of EFSA related to FOCUS groundwater models. The EFSA Journal (2004) 93, 1-20.

GW modelling with a lower interception rate for vines. (Reporting Table, points 4(79), 4(80) and 4(81)).

Summary of approach taken to address Data requirements 4.3 and 4.4

The applicant has performed new FOCUS groundwater modelling with PELMO. These simulations include lower interception rates for use on vines and also for modelling of use on potatoes, application of fluopicolide once every year and every 2 years, as well the previously assessed 1-in-3 year crop rotation pattern.

Furthermore, as a second FOCUS groundwater model was required, in accordance with the PPR opinion (EFSA Journal (2004) 93, 1-20), the applicant has performed PECgw calculations for use of fluopicolide on vines and potatoes, using the PEARL model.

In the original assessment, batch equilibrium studies (Rupprecht 2003 & Simmonds 2003) were previously evaluated in the DAR B.8.2.1 (a) and (b) and sorption of fluopicolide was correlated with organic carbon/matter content of the soil. However, these studies do not take into account kinetically controlled sorption behaviour and so may, in the view of the applicant, underestimate sorption and overestimate mobility.

Time-dependent laboratory sorption studies (Fitzmaurice, 2003, Allan, 2003b) were carried out to investigate kinetic sorption and reported in the DAR, B.8.2.1.(c) & (d), although these were not relied on for the exposure assessment presented in the original DAR. The K_{oc} was increased by a factor of *ca*. 2.1 over 23 days (Fitzmaurice, 2003) and *ca*. 2.3 over 121 days (Allan, 2003b) indicating stronger sorption of fluopicolide with time. Time-dependent sorption is proposed by the applicant, as a possible explanation for the bi-phasic behaviour of fluopicolide in some field dissipation trials. In this new assessment, the applicant has taken into account data on time-dependent sorption for fluopicolide using the PEARL model and its ability to simulate non-equilibrium sorption (PEARL NEQ).

In the PEARL NEQ model, sorption of substances in soil is described by a Freundlich type equation, with both equilibrium and non-equilibrium (kinetic) sorption being able to be considered. Sorption in the equilibrium domain of the soil system is assumed to occur instantaneously, whereas sorption in the non-equilibrium domain proceeds gradually. As pesticide is assumed to be present in both domains, 2 mass balance equations are needed.[4] The mass balance equation for sorption in the non-equilibrium domain requires additional parameters i.e. the desorption rate coefficient (k_d) and a

[4] From RIVM report 711401 008. Alterra report 28. Manual of FOCUS PEARL v 1.1.1. November 2000. A. Tiktak, F. van den Berg, J.j.T.I Doesten, D.van Kraalingen, M.Leistra & A.M.A. van der Linden:
2.5.3. …..two mass balances apply:
$\partial c^*_{eq} / \partial t = -R_s - \partial J_{p,L} / \partial z - \partial J_{p,g} / \partial z - R_t - R_u - R_d$ and

$\partial c^*_{neq} / \partial t = R_s$

where c^*_{eq} $(kg\ m^{-3})$ and c^*_{neq} $(kg\ m^{-3})$ are the pesticide concentrations in the equilibrium and non-equilibrium domains of the soil system, respectively. R_s (kg m^{-3} d^{-1}) is the volumic mass rate of pesticide sorption. $J_{p,L}$ and $J_{p,g}$ (kg m^{-2} d^{-1}) are the mass flux of pesticide in the liquid and gas phases, respectively. R_t and R_t (kg m^{-3} d^{-1}) are the transformation and formation rates , respectively. R_u (kg m^{-3} d^{-1}) is the rate of pesticide uptake by plant roots and R_d (kg m^{-3} d^{-1}) is the lateral discharge rate of pesticides.

factor describing the ratio (F_{NE}) between the Freundlich coefficients at the equilibrium ($_{EQ}$) and non-equilibrium ($_{NEQ}$) sites i.e.
($F_{NE} = Kf,_{NEQ}/ Kf,_{EQ}$).

In the PEARL NEQ model, it is assumed that transformation of a pesticide only occurs in the equilibrium domain. Therefore, as the transformation half-life can only apply to the equilibrium domain, it must be obtained using an alternative approach for this purpose, (whereas DT50 values commonly reported for pesticides usually refer to the total mass content of pesticide).

An example of such an approach for transformation of compound in case of sorption/ desorption kinetics is described in section 3.2.10 of the RIVM report 711401 008.[5]

To take into account time-dependent sorption in the PEARL model, new parameters were needed for degradation rate constant for a.s. in equilibrium phases, desorption rate constant (k_d) and ratio of Freundlich coefficients for equilibrium and non-equilibrium sites (f_{NE}). Two new studies have been submitted (Kley, 2004 MEF-04/346 and MEF - 04/347) in which the applicant has used a kinetic sorption model to describe the kinetic processes influencing sorption of fluopicolide and to obtain parameter values that could be used in a higher tier assessment. (Metabolites were not considered).

The applicant has evaluated the time-dependent sorption of fluopicolide using batch equilibrium data (Kley, 2004, MEF-04/346), to obtain the necessary parameters and then also applied the results from this approach to an evaluation of field dissipation data to obtain a suitable field DT50, for use in the PEARL kinetic sorption model, (Kley, 2004, MEF 04/347).

These kinetic sorption parameters have then been implemented into the FOCUS PEARL modelling (Kley, C. & Ellerich C. 2007 (a) and (b)). PECgw estimates conducted with the FOCUS PEARL model, using standard degradation parameters as a first step, before implementing kinetic sorption parameters, have not been provided. It was not possible to implement sorption kinetics in the PELMO model, which was instead performed using standard degradation kinetic and sorption parameters.

Each of these studies is assessed in more detail below.

RMS Evaluation of new data – Kley, C. 2004 (MEF-04/346)
Kinetic evaluation of batch equilibrium data considering time-dependent sorption:

This report describes a kinetic sorption model used by the applicant to derive degradation rates for use in FOCUS PEARL groundwater modelling. The study author claims that "*it is equivalent to the one implemented in the PEARL model, which is used to calculate predicted environmental concentrations in groundwater*".

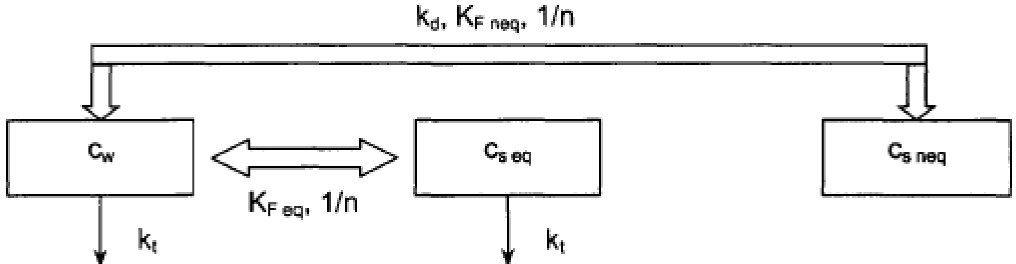
The following description of this kinetic evaluation is complex and as such is largely reproduced from the study report. (The complete reports are also appended for information). In order to conclude whether this is a valid approach, the RMS considers

[5] RIVM report 711401 008. Alterra report 28. Manual of FOCUS PEARL v 1.1.1. November 2000. A. Tiktak, F. van den Berg, J.J.T.I Boesten, D.van Kraalingen, M.Leistra & A.M.A. van der Linden.

that it will be important to determine whether this is an acceptable interpretation of how the PEARL model simulates non-equilibrium sorption.

Three compartments were considered in the kinetic sorption model for a compound in a soil system: a dissolved phase (C_w); equilibrium sorbed phase ($C_{s\,eq}$) and non-equilibrium sorbed ($C_{s\,neq}$) phase. In the kinetic sorption model, only the part of the compound in the equilibrium domain (dissolved and sorbed) is considered available for degradation, so corresponding degradation rates have to be determined.

Figure 8.24 3-compartment sorption kinetic approach.



The relation between the dissolved and equilibrium sorbed phase was characterised by instantaneous equilibrium between both phases, described by the Freundlich isotherm:

$$C_{s\,eq} = K_{f\,eq} \cdot C_w^{1/n} \qquad (1)$$

where:

$C_{s\,eq}$ concentration in the equilibrium sorbed phase, mg/kg dry soil,
$C_{s\,neq}$ concentration in the non-equilibrium sorbed phase, mg/kg dry soil,
C_w concentration in the dissolved phase, mg/L water,
$K_{f\,eq}$ Freundlich distribution coefficient for equilibrium domain, L/kg,
$K_{f\,neq}$ Freundlich distribution coefficient for non-equilibrium domain, L/kg,
1/n Freundlich exponent

The concentration in the non-equilibrium phase ($C_{s\,neq}$) was defined as non-equilibrium sorbed mass of substance / mass of dry soil, related to $C_{s\,eq}$ by:

$$\frac{d\,C_{s\,neq}}{dt} = k_d \cdot \left(\underbrace{\frac{K_{f\,neq}}{K_{f\,eq}}}_{f_{ne}} \cdot C_{s\,eq} - C_{s\,neq} \right) \qquad (2)$$

where:

K_d kinetic sorption rate constant,
$K_{f\,neq}$ I Freundlich coefficient for non-equilibrium phase,
1/n assumed valid for both the equilibrium and non-equilibrium domain

and the terms in the above equation (2):
$K_{f\,neq}$ / $K_{f\,eq}$ } f_{ne} means $K_{f\,neq}$ / $K_{f\,eq}$ = f_{ne} (i.e. the ratio of Freundlich coefficient for non-equilibrium phase to }Freundlich coefficient for equilibrium phase, which is larger the greater the sorption 'capacity' of the non-equilibrium domain).

$C_{s\,eq} \cdot K_{f\,neq} / K_{f\,eq}$ describes concentration in non-equilibrium phase after sufficiently long or infinite time at which $C_{s\,neq} = C_{s\,eq} \cdot K_{f\,neq} / K_{f\,eq}$ and the concentration gradient of $C_{s\,neq}$ is 0.

A number of further transformations are reproduced below (and described in further detail in the report, Kley 2004), which lead to the differential equation (12) for $C_{s\,eq}$. Equations (2) and (12) are reported by the study author to completely define concentrations of pesticide in all three phases.

Equation (3) represents total concentration in the dissolved and equilibrium sorbed phase:

$$C_{t\,eq} = \frac{\theta_g}{\rho_w} C_w + C_{s\,eq} \qquad (3)$$

where:

θ_g gravimetric water content (g water/g dry soil, set by experimenter)

ρ_w density of water (assumed as 1 kg/L)

Or using the isotherm, C_w (concentration in the dissolved phase) is substituted by $(C_{s\,eq} / K_{f\,eq})^{-1/n}$ to give equation (4):

$$C_{t\,eq} = \frac{\theta_g}{\rho_w} \cdot K_{f\,eq}^{-n} \cdot C_{s\,eq}^{n} + C_{s\,eq} \qquad (4)$$

Equation (5) derived from equation (3) differentiated with respect to time:

$$\frac{d\,C_{t\,eq}}{d\,t} = \frac{\theta_g}{\rho_w} \cdot \frac{d\,C_w}{d\,t} + \frac{d\,C_{s\,eq}}{d\,t} \qquad (5)$$

To derive equation (6) the differential dC_w / d_t is removed by use of the chain rule[6]

$dC_{s\,eq} / dt = dC_{s\,eq} / dC_w \cdot dC_w / dt$

(as C_w and $C_{s\,eq}$ are related via the isotherm) and substituted by $dC_{s\,eq}/dt \cdot (dC_{s\,eq}/dC_w)^{-1}$:

$$\frac{d\,C_{t\,eq}}{d\,t} = \frac{\theta_g}{\rho_w} \cdot \frac{d\,C_{s\,eq}}{d\,t} \cdot \left(\frac{d\,C_{s\,eq}}{d\,C_w}\right)^{-1} + \frac{d\,C_{s\,eq}}{d\,t} \qquad (6)$$

Using the sorption isotherm $dC_{s\,eq} / dC_w$ is written as:

[6] The chain rule is a formula for the derivative of the composite of two functions. If a variable, *y* depends on a second variable, *u* which in turn depends on a third variable, *x* then the rate of change of *y* with respect to *x* can be computed as the rate of change of *y* with respect to *u,* multiplied by the rate of change of *u* with respect to *x.* In Leibniz notation the chain rule is *df /dx = df /dg · dg /dx*.

$$\frac{d\,C_{s\,eq}}{d\,C_w} = \frac{1}{n} \cdot K_{f\,eq} \cdot C_w^{1/n - 1} \tag{7}$$

C_w is removed to give equation (8)

$$\frac{d\,C_{s\,eq}}{d\,C_w} = \frac{1}{n} \cdot K_{f\,eq} \cdot \left(\frac{C_{s\,eq}}{K_{f\,eq}}\right)^{1-n} \tag{8}$$

which is then used to rewrite equation (6) as equation (9):

$$\frac{d\,C_{t\,eq}}{d\,t} = \frac{d\,C_{s\,eq}}{d\,t} \cdot \left(\frac{\theta_g}{\rho_w} \cdot n \cdot K_{f\,eq}^{-n} \cdot C_{s\,eq}^{n-1} + 1\right) \tag{9}$$

The relationship between the equilibrium and non-equilibrium domain is described by equation (10), with k_t the first order, rate constant for degradation, (in the equilibrium domain only):

$$\frac{d\,C_{t\,eq}}{d\,t} = -k_t \cdot C_{t\,eq} - \frac{d\,C_{s\,neq}}{d\,t} \tag{10}$$

Equation (10) combined with equation (2) gives equation (11):

$$\frac{d\,C_{t\,eq}}{d\,t} = -k_t \cdot C_{t\,eq} - k_d \cdot \left(f_{ne} \cdot C_{s\,eq} - C_{s\,neq}\right) \tag{11}$$

Equation (12) is the differential equation for $C_{s\,eq}$ (concentration in the equilibrium sorbed phase). It results from equating equations (9) and (11) and using equation (4) to substitute $C_{t\,eq}$ with $C_{s\,eq}$:

$$\frac{d\,C_{s\,eq}}{d\,t} = \frac{-k_t \cdot \left(\frac{\theta_g}{\rho_w} \cdot K_{f\,eq}^{-n} \cdot C_{s\,eq}^{n} + C_{s\,eq}\right) - k_d \cdot \left(f_{ne} \cdot C_{s\,eq} - C_{s\,neq}\right)}{1 + \frac{\theta_g}{\rho_w} \cdot n \cdot K_{f\,eq}^{-n} \cdot C_{s\,eq}^{n-1}} \tag{12}$$

where:

f_{ne} ratio between Freundlich coefficients, ($k_{f\,neq}/k_{f\,eq}$)
θg gravimetric water content (g water / g dry soil)
ρ_w density of water
k_t degradation rate constant in equilibrium phase

Equations (2) and (12) were then fitted to the kinetic-sorption model by the applicant using ACSL Optimize 1.2 software. The 3 parameters required by the model of k_d (kinetic-sorption rate constant), k_t (degradation rate constant in the equilibrium domain) and $f_{ne,}$ (ratio between the Freundlich coefficients in the non-equilibrium and

in the equilibrium domain), were optimised by simultaneous fits to the experimental data ($C_{s\ eq}$ and $C_{s\ neq}$) as described below. Initial value for non-equilibrium sorbed concentration ($C_{s\ neq\ 0}$) was set to 0.

Processing of the experimental data:

Concentrations in the dissolved, equilibrium sorbed and non-equilibrium sorbed phases for use in the kinetic sorption model were calculated from the experimental data (from Fitzmaurice, 2003 and Allan, 2003b). These data were pre-processed to calculate concentrations as valid during the ageing period, i.e. at just after application without any dilution by aqueous or organic solvent and before removing supernatant in single or multiple extraction steps. The equations used are summarised below, full details and input values are described in Kley, 2004, (MEF-04/346).

The total mass of compound recovered is given by equation (13)

$$m_t = m_{OrgExtract} + \sum_{i}^{n} C_{w\,i} \cdot V_{w\,i} \qquad (13)$$

where:

m_t	total mass of compound recovered
$m_{OrgExtract}$	substance mass in organic solvent (sum of substance in organic supernatant + in pore volume filled with organic solvent)
m_0	mass of dry soil
n	number of extraction steps
i	supernatant
C_{wi}	concentration in dissolved phase
V_{wi}	volume of water in supernatant
V_P	volume of water in soil (water in pore volume)

The aged sorption study in Allan (2003) involved a single aqueous extraction step, (the system after a single extraction and centrifugation with $CaCl_2$ is denoted by $_1$ in equation (14) below). The Freundlich co-efficient $K_{f\ eq}$ (valid for desorption) was calculated using day 0 values, where t = 0, $C_{s\ eq\ 1}$ is calculated as difference between total mass and mass dissolved (Equation 14a), as the non-equilibrium concentration was defined as $C_{s\ neq} = 0$. The mean Freundlich exponent (1/n) from the standard batch equilibrium studies (0.9028) was used, (DAR, Table B.8.190).

$$K_{f\,eq} = \left.\frac{C_{s\,eq\,1}}{C_{w\,1}^{1/n}}\right|_{t=0} \qquad (14)$$

$$C_{s\ eq\ 1} = m_t / m_0 - C_{w1}\ (V_{w1} + V_{P1}) / M_0 \qquad (14a)$$

The aged sorption study in Fitzmaurice (2003) involved multiple aqueous desorption steps. The mean $K_{f\ eq,\ des}$ and 1/n for the 3 desorption steps at day 0 were used, with $K_{f\ eq,\ des}$ (4.363, 4.287, 18.303 and 4.623) and corresponding 1/n (0.9237, 0.888, 0.9813 and 0.904) for each of the 4 soils.

Total substance mass in the dissolved and equilibrium sorbed phase ($m_{t\,eq}$) before and after the aqueous desorption steps has to be equal.

$$m_{teq} = \underbrace{C_w \cdot V_{p0} + C_{s\,eq} \cdot m_0}_{\text{before desorption}} = \underbrace{\sum_i^n C_{wi} \cdot V_{wi} + C_{wn} \cdot V_{pn} + C_{s\,eq\,n} \cdot m_0}_{\text{after desorption}} \quad (15)$$

The volume of water in soil pores after nth extraction step (V_{Pn}) and centrifugation is calculated by equation (16).

$$V_{Pn} = V_{P0} + V_{w\,\text{extraction solution added}} - V_{wn} \quad (16)$$

Equation (17) is derived from equation (15), using the sorption isotherm ((equation (1) $C_{s\,eq} = K_{f\,eq} \cdot C_w^{\ 1/n}$).

$$m_{teq} = C_w \cdot V_{p0} + K_{f\,eq} \cdot C_w^{1/n} \cdot m_0 = \sum_i^n C_{wi} \cdot V_{wi} + C_{wn} \cdot V_{pn} + K_{f\,eq} \cdot C_{wn}^{1/n} \cdot m_0 \quad (17)$$

The value for C_w was calculated iteratively using Microsoft Excel® Add-in Solver and $C_{s\,eq}$ calculated from C_w using equation (18), (also shown as equation 1).

$$C_{s\,eq} = K_{f\,eq}\, C_w^{1/n} \quad (18)$$

Equation (19) describes the non-equilibrium sorbed concentration (i.e. from mass of substance in organic extracts minus remainders of other phases in soil after aqueous desorption steps):

$$C_{s\,neq} = \frac{m_{OrgExtract}}{m_0} - \frac{C_{wn} \cdot V_{Pn}}{m_0} - K_{f\,eq} \cdot C_{wn}^{1/n} \quad (19)$$

The total substance mass ($m_{t\,eq}$) in the dissolved and equilibrium phase is calculated with equation (20):

As in the original experiment, degradation and sorption could occur during the 24 h shaking process, as well as during the ageing process, this shaking period was treated by the study author as additional ageing time and 1 d added to the time points. Day 0 values were therefore calculated with equation (17), assuming non-equilibrium sorbed concentration at day 0 is zero ($C_{s\,neq}$ (t=0) = 0) and that applied substance is distributed in both the dissolved (C_w) and equilibrium sorbed ($C_{s\,eq}$) phase.

$$m_{teq} = C_w \cdot \frac{\theta_g}{\rho_w} \cdot m_0 + K_{f\,eq} \cdot C_w^{1/n} \cdot m_0 \quad (20)$$

where:

$m_{t\,eq}$ total substance mass in the dissolved and equilibrium phase
C_W concentration in dissolved phase (from iterative calculations in Excel Solver)
θg gravimetric water content (g water / g dry soil)
ρ_w density of water
$m0$ mass of dry soil
$K_{f\,eq}$ Freundlich distribution co-efficient for equilibrium domain
$1/n$ Freundlich exponent
$C_{s\,eq}$ $= (K_{f\,eq} \cdot C_w^{\ 1/n})$

Results of the kinetic sorption model:

Material in the dissolved and equilibrium sorbed phases (‘equilibrium domain’) is in instantaneous equilibration with sorption described by the Freundlich isotherm. The kinetic sorption model assumes that fluopicolide is transferred to the non-equilibrium domain (NEQD) and vice versa by the concentration gradient between the equilibrium (EQD) and non-equilibrium (NEQD) domains. At day 0 the non-equilibrium sorbed concentration is assumed to be zero so maximum transfer to the NEQD is predicted. Once NEQD sorbed concentrations reach the same as EQD sorbed concentrations the transfer is reversed. Degradation is assumed to occur in the equilibrium domain only and is described by first-order kinetics (rate constant k_d).

Table 8.18 Parameters of the kinetic sorption model for all soils

trial	k_t d^{-1}	k_d d^{-1}	f_{ne}
Philippsburg, mean	0.0121	0.0589	0.4692
Rödelsee, mean	0.0074	0.0835	0.3701
Huntlosen, mean	ns.	0.1950	0.3657
Senas, mean	0.0118	0.1075	0.3230
Abington, both labels	0.00317	0.0362	0.4485
arithm. mean			**0.3953**
geo. mean		**0.08211**	

ns. Not significant

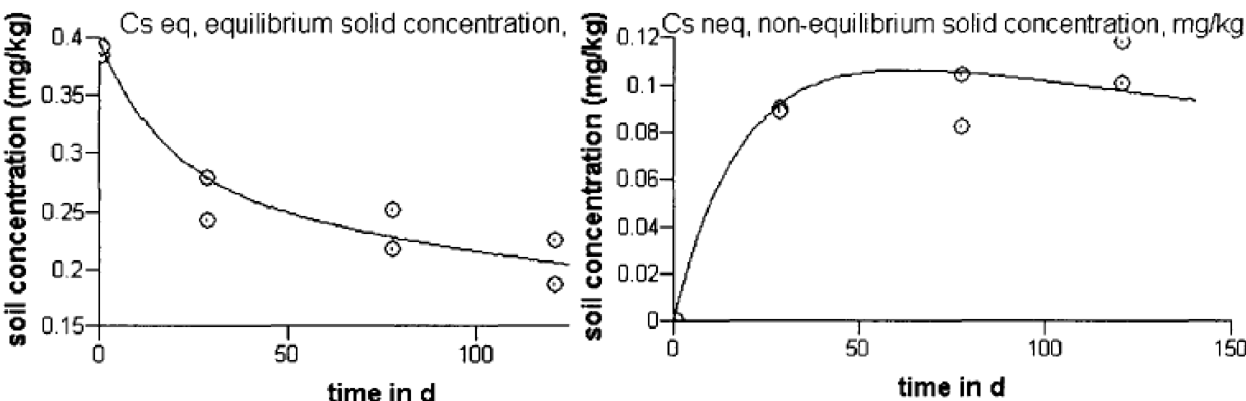
The ‘capacity’ of the non-equilibrium sorbed phase is stated to be characterised by the parameter f_{ne}, (defined as the ratio between the Freundlich coefficients in the non-equilibrium and the equilibrium phase). The applicant stated that mean f_{ne} ratio of 0.395 indicates moderate kinetic effects on sorption i.e. kinetically controlled ‘sorption capacity’ about 40% of instantaneous ‘sorption capacity’.

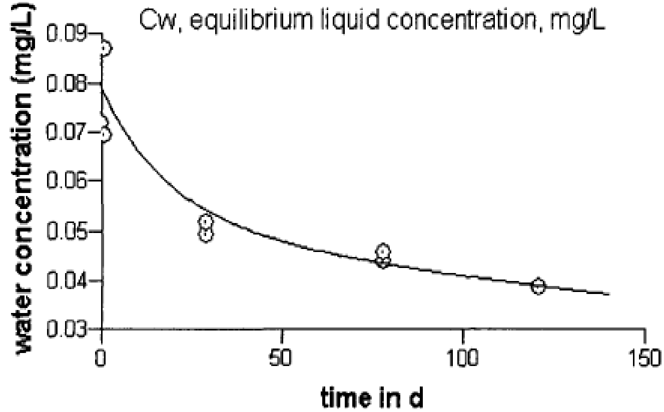
The above rate constants (k_d) indicated ‘pseudo half-lives’ of 2.8-19.1 days for sorption of parent representing exchange between the equilibrium and non-equilibrium phases as shown in table 8.19. The resulting degradation rates in the equilibrium domain (k_t) based on laboratory data indicated DT50 values of 49-286 days. These degradation

rates differentiate between degradation in specific phases, so these are only valid for use with the kinetic sorption model.

Curves of $C_{s\ eq}$ and $C_{s\ neq}$ concentration were provided. The applicant concluded that the kinetic-sorption model provided a good visual fit with the experimental data and sufficient goodness of fit. With regards to Table 8.19, the RMS notes that scaled errors (ε) for Chi^2 were >15% in only 3 cases, with r^2 of 0.87-0.99 and t-test values <0.05 in all but in 2 cases (k_t in Huntlosen soil), attributed by the study author to the DT50 being extrapolated beyond the short study duration of 23 d.

Figure 8.25 Curves of simulated (solid line) and measured (symbols) equilibrium and non-equilibrium sorbed, and liquid concentrations of fluopicolide, for Abington soil.





As correlation matrices showed no significant correlations between the parameters k_d, k_t and $f_{ne,}$ the applicant claimed that these parameters may be applied to other studies to calculate kinetic sorption compatible half lives. Therefore, the applicant proposed that this kinetic sorption model be applied to the field dissipation data to provide more realistic degradation rates. This approach was reported in Kley, 2004, (MEF- 04/347).

Table 8.19 Results of kinetic sorption model and statistical parameters.

	Philippsburg			Rödelsee		
mg/kg	1.793	0.448	0.109	1.793	0.448	0.109
DT50 sorption (d)	14.1	14.8	7.8	13.8	6.1	6.8
DT50 degrad'n (d)	49.6	59.4	63.6	84.4	80.6	122.1
Statistical parameters:						
χ^2 ε of $C_{s\ eq}$ (%)	3.2	2.4	1.8	4.5	0.5	2.4
χ^2 ε of $C_{s\ neq}$ (%)	8.3	3.9	4.3	19.8	6.9	11.0
r^2	0.952	0.984	0.992	0.870	0.993	0.961
T-probability of:						
k_t	4.3×10^{-9}	3.9×10^{-9}	6.9×10^{-10}	2.3×10^{-4}	3.4×10^{-13}	5.8×10^{-5}
k_d	2×10^{-4}	3.1×10^{-7}	4.5×10^{-9}	5.2×10^{-3}	7.7×10^{-11}	2.1×10^{-4}
f_{ne}	1.3×10^{-6}	1.4×10^{-9}	3.6×10^{-13}	5.9×10^{-4}	4.7×10^{-14}	8.5×10^{-9}

	Huntlosen			Senas		
mg/kg	1.793	0.448	0.109	1.793	0.448	0.109
DT50 sorption (d)	2.95	2.77	5.5	6.4	7.1	5.95
DT50 degrad'n (d)	286.3	349.7	$6.9 \times 10^{+07}$	48.9	52.2	79.9
Statistical parameters:						
χ^2 ε of $C_{s\ eq}$ (%)	2.8	3.1	3.3	2.2	4.2	2.8
χ^2 ε of $C_{s\ neq}$ (%)	17.3	13.5	12.5	21.0	6.6	9.2
r^2	0.872	0.907	0.939	0.955	0.932	0.963
T-probability of:						
k_t	4.2×10^{-2}	0.14	nd	9×10^{-10}	4.7×10^{-7}	2.4×10^{-6}
k_d	1.8×10^{-4}	1.7×10^{-5}	1.5×10^{-5}	2.8×10^{-3}	1.4×10^{-6}	4.9×10^{-5}
f_{ne}	9.6×10^{-9}	1.2×10^{-10}	1.1×10^{-12}	1.9×10^{-6}	1.1×10^{-10}	1.7×10^{-9}

nd "parameter could not be evaluated reliably"

	Abington
mg/kg	0.41
DT50 sorption (d)	19.2
DT50 degrad'n (d)	218.5
Statistical parameters:	
χ^2 ε of $C_{s\ eq}$ (%)	4.7
χ^2 ε of $C_{s\ neq}$ (%)	14.8

r^2	0.958
T-probability of:	
k_t	2.7×10^{-7}
k_d	3.5×10^{-4}
f_{ne}	1.2×10^{-11}

RMS Evaluation of new data – Kley, C. 2004 (MEF -04/347)
Kinetic evaluation of field dissipation data considering time-dependent sorption:

For the kinetic sorption model, it is assumed that only fluopicolide in the equilibrium domain is available for degradation, so new degradation rates reflecting this are required. In this report, the kinetic sorption model described above (Kley, 2004, MEF-04/346) was applied to field dissipation data from 6 trials, (previously reported in the DAR, B.8.1.5) to determine degradation rates (k_t), that could be used with the kinetic sorption rate constant (k_d) and the ratio between the Freundlich coefficients for equilibrium and non-equilibrium domain (f_{ne}). Both the k_d and f_{ne} parameters were previously derived from the kinetic evaluation of laboratory data above (Kley, 2004, MEF-04/346).

The parameters k_t and $C_{s\ eq\ 0}$ (initial value of equilibrium sorbed phase) were optimised by the applicant using ACSL Optimize 1.2 software, by fitting to measured total residue (C_t) in mg/kg. The measured total residue (Ct) included residues at depth and below the LOQ, as previously assessed and reported in the DAR (at B.8.1.5). The initial concentration for non-equilibrium sorbed phase ($C_{s\ neq\ 0}$) was set to 0.

An equation was given by the applicant to describe the total soil residue (C_t) or mass balance:

$$C_t = \frac{\theta_g}{\rho_w} \cdot K_{f\,eq}^{-n} \cdot C_{s\,eq}^{n} + C_{s\,eq} + C_{s\,neq} \qquad (21)$$

where:
C_t total soil residue or concentration
θg gravimetric water content (g), *can be substituted with*
θv *volumetric water content (L)*
ρ_w density of water, *can be substituted with*
ρ_{bd} *soil bulk density (kg/L)*
$Kf^{-n}{}_{eq}$ Freundlich coefficient for non-equilibrium domain +
$C^n{}_{s\ eq}$ Concentration
$C_{s\ eq}$ Equilibrium sorbed concentration
$C_{s\ neq}$ Non-equilibrium sorbed concentration

Degradation rates (k_t) for use specifically with the kinetic sorption model were temperature and moisture normalised according to the time transformation approach (FOCUS 2000). Daily soil moisture and weather data used were available from the original trials reports, except in the case of Senas, for which values were simulated using FOCUS PEARL 1.1.1.

Table 8.20 Soil specific input parameters for fluopicolide.

	$\theta_{v\ ref}$ (100% field capacity)	ρ_{bd}	$K_{f\ eq,\ ads}$	1/n	considered max. depth of soil residues
	% v/v	kg/L	L/kg		m
Philippsburg	18.0	1.5	1.49	0.841	0.5
Rödelsee	35.9	1.5	2.59	0.859	0.5
Huntlosen	24.1	1.5	9.27	0.953	0.2
Appilly	34.7	1.5	*4.69*	0.9028	0.3
Valencia	24.6	1.5	*5.46*	0.9028	0.3
Senas	30.35	1.5	3.59	0.882	0.3

In italics: value recalculated, based on the mean K_{oc} of 321.1 L/kg and the corresponding organic carbon content of the top soils

Table 8.21 below shows $C_{t\ 0}$ (g/ha) values, which have been calculated from the fitted $C_{s\ eq\ 0,}$ alongside the nominal application rates, together with the fitted degradation rates (k_t). The kinetic sorption DT50 values range from 53-108 days (representing degradation in the equilibrium domain only, compared to the DT50 values from the standard field dissipation assessment (DAR B.8.1.5) which were 77-224 days, with a geometric mean of 138.8 days). The applicant states that total degradation is determined by both the degradation rate constant (k_t) and the transfer rate (k_d), since any substance in the non-equilibrium domain will only be degraded upon transfer to the equilibrium domain.

The applicant considered that that the geometric mean field degradation rate (k_t) of **87.8 days** above, which takes into account kinetic sorption, was more realistic than the laboratory derived values (Kley 2004, MEF -04/346). Therefore, this has been used subsequently as the appropriate degradation parameter for fluopicolide in the FOCUS PEARL model to estimate PECgw values.

The applicant considered that the SFO degradation model in, combination with sorption kinetic, was acceptable to describe the field residues of fluopicolide, with good visual fits and sufficient statistical goodness of fit between the experimental and modelled data. The results of Chi^2 statistical test, single sided t-test and coefficient of determination (r^2) were reported, see Table 8.22. Scaled errors (ε) were <15% in all but 3 cases, with r^2 of 0.81-0.97 and low t-test values, all <0.05.

Table 8.21. Results of field degradation evaluation of fluopicolide, valid for use with sorption kinetic model (20°C, 100% field capacity) and sorption kinetic input parameters.

trial	Nominal application rate	C_{t0}	k_t	DT_{50} field degradation, with kinetic sorption	used k_d	used f_{ne}
	g/ha	g/ha	d^{-1}	d	d^{-1}	
Philippsburg	400	371.4	0.006899	100.5	0.0589	0.4692
Rödelsee	400	607.3	0.007927	87.4	0.0835	0.3701
Huntlosen	400	306.8	0.00693	100.0	0.1950	0.3657
Senas, 1st year	500	298.9	*0.01051*	*66.0*	0.1075	0.3230
Senas, 2nd year	500 +	632.9	*0.01317*	*52.6*	"	"
Senas mean			0.01184	59.3		
Appilly	400	362.0	0.00846	81.9	0.0821 m	0.3953 m
Valencia	400	428.2	0.00645	107.5	0.0821 m	0.3953 m
90th percentile				104.0		
geo. mean			0.00789	**87.8**		

m = mean values from laboratory batch studies
italics = mean values of Senas used for further averaging to avoid overweighting.

Table 8.22 Results of kinetic sorption model and statistical parameters.

	Philipps-burg	Rödelsee	Huntlosen	Senas Y 1	Senas Y 2	Apilly	Valencia
DT50 degrad (d)	100.5	87.4	100	66	52.6	81.9	107.5
Statistical parameters:							
χ^2 ε of C_t (%)	16.4	14.0	15.5	9.5	13.3	14.4	15.5
r^2	0.812	0.886	0.836	0.966	0.899	0.897	0.893
T-probability of:							
k_t	4×10^{-5}	3.2×10^{-5}	5.8×10^{-5}	3.1×10^{-9}	4.9×10^{-5}	4.9×10^{-6}	1.1×10^{-5}
Cs eq 0	8×10^{-10}	7.5×10^{-9}	1.3×10^{-9}	3.6×10^{-14}	5.8×10^{-9}	7.5×10^{-11}	1.9×10^{-10}

Figure 8.26 Degradation curve and residual plot of measured vs simulated data at **Philippsburg**

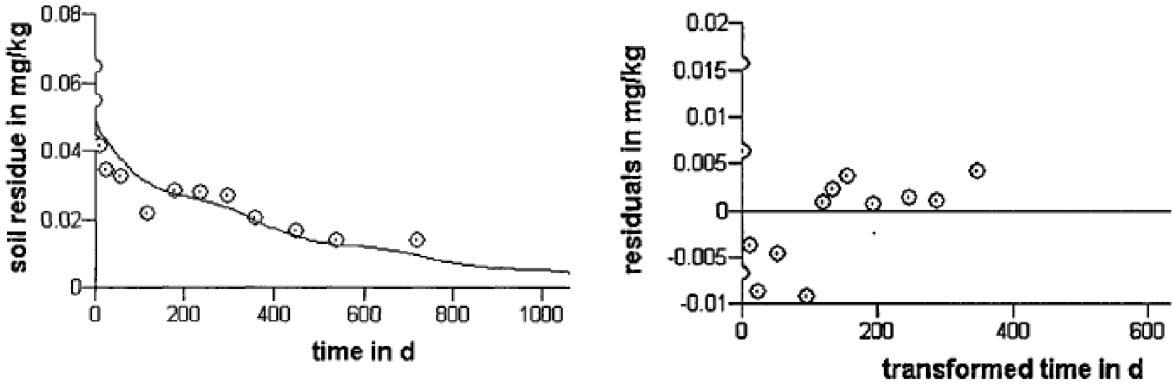


Figure 8.27 Degradation curve and residual plot of measured vs simulated data at **Rödelsee**

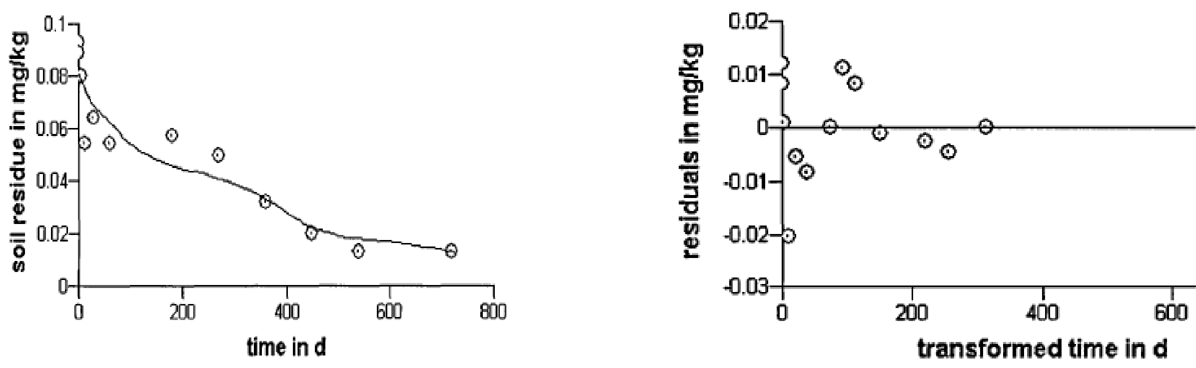


Figure 8.28 Degradation curve and residual plot of measured vs simulated data at **Huntlosen**

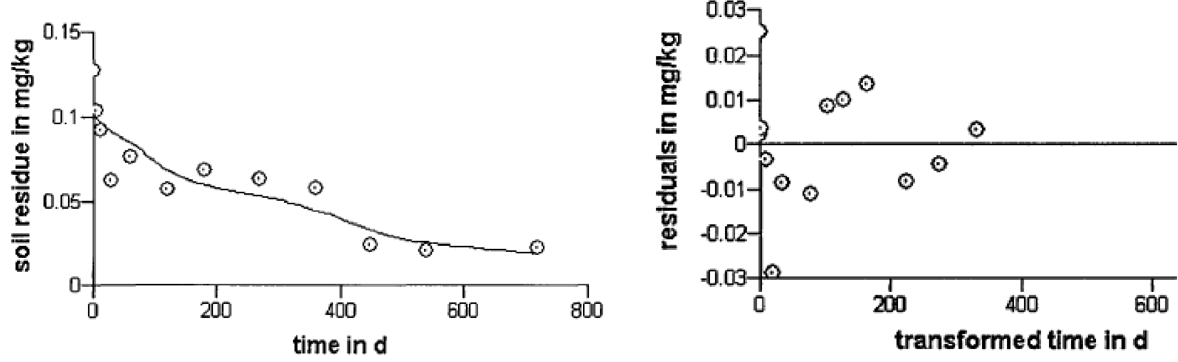


Figure 8.29 Degradation curve and residual plot of measured vs simulated data at **Senas (Yr 1)**

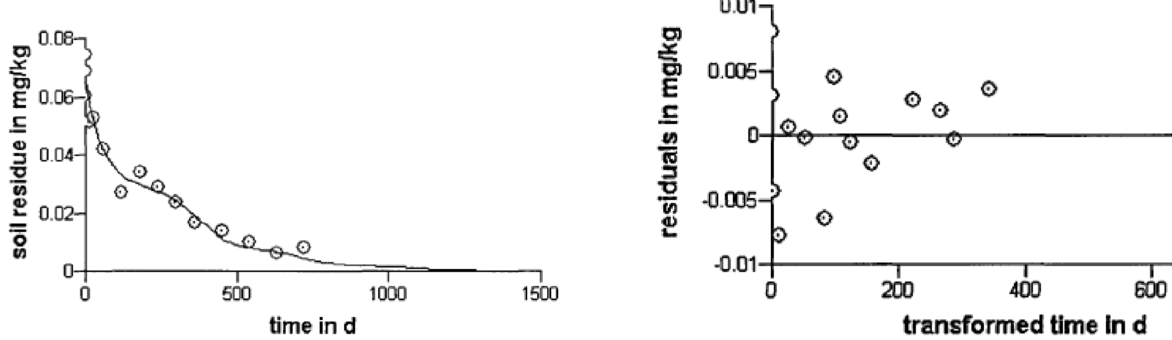


Figure 8.30 Degradation curve and residual plot of measured vs simulated data at **Senas Yr 2**

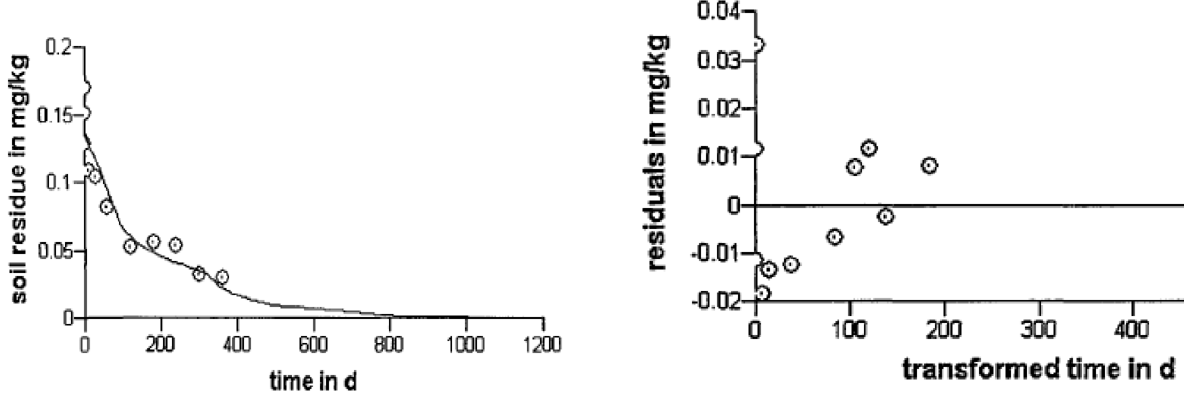


Figure 8.31 Degradation curve and residual plot of measured vs simulated data at **Apilly**

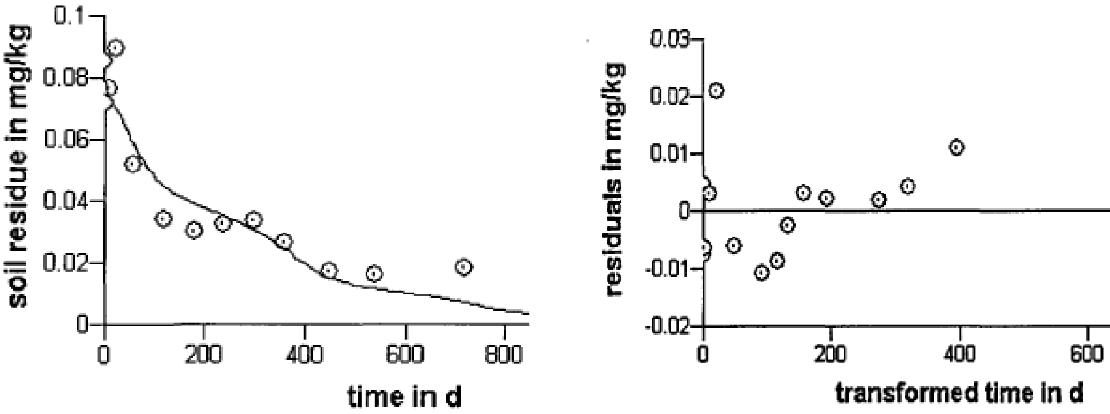
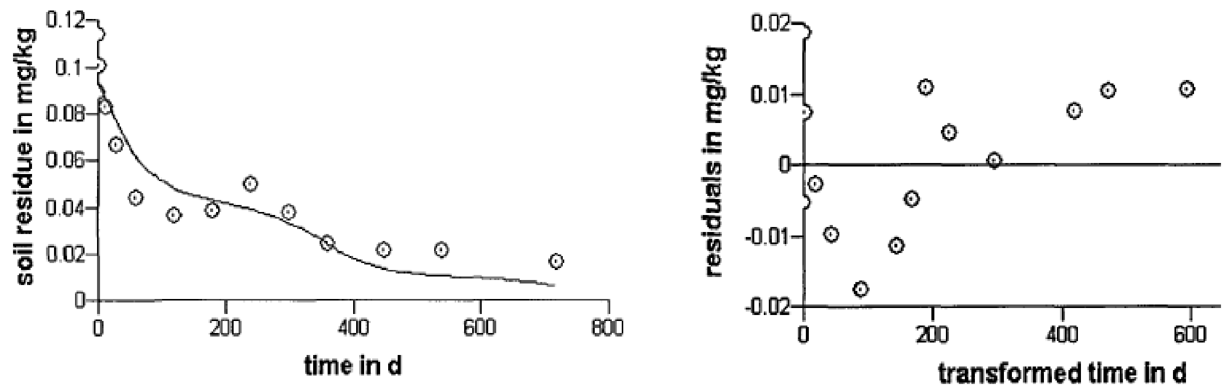


Figure 8.32 Degradation curve and residual plot of measured vs simulated data at **Valencia**



Validation of the kinetic sorption model:

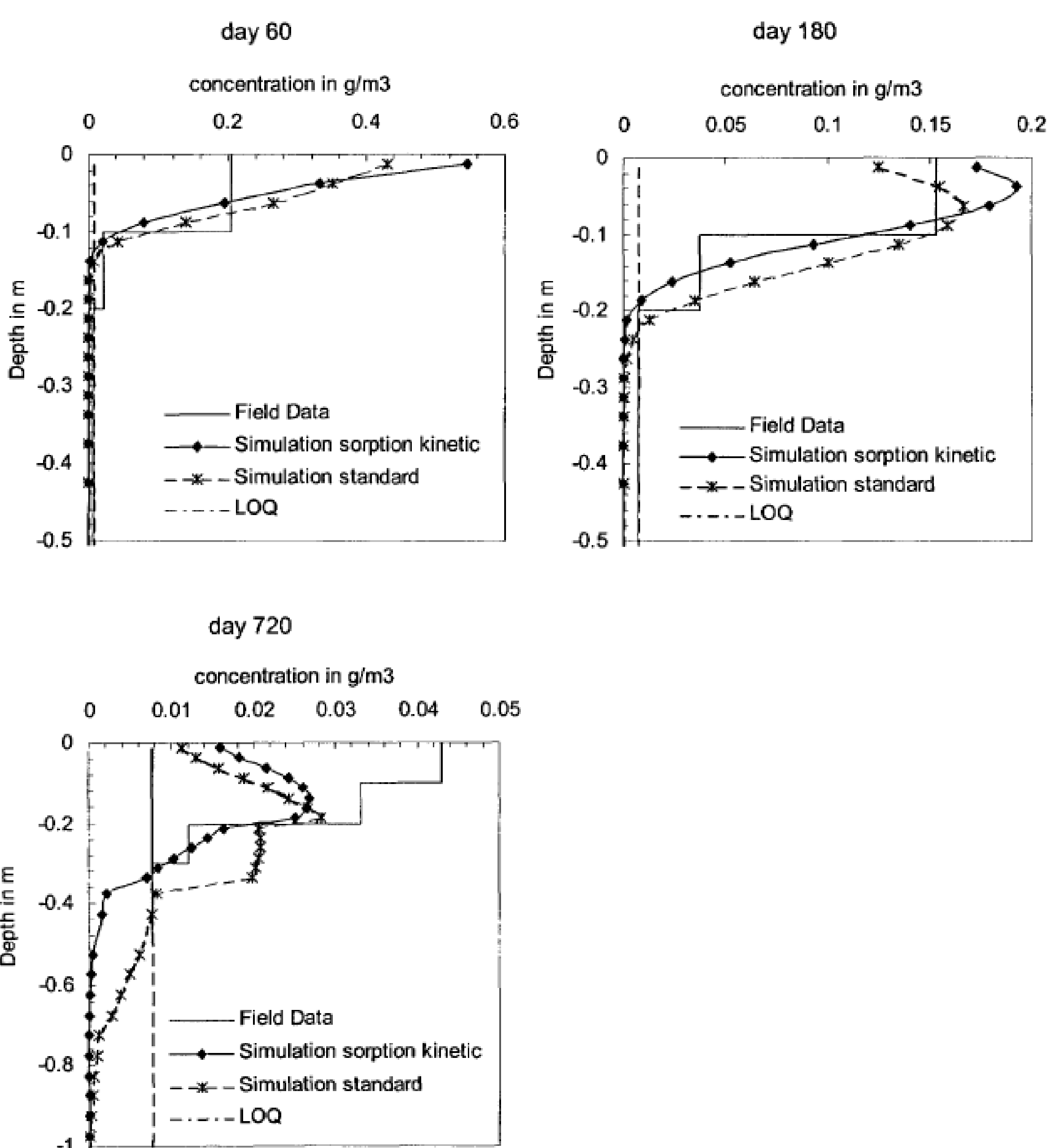
The applicant also provided validation of the kinetic sorption model, using the distribution of measured residue data with depth. The FOCUS PEARL model v 1.1.1 was used to simulate water and substance transport at four of the six field sites, (Philippsburg, Rödelsee, Huntlosen and Senas). Two PEARL simulations were run, one with the standard field degradation parameters (as assessed in the DAR) and one with the kinetic sorption parameters (k_t, k_d and f_{ne} evaluated here), to compare both approaches with the measured residue data (C_t, in mg/kg or g/m^3).

Site-specific soil, weather, irrigation, tillage, crop and application conditions, as well as hydraulic calibration were all as previously described in the DAR (B.8.1.5.1). Fitted initial soil concentrations (g/ha) were used as application rates. The input and output files for these PEARL simulations are given in Kley 2004, (MEF -04/347). Depth profiles of concentration were evaluated according to the 'method of moments' (Jury 1990[7]).

The applicant stated that both the kinetic sorption and standard degradation approaches resulted in the same overall degradation characteristics, but retardation of fluopicolide due to sorption processes lead to differences in transport velocity. The kinetic sorption simulation gave narrower depth distributions and higher peak values compared to the standard simulation. This was attributed to more substance being retained for the kinetic sorption model, with fluopicolide sorbed in the non-equilibrium domain not being available for transport. For the standard simulation, the substance is dispersed more greatly, with increasing travel depth which results in lower peak values.

[7] Jury, W.A., Roth, K (1990). Transfer functions and solute movement through soil: Theory and Applications.

Figure 8.33 Example of comparison of depth profiles for simulated and measured concentrations, from Philippsburg site. (Further concentration depth profiles provided in Kley, 2004, MEF-04/347).



From day 60-189 the standard simulation appeared to over-predict the transport velocity of fluopicolide compared to the measured data, while the kinetic sorption simulation gave a closer depth concentration profile to the measured data. The mean travel depth[8] after 2 years with the kinetic sorption simulation was also closer to the measured mean travel depth for each site, than with the standard simulation, (except, the RMS notes, for Rödelsee).

[8] mean travel depth (z_s), calculated as $z_s = \int z \cdot dm / \int dm$ where dm is substance mass at a certain depth. A description of how this was converted to soil concentration at depth is given in Kley, 2004 (MEF 04-347).

Table 8.23 Mean travel depth of fluopicolide in soil at end of trial for different soil concentration curves.

	Mean travel depth (cm) after 720 days		
	Measured	Kinetic sorption simulation	Standard simulation
Philippsburg	15.6	16.5	26.6
Rödelsee	12.5	9.4	11.6
Huntlosen	6.1	7.7	9.2
Senas	6.9	9.6	11.9

Based on these results, the applicant considered the kinetic sorption simulation was closer to the measured data than the standard simulation, and therefore more accurately described the behaviour of fluopicolide in the field.

FOCUS PEARL & PELMO modelling of PECgw for use in vines and potatoes: RMS Evaluation of New Data – Kley, C. & Ellerich C. 2007 (a) and (b)

The potential for fluopicolide and 9 of its metabolites, (M-01, M-02, M-03, M-05, M-10, M-11, M-12, M-13 and M-14) to leach to groundwater following use in Europe on vines or potatoes is assessed in the DAR (B.8.6.2), using the FOCUS PELMO 3.3.2 model.

The proposed degradation pathway (also shown in DAR, Figure B.8.6 and B.8.17) is given below. Fluopicolide is cleaved into metabolites M-01 (phenyl ring) and M-02 (pyridine ring) and the intermediate metabolite M-03, formed in acidic soils, is also cleaved into M-01 and M-02. The applicant states that where there is cleavage of a molecule, the degradation rate is equal to the formation rate for each of the resulting metabolites. Therefore, as assessed in the DAR, the same partial formation fraction k_{13} was used by the applicant for the pathways from parent to M-01 and parent to M-02 and likewise k_{23} was used for both the pathways from M-03 to M-01 and M-03 to M-02.

Figure 8.34 Applicant's proposed reaction pathways of fluopicolide in soil.

Fluopicolide (AE C638206), comp. 1

k_{13} k_{12} k_{13}

AE 0608000 (M-03), comp. 2

k_{23} k_{23}

AE C657188, M-02

AE C653711, M-01

[GSH]

k_3

Further mineralisation

M-13

AE 1344123 (M-10)

AE 1344122 (M-05)

Intermediate, MW 269

M-11 and M-12

AE 1388273 (M-14)

Further mineralisation

Further mineralisation

Degradation rate used in revised PELMO modelling:

A kinetic evaluation of the field dissipation studies was performed in the DAR (B.8.1.5.1.) using a 3-tier approach. A 2nd tier approach was based on inverse modelling of dissipation curves from three field sites with PEARL and PEST models. A further kinetic assessment using ModelMaker was performed for three additional field trial sites, where detailed soil hydrology data were not available for use in PEARL. The resulting field degradation rates for fluopicolide, normalised to 20°C and field capacity, were accepted by the RMS previously for use in the groundwater assessment and are shown below. The standard geometric mean DT50 of 138.8 days has been used here in the revised PELMO groundwater modelling, since it was not possible to incorporate sorption kinetics into PELMO.

Table 8.24 Field degradation half-lives for use in PELMO exposure assessment.

	application	Estimated initial soil concentration c_0	**DT_{50} Fluopicolide**
	g a.i. / ha	g a.i. /ha	d
Philippsburg	400	400	177.7
Rödelsee	400	610	123.3
Huntlosen	400	400	117.5
Appilly	400	400	161.2
Valencia	400	400	223.6
Senas mean	500		77.0
90th percentile			200.7
arith. mean			146.7
geom. mean			**138.8**

The degradation rate constants, calculated to take into account kinetic sorption (geometric mean $DT50_{field, norm}$ 87.8 days for the equilibrium phase, Kley, 2004, MEF -04/347), reported in this Addendum, have been implemented into the groundwater modelling with FOCUS PEARL as described below.

Parameters assumed for the metabolites in new PEARL and PELMO modelling:

For the metabolites, almost all the parameters for degradation and sorption and formation fractions used in the modelling, were as previously accepted by the RMS in the original PELMO groundwater assessment (DAR B.8.6.2). These are summarised below.

Metabolite M-03 (AE 060800) was not detected in alkaline soils (pH>6) in field dissipation trials but was observed at up to 6.1% applied (parent equivalents) in one acidic field trial. Laboratory studies (DAR, B.8.1.2.b.) confirmed that its degradation was pH-dependent.

For alkaline scenarios (pH>6) fluopicolide was assumed to be completely degraded via M-03, with the geometric mean DT50 $_{lab, norm}$ of 0.09 days. For acidic scenarios (pH<6) parallel degradation of fluopicolide to M01 and M-02 directly (k_{13}) and also via M-03 (k_{12}) was assumed, with the geometric mean DT50 $_{field, norm}$ of 55.5 days for M-03.

Formation fractions assumed were 0.288 for M-03 and 0.712 for M-01/M-02. An arithmetic mean Koc of 108.8 L/kg, with mean 1/n of 0.971 was previously estimated and used in the groundwater assessment. M-03 was also rapidly hydrolysed in laboratory studies to form M-01/M-02 with DT50 from 8.1 minutes (pH 8) to 45.5 hours (pH 5), (DAR, B.8.4.1.d.).

Metabolite M-01 (AE C653711) reached up to 24.1% (parent equivalents, excluding Senas, 2nd year data) and 40.2% applied (parent equivalents) in the field and laboratory, respectively. A geometric mean $DT50_{field, norm}$ of 137.7 days, arithmetic mean Koc of 40.9 L/kg and 1/n of 0.9158 were previously accepted for use in the original PELMO groundwater assessment.

Metabolite M-02 (AE C657188) reached a maximum of 16.4% (parent equivalents, excluding Senas, 2nd year data) and 7.3% applied (parent equivalents) in field and laboratory studies, respectively. It was not possible to calculate reliable field degradation rates for M-02, as residues were only detected at low levels and early time points, so a geometric mean, $DT50_{lab, norm}$ of 2.82 days (using ModelMaker), together with an arithmetic mean Koc of 5.99 L/kg and 1/n of 0.7737, was previously used for the original PELMO groundwater assessment.

Metabolite M-05 (AE 1344122) reached a maximum of 17.99% of applied M-02 in a laboratory soil degradation study with M-02 (DAR, B.8.1.2.c). A geometric mean, $DT50_{lab, norm}$ of 42.6 days, arithmetic mean Koc of 25.9 L/kg and 1/n of 0.9182 were previously accepted for use in the original PELMO groundwater assessment. As no M-14 was formed in one of the soils tested, (attributed by the applicant as possibly due to slow degradation of M-05), the worst case formation fraction of 0.384 was selected by the applicant for M-05 into M-14. The mean formation fraction of 0.252 was used in the DAR. This also gives a slightly different partial reaction rate of 0.006248 d^{-1}, (compared to 0.0041 d^{-1} in the DAR).

Metabolite M-10 (AE 1344123) reached up 4.97% of applied M-02 in a soil degradation study with M-02 (DAR, B.8.1.2.c). A geometric mean, $DT50_{lab, norm}$ of 26.4 days, arithmetic mean Koc of 6.3 L/kg and 1/n set to 0.9 were previously accepted for use in the original PELMO groundwater assessment.

Metabolite M-11/12 (P2a/P2b) are two isomers (60:40 ratio) formed at up to 6.55% of applied M-02 in a soil degradation study with M-02 (DAR, B.8.1.2.c). A geometric mean, $DT50_{lab, norm}$ of 35.95 days, was previously accepted for use in the original PELMO groundwater assessment. No reliable Koc value could be determined and this was set at 0 (with 1/n of 0.9) as a worst case.

Metabolite M-13 (P3) reached up to 4.38% of applied M-02 in a soil degradation study with M-02 (DAR, B.8.1.2.c). A geometric mean, $DT50_{lab, norm}$ of 11.8 days was previously accepted for use in the original PELMO groundwater assessment. At pH 6

only very low sorption was observed, Koc of 0.003 L/kg so this was set at 0 L/kg, (1/n set to 0.9).

Metabolite M-14 (AE 1388273) reached up to 1.56% of applied M-02 in a soil degradation study with M-02 (DAR, B.8.1.2.c). A geometric mean, $DT50_{lab, norm}$ of 5.2 days was previously accepted for use in the original PELMO groundwater assessment. At pH 6 sorption was moderate, a Koc of 19.2 L/kg (Kom of 11.14 L/kg) were used with 1/n set to 0.9.

The parameters for fluopicolide and its metabolites input into the revised groundwater modelling with FOCUS PEARL and PELMO are summarised below.

Table 8.25 Summary of degradation and sorption parameters used in FOCUS groundwater scenarios

Compound	FOCUS scenario	DT_{50} (days)	K_{oc} (L/kg)	K_{om} (L/kg)	Freundlich exponent (1/n)
Fluopicolide	All	138.8 [a]	321.1	186.2	0.9028
		87.8 [b]			
M-03	pH < 6	55.5 [c]	108.8	63.1	0.9707
	pH > 6	0.09 [d]			
M-01	All	137.7	40.9	24	0.9158
M-02	All	2.82	5.99	3.47	0.7737
M-05 (P1x)	All	42.6	25.9	15	0.9182
M-10 (P4)	All	26.4	6.3	3.7	0.9*
M-14 (P7)	All	5.2	19.2	11.14	0.9*
M-11 and M-12	All	35.95	0	0	0.9*
M-13	All	11.8	0	0	0.9*

[a] standard overall degradation half-life used in PELMO
[b] DT50 valid only with kinetic sorption parameters K_d = 0.08211 d-1, f_{ne} = 0.3953 used in PEARL
[c] in acidic soils (Hamburg, Jokioinen, Okehampton, Porto)
[d] in alkaline soils (Châteaudun, Kremsmünster, Piacenza, Sevilla, Thiva)
[*] default 1/n

Table 8.26 Formation fractions used for FOCUS PEARL and PELMO groundwater scenarios

Compound	FOCUS scenario	Formation fraction	k_{ij} (d^{-1})
f (fluopicolide → M-02)	pH < 6	0.712	0.00356
	pH > 6	0	0
f (fluopicolide → M-03)	pH < 6	0.288	0.00144
	pH > 6	1	0.00499
f (M-03 → M-02)	pH < 6	1	0.01249
	pH > 6	1	7.7016
f (M-02 → M-05)	all	0.203	0.05
f (M-02 → M-10)	all	0.095	0.0233
f (M-02 → M-13)	all	0.062	0.0152
f (M-02 → CO_2)*	all	0.587	0.1444
f (M-02 → M-14)	all	0.053	0.013
f (M-05 → M-14)	all	0.384#	0.006248[#]
f (M-05 → CO_2)	all	0.748	0.01002[#]
f (M-14 → CO_2)	all	1	0.1333
f (M-10→ CO_2)	all	1	0.02622
f (M-13 → CO_2)	all	1	0.05864

*The formation fraction for f (M-02 → CO_2) was 0.640 with K_{ij} of 0.1574 d^{-1} in the DAR, the applicant appears to have divided this into formation fractions for f (M-02 → M-11/M-12 and → CO_2) as shown.
[#] In the DAR, K_{ij} for f (M-05 → M14) and f (M-05 → CO_2) were 0.0041 d^{-1} and 0.0122 d^{-1} respectively. The worst case formation fraction of 0.384 has been used for formation into M-14, instead of the mean of 0.252 used in the DAR.

Plant Uptake

For fluopicolide and metabolites M-01, M-02 and M-05, which were considered to be systemic, the plant uptake factor was set to 0.5 (default). For metabolites, M-03, M-10, M-11/-12, M-13 and M-14, which were not detected in plants, the uptake factor was set to 0.

GW modelling Assumptions

Simulations were performed for use of fluopicolide on vines, with a lower crop interception rate than assumed in the DAR (B.8.6). In vines, a scenario of 3 applications of 133 g fluopicolide per hectare at 10 day intervals each year was chosen as a worst-case. The applicant assumed crop interception of 60% + 70% + 70% in accordance with FOCUS (2000) and a crop growth stage of BBCH 53-77. This is, as requested, lower and more worst case than previously used in the DAR, (70%+70%+85% for Hamburg, Kremsmunster and Sevilla or 70% for other scenarios).

Groundwater modelling of use of fluopicolide on potatoes was performed assuming use on potatoes every year, once every 2 years and once every 3 years. In potatoes, a scenario of 4 applications of 100 g fluopicolide per hectare at 5 day intervals was used, with the product applied every 1, 2 or 3 years. The applicant stated three year rotation was commonly practised in many European MSs to avoid build up of potato cyst nematodes, with two year rotation possible in specific cases, but that application

every year to potatoes was unlikely. For this modelling, the applicant used the same crop interception as previously assessed in the DAR of 50, 50, 80 and 80% for the first, second, third and fourth applications, respectively. This is in accordance with FOCUS 2000 guidance of 50% interception at BBCH 20-39 and 80% at BBCH 40-89. (Although the applicant referred to application being within a slightly narrower crop growth stage band (BBCH 35-89), than reported previously in the DAR, (BBCH 20-91, for which interception was as above, but declined to 50% for BBCH 90-99)).

Simulations were performed over 26 years (including 6 year warm up period) for vines and potatoes (with application every year) and for a total period of 46 or 66 years for potatoes (applications every 2 or 3 years).

The earliest application was assumed to be 5 weeks after leaf emergence for vines and 3 weeks after emergence for potatoes, with application dates shown below.

Table 8.27 Plant development in FOCUS GW scenarios and application dates – vines.

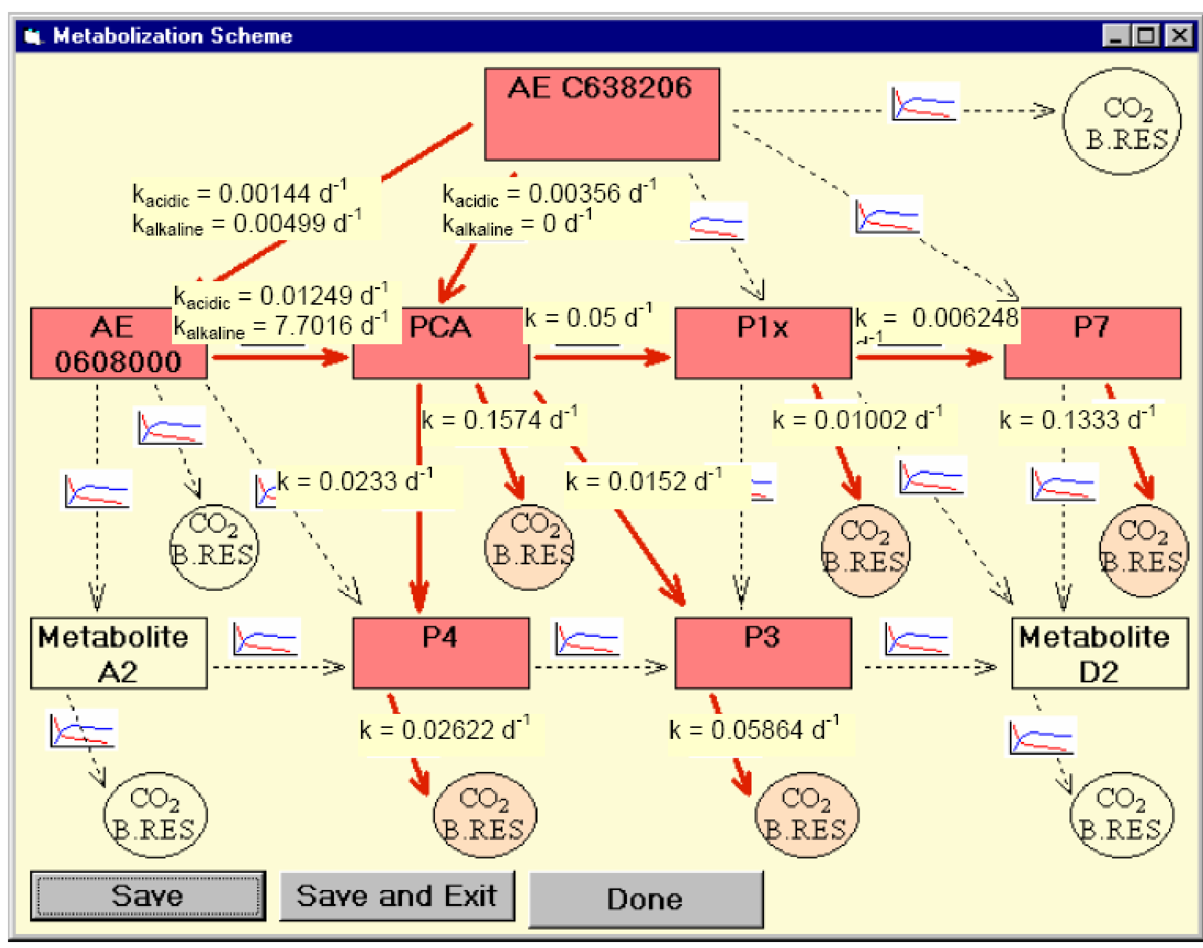
Scenario	Leaf emergence vine	LAI_{max} vine	selected application dates		
Châteaudun	1st April	31st July	6.5. (60 % Int.)	16.5. (70%)	26.5. (70%)
Hamburg	1st May	15th July	5.6. (60%)	15.6. (70%)	25.6. (70%)
Kremsmünster	1st May	15th July	5.6. (60%)	15.6. (70%)	25.6. (70%)
Piacenza	1st April	31st July	6.5. (60 %)	16.5. (70%)	26.5. (70%)
Porto	15th March	31st July	19.4. (60%.)	29.4. (70%)	9.5. (70%)
Sevilla	31st March	15th June	5.5. (60%)	15.5. (70%)	25.5. (70%)
Thiva	15th March	30th June	19.4. (60%.)	29.4. (70%)	9.5. (70%)

Table 8.28 Plant development in FOCUS GW scenarios and application dates – potatoes.

Scenario	emergence potatoes	LAI_{max} potatoes	harvest potatoes	selected application dates			
Châteaudun	30th April	15th June	1st September	21.5.	26.5.	31.5.	5.6.
Hamburg	10th May	20th July	15th September	31.5.	5.6.	10.6.	15.6.
Jokioinen	5th June	30th August	25th September	26.6.	1.7.	6.7.	11.7.
Kremsmünster	10th May	20th July	15th September	31.5.	5.6.	10.6.	15.6.
Okehampton	30th April	15th July	1st September	21.5.	26.5.	31.5.	5.6.
Piacenza	20th April	1st June	10th September	11.5.	16.5.	21.5.	26.5.
Porto	15th March	30th May	15th June	5.4.	10.4.	15.4.	20.4.
Sevilla	31st January	31st March	31st May	21.2.	26.2.	2.3.	7.3.
Thiva	1st March	30th April	30th July	22.3.	27.3.	1.4.	6.4.

As described previously in the DAR (B.8.6.2) the degradation schemes had to be implemented into FOCUS PELMO 3.3.2 in separate parts to predict the groundwater concentrations of M-01 and M-02 arising from cleavage of fluopicolide and to reflect the extensive metabolism of M-02 in soil (DAR, Figures B.8.32-34). Estimated formation fractions and partial reaction rates were used as before, except where indicated in the footnotes to the table 8.26 above.

Figure 8.35 PELMO metabolism scheme for transport of fluopicolide, M-02 and its M-02 metabolites (excluding M-11/M-12) in acidic and alkaline soils.



Where AE C638206 = fluopicolide, AE0608000 = M-03, PCA = M-02, P1x = M-05, P4 = M-10, P7 = M-14, P3 = M-13.

For both the simulation run with the FOCUS PEARL model (implementing kinetic sorption behaviour) and the simulation with the FOCUS PELMO model (no sorption kinetic included), the 80th percentile annual average concentrations of fluopicolide and its metabolites at 1 m depth are shown below.

RESULTS FOR VINES

Table 8.29 Predicted 80th percentile annual average concentrations in groundwater at 1 m depth following use to **vines** (**PEARL, including sorption kinetics)**

Scenario	Annual PEC$_{gw}$ in µg/L									
	Fluo-picolide	M-03, AE 0608000	M-01, AE C653711	M-02, AE C657188	M-05, AE 1344122	M-14, AE 1388273	M-11, P2a	M-12, P2b	M-13, P3	M-10, AE 1344123
Châteaudun	0.018	< 0.001	4.887	0.002	0.510	0.025	0.241	0.161	0.083	0.313
Hamburg [a]	0.010	0.423	5.879	0.019	0.672	0.032	0.371	0.247	0.181	0.444
Krems-münster	0.008	< 0.001	4.389	0.001	0.411	0.020	0.218	0.145	0.077	0.257
Piacenza	0.147	< 0.001	4.515	0.017	0.571	0.027	0.187	0.124	0.085	0.287
Porto [a]	< 0.001	0.013	1.553	< 0.001	0.082	0.004	0.127	0.084	0.043	0.092
Sevilla	0.006	< 0.001	3.630	0.001	0.329	0.015	0.168	0.112	0.045	0.202
Thiva	0.019	< 0.001	3.875	0.002	0.339	0.016	0.132	0.088	0.032	0.163

a acidic soil, corresponding metabolism pathway used.

Table 8.30 Predicted 80th percentile annual average concentrations in groundwater at 1 m depth following use to **vines** (**PELMO, no sorption kinetic**)

Scenario	Annual PEC$_{gw}$ in µg/L									
	Fluo-picolide	M-03, AE 0608000	M-01, AE C653711	M-02, AE C657188	M-05, AE 1344122	M-14, AE 1388273	M-11, P2a	M-12, P2b	M-13, P3	M-10, AE 1344123
Châteaudun	0.173	< 0.001	5.003	0.012	0.554	0.027	0.261	0.174	0.090	0.343
Hamburg [a]	0.067	0.525	6.265	0.036	0.715	0.033	0.516	0.344	0.216	0.586
Krems-münster	0.089	< 0.001	4.862	0.007	0.474	0.023	0.302	0.202	0.117	0.363
Piacenza	0.519	< 0.001	4.891	0.038	0.607	0.029	0.246	0.164	0.096	0.353
Porto [a]	< 0.001	0.018	1.981	0.001	0.126	0.006	0.208	0.138	0.069	0.140
Sevilla	0.001	< 0.001	4.118	0.001	0.236	0.011	0.296	0.197	0.037	0.203
Thiva	0.087	< 0.001	4.645	0.007	0.388	0.018	0.218	0.145	0.041	0.238

a acidic soil, corresponding metabolism pathway used

Table 8.31 Maximum 80th percentile annual average concentrations and exceedance of 0.1 µg/l limit following use to **vines – new assessment**.

	Highest 80th percentile concentrations (µg/l, *scenario*)		No. of scenarios > 0.1 µg/l (out of 7 simulated).	
	PEARL	**PELMO**	**PEARL**	**PELMO**
Parent	**0.147** (*P*)	**0.519** (*P*)	1	2
M-03	**0.423** (*H*)	**0.525** (*H*)	1	1
M-01	**5.879** (*H*)	**6.265** (*H*)	7	7
M-02	0.019 (*H*)	0.038 (*P*)	-	-
M-05	**0.672** (*H*)	**0.715** (*H*)	6	7
M-14	0.032 (*H*)	0.033 (*H*)	-	-
M-11	**0.371** (*H*)	**0.516** (*H*)	7	7
M-12	**0.247** (*H*)	**0.344** (*H*)	5	7
M-13	**0.181** (*H*)	**0.216** (*H*)	1	2
M-10	**0.444** (*H*)	**0.586** (*H*)	6	7

bold font denotes > 0.1 µg/l.
P = Piacenza, H= Hamburg, J= Jokioinen.

For comparison, Table 8.32 below provides the same results from the original groundwater assessment (reported in the DAR at B.8.6.2) using FOCUS PELMO, but assuming greater crop interception.

Table 8.32 Maximum 80th percentile annual average concentrations and exceedances of 0.1 µg/l limit following use to **vines – original assessment**

	Highest 80th percentile concentrations (µg/l, *scenario*)	No. of scenarios > 0.1 µg/l (out of 7 simulated).
	PELMO	**PELMO**
Parent	**0.452** (*P*)	2
M-03	**0.381** (*H*)	1
M-01	**4.614** (*H*)	7
M-02	0.033 (*P*)	-
M-05	**0.540** (*P*)	7
M-14	0.016 (*P*)	-
M-11	**0.386** (*H*)	7
M-12	**0.258** (*H*)	7
M-13	**0.160** (*H*)	1
M-10	**0.430** (*H*)	7

bold font denotes > 0.1 µg/l.

P = Piacenza, H= Hamburg, J= Jokioinen.

The maximum 80th percentile concentrations of parent compound and metabolites predicted in groundwater, following use on vines are shown in Table 8.31. The 80th percentile PECgw value for fluopicolide exceeded the maximum acceptable concentration of 0.1 µg/l at the Châteaudun (PEARL and PELMO) and Piacenza (PELMO) scenarios.

For metabolites M-01 and M-11, PECgw values were predicted to exceed 0.1 µg/l at every scenario, with both the FOCUS PEARL and PELMO models. Metabolites M-03, M-05, M-12 and M-13 also exceeded 0.1 µg/l at some scenarios, (shown in Table 8.31). Only metabolites M-02 and M-14 were predicted to be below 0.1 µg/l in groundwater at all 7 scenarios simulated with both models.

For both the models run, predicted concentrations of M-01 following use on vines were between >0.75 µg/l and <10 µg/l. Predicted concentrations of the other metabolites simulated were all <0.75 µg/l.

RESULTS FOR POTATOES

Table 8.33 Predicted 80th percentile annual average concentrations in groundwater at 1 m depth following use to **potatoes every year PEARL**, with sorption kinetics)

Scenario				Annual	PEC_{gw}	in µg/L				
	Fluo-picolide	M-03, AE 0608000	M-01, AE C653711	M-02, AE C657188	M-05, AE 1344122	M-14, AE 1388273	M-11, P2a	M-12, P2b	M-13, P3	M-10, AE 1344123
Châteaudun	0.004	< 0.001	5.100	< 0.001	0.440	0.021	0.232	0.155	0.060	0.260
Hamburg [a]	0.007	0.386	6.628	0.019	0.697	0.033	0.490	0.327	0.202	0.508
Jokioinen [a]	< 0.001	0.146	5.658	0.007	0.477	0.021	0.669	0.446	0.312	0.525
Krems-münster	0.004	< 0.001	4.761	< 0.001	0.421	0.020	0.254	0.169	0.080	0.272
Okehamp-ton [a]	0.007	0.382	5.394	0.020	0.594	0.027	0.278	0.186	0.113	0.352
Piacenza	0.104	< 0.001	4.756	0.013	0.531	0.025	0.191	0.127	0.077	0.290
Porto [a]	< 0.001	0.011	1.576	< 0.001	0.082	0.003	0.119	0.080	0.044	0.090
Sevilla	< 0.001	< 0.001	3.448	< 0.001	0.161	0.007	0.089	0.059	0.022	0.086
Thiva	0.003	< 0.001	3.961	< 0.001	0.250	0.012	0.125	0.084	0.022	0.119

a acidic soil, corresponding metabolism pathway used.

Table 8.34 Predicted 80th percentile annual average concentrations in groundwater at 1 m depth following use to **potatoes every 2 years (PEARL**, with sorption kinetics)

Scenario	Annual PEC$_{gw}$ in µg/L									
	Fluo-picolide	M-03, AE 0608000	M-01, AE C653711	M-02, AE C657188	M-05, AE 1344122	M-14, AE 1388273	M-11, P2a	M-12, P2b	M-13, P3	M-10, AE 1344123
Châteaudun	0.001	< 0.001	2.428	< 0.001	0.199	0.009	0.119	0.079	0.030	0.127
Hamburg [a]	0.002	0.201	3.153	0.009	0.315	0.015	0.227	0.151	0.099	0.242
Jokioinen [a]	< 0.001	0.065	2.609	0.003	0.208	0.009	0.333	0.222	0.142	0.242
Krems-münster	0.001	< 0.001	2.281	< 0.001	0.190	0.009	0.131	0.087	0.039	0.126
Okehamp-ton [a]	0.002	0.186	2.554	0.008	0.272	0.012	0.131	0.088	0.057	0.172
Piacenza	0.036	< 0.001	2.274	0.005	0.269	0.013	0.092	0.061	0.038	0.140
Porto [a]	< 0.001	0.004	0.700	< 0.001	0.032	0.001	0.062	0.041	0.022	0.041
Sevilla	< 0.001	< 0.001	1.546	< 0.001	0.072	0.003	0.049	0.032	0.012	0.045
Thiva	0.001	< 0.001	1.950	< 0.001	0.106	0.005	0.065	0.044	0.008	0.055

a acidic soil, corresponding metabolism pathway used.

Table 8.35 Predicted 80th percentile annual average concentrations in groundwater at 1 m depth following use to **potatoes every 3 years (PEARL**, with sorption kinetics)

Scenario	Annual PEC$_{gw}$ in µg/L									
	Fluo-picolide	M-03, AE 0608000	M-01, AE C653711	M-02, AE C657188	M-05, AE 1344122	M-14, AE 1388273	M-11, P2a	M-12, P2b	M-13, P3	M-10, AE 1344123
Châteaudun	0.001	< 0.001	1.602	< 0.001	0.130	0.006	0.080	0.053	0.021	0.086
Hamburg [a]	0.001	0.116	2.100	0.005	0.210	0.010	0.142	0.094	0.067	0.158
Jokioinen [a]	< 0.001	0.040	1.597	0.001	0.122	0.005	0.206	0.137	0.091	0.141
Krems-münster	0.001	< 0.001	1.553	< 0.001	0.118	0.006	0.09	0.06	0.025	0.084
Okehamp-ton [a]	0.001	0.118	1.701	0.005	0.172	0.008	0.087	0.058	0.037	0.111
Piacenza	0.024	< 0.001	1.582	0.003	0.182	0.008	0.061	0.040	0.026	0.094
Porto [a]	< 0.001	0.003	0.436	< 0.001	0.018	0.001	0.039	0.026	0.015	0.025
Sevilla	< 0.001	< 0.001	0.910	< 0.001	0.043	0.002	0.034	0.023	0.008	0.030
Thiva	< 0.001	< 0.001	1.292	< 0.001	0.073	0.003	0.044	0.030	0.006	0.041

[a] acidic soil, corresponding metabolism pathway used

Table 8.36 Predicted 80th percentile annual average concentrations in groundwater at 1 m depth following use to **potatoes every year** (**PELMO**, no sorption kinetic)

Scenario	Annual PEC$_{gw}$ in µg/L									
	Fluo-picolide	M-03, AE 0608000	M-01, AE C653711	M-02, AE C657188	M-05, AE 1344122	M-14, AE 1388273	M-11, P2a	M-12, P2b	M-13, P3	M-10, AE 1344123
Châteaudun	0.001	< 0.001	3.995	< 0.001	0.160	0.008	0.206	0.137	0.029	0.154
Hamburg [a]	0.010	0.275	6.733	0.016	0.592	0.027	0.496	0.334	0.199	0.525
Jokioinen [a]	< 0.001	0.028	4.536	0.002	0.240	0.011	0.813	0.542	0.369	0.534
Krems-münster	0.001	< 0.001	4.181	< 0.001	0.206	0.010	0.292	0.195	0.059	0.212
Okehamp-ton [a]	0.008	0.175	5.392	0.008	0.429	0.020	0.319	0.212	0.111	0.352
Piacenza	0.212	< 0.001	4.867	0.018	0.501	0.024	0.205	0.137	0.071	0.284
Porto [a]	< 0.001	0.001	1.079	< 0.001	0.021	0.001	0.165	0.11	0.055	0.093
Sevilla	< 0.001	< 0.001	0.114	< 0.001	0.001	< 0.001	0.041	0.027	0.005	0.009
Thiva	< 0.001	< 0.001	1.951	< 0.001	0.027	0.001	0.074	0.049	0.008	0.029

a acidic soil, corresponding metabolism pathway used.

Table 8.37 Predicted 80th percentile annual average concentrations in groundwater at 1 m depth following use to **potatoes every 2 years** (**PELMO**, no sorption kinetic)

Scenario	Annual PEC$_{gw}$ in µg/L									
	Fluo-picolide	M-03, AE 0608000	M-01, AE C653711	M-02, AE C657188	M-05, AE 1344122	M-14, AE 1388273	M-11, P2a	M-12, P2b	M-13, P3	M-10, AE 1344123
Châteaudun	< 0.001	< 0.001	1.913	< 0.001	0.072	0.003	0.097	0.064	0.014	0.068
Hamburg [a]	0.003	0.119	3.152	0.006	0.271	0.012	0.242	0.162	0.099	0.243
Jokioinen [a]	< 0.001	0.013	2.073	0.001	0.097	0.004	0.371	0.247	0.177	0.214
Krems-münster	< 0.001	< 0.001	1.986	< 0.001	0.089	0.004	0.136	0.090	0.029	0.100
Okehamp-ton [a]	0.003	0.079	2.542	0.004	0.183	0.008	0.157	0.104	0.051	0.157
Piacenza	0.076	< 0.001	2.357	0.005	0.247	0.012	0.098	0.065	0.036	0.139
Porto [a]	< 0.001	< 0.001	0.471	< 0.001	0.009	0.001	0.079	0.053	0.027	0.034
Sevilla	< 0.001	< 0.001	0.056	< 0.001	< 0.001	< 0.001	0.020	0.014	0.002	0.004
Thiva	< 0.001	< 0.001	0.830	< 0.001	0.010	< 0.001	0.029	0.019	0.003	0.013

a acidic soil, corresponding metabolism pathway used

Table 8.38 Predicted 80th percentile annual average concentrations in groundwater at 1 m depth following use to **potatoes every 3 years (PELMO**, no sorption kinetic)

Scenario	Annual PEC$_{gw}$ in µg/L									
	Fluo-picolide	M-03, AE 0608000	M-01, AE C653711	M-02, AE C657188	M-05, AE 1344122	M-14, AE 1388273	M-11, P2a	M-12, P2b	M-13, P3	M-10, AE 1344123
Châteaudun	< 0.001	< 0.001	1.223	< 0.001	0.043	0.002	0.065	0.044	0.009	0.043
Hamburg [a]	0.002	0.079	2.003	0.004	0.170	0.008	0.151	0.101	0.068	0.160
Jokioinen [a]	< 0.001	0.008	1.331	< 0.001	0.060	0.003	0.249	0.166	0.117	0.131
Krems-münster	< 0.001	< 0.001	1.224	< 0.001	0.051	0.003	0.091	0.060	0.019	0.061
Okehamp-ton [a]	0.001	0.049	1.627	0.002	0.113	0.005	0.104	0.069	0.034	0.099
Piacenza	**0.041**	< 0.001	1.526	0.003	0.166	0.008	0.065	0.043	0.024	0.093
Porto [a]	< 0.001	< 0.001	0.303	< 0.001	0.005	< 0.001	0.053	0.035	0.017	0.018
Sevilla	< 0.001	< 0.001	0.034	< 0.001	< 0.001	< 0.001	0.010	0.007	0.001	0.002
Thiva	< 0.001	< 0.001	0.559	< 0.001	0.006	< 0.001	0.019	0.013	0.002	0.008

[a] acidic soil, corresponding metabolism pathway used

Table 8.38 Maximum 80th percentile annual average concentrations and exceedance of 0.1 µg/l limit following use to **potatoes with PEARL**.

	Highest 80th percentile concentrations (µg/l, *scenario*)			No. of scenarios > 0.1µg/l (out of 9 simulated).		
Application:	**Every yr**	**1 in 2 yrs**	**1 in 3 yrs**	**Every yr**	**1 in 2 yrs**	**1 in 3 yrs**
Parent	**0.104** (*P*)	0.036 (*P*)	0.024 (*P*)	1	-	-
M-03	**0.386** (*H*)	**0.201** (*H*)	**0.118** (*N*)	3	2	2
M-01	**6.628** (*H*)	**3.153** (*H*)	**2.10** (*H*)	9	9	9
M-02	0.02 (*N*)	0.009 (*H*)	0.005 (*H/N*)	-	-	-
M-05	**0.697** (*H*)	**0.315** (*H*)	**0.210** (*H*)	8	7	6
M-14	0.033 (*H*)	0.015 (*H*)	0.01 (*H*)	-	-	-
M-11	**0.669** (*J*)	**0.333** (*J*)	**0.206** (*J*)	8	5	2
M-12	**0.446** (*J*)	**0.222** (*J*)	**0.137** (*J*)	6	2	1
M-13	**0.312** (*J*)	**0.142** (*J*)	0.091 (*J*)	3	1	-
M-10	**0.525** (*J*)	**0.242** (*H/J*)	**0.158** (*H*)	7	6	3

bold font denotes > 0.1 µg/l

Table 8.39 Maximum 80th percentile annual average concentrations and exceedance of 0.1 µg/l limit following use to **potatoes with PELMO**.

	Highest 80th percentile concentrations (µg/l, *scenario*)			No. of scenarios > 0.1µg/l (out of 9 simulated).		
Application:	**Every yr**	**1 in 2 yrs**	**1 in 3 yrs**	**Every yr**	**1 in 2 yrs**	**1 in 3 yrs**
Parent	**0.212** (*P*)	0.076 (*P*)	0.041 (*P*)	1	-	-
M-03	**0.275** (*H*)	**0.119** (*H*)	0.079 (*H*)	2	1	-
M-01	**6.733** (*H*)	**3.152** (*H*)	**2.003** (*H*)	8	8	7
M-02	0.018 (*P*)	0.006 (*H*)	0.004 (*H*)	-	-	-
M-05	**0.592** (*H*)	**0.271** (*H*)	**0.170** (*H*)	6	3	3
M-14	0.027 (*H*)	0.012 *H/P*)	0.008 (*H/P*)	-	-	-
M-11	**0.813** (*J*)	**0.371** (*J*)	**0.249** (*J*)	7	4	3
M-12	**0.542** (*J*)	**0.247** (*J*)	**0.166** (*J*)	7	3	2
M-13	**0.369** (*J*)	**0.177** (*J*)	**0.117** (*J*)	3	1	1
M-10	**0.534** (*J*)	**0.243** (*H*)	**0.160** (*H*)	6	5	2

bold font denotes > 0.1 µg/l.
P = Piacenza, H= Hamburg, J= Jokioinen.
The results of Table 8.39 for application 1 in 3 years are equivalent to those reported for the original groundwater assessment in the DAR, Table B.8.260, with very slight differences for metabolite M-14 (highest 80th percentile concentration was 0.005 µg/l).

The maximum predicted 80th percentile concentrations of fluopicolide and metabolites in groundwater following use on potatoes, and the number of scenarios where 0.1 µg/l is exceeded, are shown in Tables 8.38 and 8.39.

Following use to potatoes, PECgw values for fluopicolide exceeded the maximum acceptable concentration of 0.1 µg/l at Piacenza (PEARL and PELMO), when application was assumed every year. If crop rotation was taken into account (application assumed every 2 or 3 years), then PECgw values for fluopicolide were less than 0.1 µg/l.

PECgw values of metabolite M-01 exceeded 0.1 µg/l at every scenario (PEARL and PELMO) when application was assumed every year. When application to potatoes was assumed every 2 or 3 years instead, M-01 still exceeded 0.1 µg/l at every scenario (PEARL) and all but Sevilla (PELMO).

Only metabolites M-02 and M-14 were predicted to be below 0.1 µg/l in groundwater at every scenario (and application regime simulated), for both models.

For both models, following use in potatoes, PECgw for all the metabolites simulated were <0.75 µg/l, with these exceptions, which were between >0.75 µg/l and <10 µg/l:

M-11 at Jokioinen (PELMO, application every year)
M-01 at every scenario/ application regime simulated, except Sevilla (PELMO, application every 1, 2 and 3 years which were <0.75 µg/l).

Comparison of results with original groundwater assessment in DAR, B.8.6.2.

Metabolites exceeding 0.1 µg/l

The original groundwater assessment for fluopicolide and its metabolites (reported in the DAR, B.8.6.2) was carried out using FOCUS PELMO with standard degradation and sorption parameters and for use on vines, assumed greater crop interception than considered here. The results indicated that parent and the metabolites, M-01, M-03, M-05, M-10, M-11, M-12 and M-13 had potential to exceed 0.1 µg/l at various scenarios (see Table 8.32).

The new groundwater modelling with PELMO (assuming less crop interception for vines) and PEARL (incorporating sorption kinetics), results in the same metabolites being predicted to have potential to contaminate groundwater above 0.1 µg/l. No additional metabolites are predicted to exceed 0.1 µg/l, following proposed use of fluopicolide to vines.

The original groundwater assessment (DAR, B.8.6.2) with FOCUS PELMO assumed application to potatoes, once every 3 years. It resulted in predicted concentrations of fluopicolide being < 0.1 µg/l, but metabolites M-01, M-5, M-10, M-11, M-12 and M-13 were predicted to have potential to contaminate groundwater > 0.1 µg/l.

The new groundwater modelling with PELMO (assuming application to potatoes also every 2 and every 3 years) and with PEARL (incorporating sorption kinetics), results in the same metabolites being predicted to have potential to contaminate groundwater above 0.1 µg/l. However, for application every year, parent compound and M-03 are also predicted to exceed 0.1 µg/l for certain scenarios.

Predicted concentrations of M-03 exceed 0.1 µg/l in both the PEARL and PELMO models, following application to potatoes every 2 years, and also in PEARL after application every 3 years, (though not in PELMO). Following application to potatoes every 3 years, M-13 did not exceed 0.1 µg/l in PEARL, though it did at one scenario in PELMO.

Number of scenarios where 0.1 µg/l is exceeded

For use of fluopicolide on vines, the number of scenarios where 0.1 µg/l was exceeded by parent or metabolites is almost the same, when comparing the results of new and previous PELMO modelling. Incorporation of sorption kinetics in PEARL modelling, gave slightly fewer scenarios exceeding 0.1 µg/l for parent, M-05, M-10 and M-12, but otherwise was similar.

For use of fluopicolide on potatoes, the results of PELMO modelling for application once every 3 years are essentially the same as previously reported in the DAR. Assuming more frequent application, i.e. every year or every 2 years, modelling with PELMO gave a greater number of scenarios where 0.1 µg/l was exceeded, as shown in Table 8.39.

Incorporation of sorption kinetics in PEARL modelling for use on potatoes generally gave an increased number of scenarios at which concentrations of metabolites exceeded 0.1 µg/l, (increasing with frequency of application). There were some exceptions: for M-13, the number of scenarios with concentrations > 0.1 µg/l were similar to those with PELMO and for application every 3 years were all <0.1 µg/l in PEARL. For M-12, the number of scenarios with concentrations >0.1µg/l were slightly fewer in PEARL, than those with PELMO. For M-11, the number of scenarios with concentrations >0.1µg/l were one less than in PEARL, for application once every 3 years).

Differences in 80th percentile concentrations of parent and metabolites

For use of fluopicolide on vines, the assumption of less crop interception in PELMO modelling resulted in higher 80th percentile annual average concentrations for parent and metabolites, as would be expected. The incorporation of sorption kinetics in PEARL modelling gave lower PECgw values for parent fluopicolide, than in the original PELMO assessment, but in some cases concentrations of metabolites were higher (e.g. M-01, M-03, M-05, M-10, M-11, M-13 and M-14. Compare Tables 8.31 and 8.32).

For use of fluopicolide on potatoes, revised PELMO modelling assuming more frequent application (every year or every 2 years) gave higher PECgw values for parent and metabolites, as would be expected. Incorporating sorption kinetics into PEARL modelling generally gave similar or slightly lower PECgw, compared to the results of PELMO modelling, with application every 3 years. (See Table 8.38 compared with the

column for "application 1 in 3 years" of Table 8.39, the results of which are equivalent to those originally reported in the DAR).

For application to potatoes every 2 years, PEARL modelling gave a slightly higher 80th percentile concentration for M-05, but similar or lower concentrations for parent and other metabolites, compared to corresponding results with PELMO. For application every 3 years, PEARL gave higher 80th percentile concentrations for M-03, M-05 and M-14, but similar or lower concentrations for parent and the other metabolites, compared to corresponding results with PELMO.

RMS Risk Assessment and Conclusions:

For use on vines, fluopicolide is predicted to contaminate groundwater above the maximum acceptable concentration (0.1 µg/l) at one or two of the 7 scenarios modelled, (Châteaudun and or Piacenza). Concentrations of the metabolites M-01, M-05, M-10, M-11, M-12 and M-13 were predicted to exceed 0.1 µg/l in groundwater. Of these, M-01, M-05, M-10, M-11 and M-12 exceeded 0.1 µg/l in all, or almost all of the scenarios simulated in both PELMO and PEARL. In particular, predicted concentrations of M-01 were many orders of magnitude higher than this limit (range 1.6-6.3 µg/l). Metabolites M-03 and M-13 only exceeded 0.1 µg/l at a couple of scenarios, (and for M-03 the scenarios were those with acidic soils). Therefore, the relevance of these metabolites needs to be assessed further, in accordance with the EU Guidance Document on the assessment of the relevance of metabolites in groundwater.[9]

In the view of the RMS, application every year to potatoes is considered to be extreme and not representative in the vast majority of cases. For use of fluopicolide as proposed on potatoes, assuming application every 2 or 3 years, fluopicolide was not predicted to contaminate groundwater above 0.1 µg/l. However, M-01 exceeded 0.1 µg/l in all or almost all of the modelled scenarios (up to 2 µg/l and 3.2 µg/l, for application every 2 and 3 years, respectively). Metabolites M-03, M-05, M-10, M-11, M-12 and M-13 also exceeded the 0.1 µg/l limit for various scenarios. Therefore, as above for vines, the relevance of these metabolites need to be assessed further, in accordance with the EU Guidance Document.

Overall, it can be seen that the revised modelling has not resulted in any additional metabolites being predicted to occur at >0.1 µg/l on an annual average basis. The highest concentrations of fluopicolide metabolites from either modelling or lysimeter study seen in the original DAR compared to the highest results from modelling in this addendum are presented below. These have been tabulated simply on the basis of concentration and ignore the GAP used to produce the PEC values and the model used. However, it should be noted that some of the highest concentrations from modelling in this addendum are from use every year on potatoes which the RMS considers to be extreme worst-case and inappropriate as a regulatory scenario.

[9] EU Guidance Document on the assessment of the relevance of metabolites in groundwater of substances regulated under Council Directive 91/414/EEC – Sanco/221/2000-rev 10, 25 February 2003.

Table B.8.40 Comparison of highest metabolite groundwater PEC values from original DAR and this addendum for regulatory decision-making (µg/l)

	Highest concentrations in original DAR	Highest concentrations in addendum
M-03	0.381 (H)	**0.525** (H)
M-01	4.614 (H)	**6.733** (H)
M-02	0.033 (P)	0.038 (P)
M-05	0.90 (L)	0.715 (H)
M-14	0.19 (L)	0.033 (H)
M-11	0.55 (L)	**0.813** (J)
M-12	0.36 (L)	**0.542** (J)
M-13	0.160 (H)	**0.369** (J)
M-10	0.83 (L)	0.586 (H)

Values in **bold** are increases from the original DAR values
P = Piacenza; H = Hamburg; L = lysimeter; J = Jokioinen

Thus it can be seen that the highest concentrations of regulatory significance for most metabolites have increased as a result of this new assessment. It should be noted that for M-11, the revised concentration is >0.75 µg/l, whereas in the original DAR the concentration was <0.75 µg/l. This has implications for the relevance assessment. However, it must be realised that the highest concentration occurred on potatoes assuming that the crop was grown every year. In the opinion of the RMS, this is an extreme and unrepresentative GAP for potato, and in GAP assuming a rotation of 1 in 2 years or longer, 0.75 µg/l was not exceeded.

Implications for Ecotoxicogical Assessment:

Based on assessment of new FOCUS groundwater modelling, the following metabolites are predicted to have potential to exceed 0.1 µg/l in groundwater: M-01, M-03 (acidic soils), M-05, M-10, M-11, M-12 and M-13. (M-02 and M-14 were predicted at concentrations less than 0.1 µg/l).

The only metabolite predicted to contaminate groundwater at > 0.1 µg/l, not previously identified in the original assessment (DAR, B.8.6.2) is M-03 following use on potatoes. However, M-03 was considered in the previous assessment to exceed the 0.1 µg/l limit, following use of fluopicolide on vines.

Table 8.41 Summary of predicted potential of fluopicolide and metabolites to contaminate groundwater above 0.1 µg/l.

	Vines	Potatoes (every yr)	Potatoes (every 2 yr)	Potatoes (every 3 yr)
Parent	X	X	-	-
M-01	X	X	X	X
M-02	-	-	-	-
M-03	X	X	X	X
M-05	X	X	X	X
M-10	X	X	X	X
M-11	X	X	X	X
M-12	X	X	X	X
M-13	X	X	X	X
M-14	-	-	-	-

X 80th percentile annual average concentration > 0.1 µg/l for at least one FOCUS scenario in PEARL and/or PELMO model(s).

\- 0.1 µg/l not exceeded at any FOCUS scenario modelled.

Concentrations of parent and metabolites following use on vines have been modelled assuming less crop interception, so the results from the PELMO model are higher (Table 8.30) than previously assessed. The concentrations predicted from the PEARL model (for vines), taking into account sorption kinetics, were higher for metabolites M-01, M-03, M-05, M-10, M-11, M-13 and M-14.

Concentrations of parent and metabolites have been modelled using the FOCUS PELMO and PEARL models assuming application to potatoes every year, every 2 and every 3 years. The previous assessment (DAR, B.8.6.2) used only the PELMO model and assumed application only once every 3 years. Therefore, concentrations from the PELMO model (application every 3 years) are equivalent to those previously assessed (Table 8. 39). However, taking sorption kinetics into account in the PEARL model (Table 8.38) gave higher 80th percentile concentrations for the metabolites M-01, M-03, and M-05, (and only very fractionally higher for M-02 and M-14).

Assuming more frequent application to potatoes, every year or every 2 years, the predicted 80th percentile concentrations of all the metabolites are increased over those previously considered in the DAR. (The RMS considers that application every year to potatoes is extreme and not representative in the vast majority of cases).

(Kley, C. & Ellerich, C. 2007 (a) & (b), Kley 2004, MEF-04/346 and MEF-04/347)

B.8.10 Additional References Relied On:

Annex Point/ Location in Dossier	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Data protect. claimed	Owner
Doc K AII 7.1.1.2.2	Kley, C; Mackenzie, E.	2007	Fluopicolide – Relevance of photolysis in soil degradation studies. Bayer CropScience AG Report no. MEF-06/495 Edition no. M-286182-01-1 Date: 2007-03-28 No GLP, unpublished	Yes	BCS
Doc K AII 7.1.1.2.2.3	Kley, C; Mackenzie, E.	2006	An evaluation of the potential accumulation of fluopicolide in the field Bayer CropScience AG, Edition Number: M-267721-01-1 Date: 2006-03-09 Non GLP, unpublished	Yes	BCS
Doc Kb Position Papers	Kley, C; Mackenzie, E.	2006	Distribution of fluopicolide (AE C638206) in soil under zero tillage conditions Bayer CropScience AG, Report No.: MEF-06/021 Edition Number: M-268742-01-1 Date: 2006-03-09 Non GLP, unpublished	Yes	BCS
AII IIA 7.1.1 Position Papers	Kley, C; Mackenzie, E	2007	Evaluation of Soil Degradation Parameters for Fluopicolide (AE C638206) for use as Trigger Values Bayer CropScience AG, Report No.: MEF-07/265 Project ID: MEACX083 Date: 2007-11-12 Non GLP, unpublished	Yes	BCS
AII IIA 7.1.1 Position Papers	Kley, C; Mackenzie, E	2007	Evaluation of Soil Degradation Parameters for Fluopicolide AND ITS METABOLITES FROM Laboratory and Field Trials for Modelling Purposes Bayer CropScience AG, Report No.: MEF-07/266 Project ID: MEACX083 Date: 2007-11-12 Non GLP, unpublished	Yes	BCS

Plant Protection Product - EXP11074B

Annex Point/ Location in Dossier	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Data protect. claimed	Owner
Doc K AIIIa 9.2.1	Kley, C; Ellerich, C.	2007	Predicted environmental concentrations in groundwater (PECgw) for fluopicolide and its metabolites calculated with FOCUS PEARL and FOCUS PELMO, Use in vines in Europe Bayer CropScience Report no. MEF-07/166 Edition No. M-287350-01-1 Date: 2007-04-25 no GLP, unpublished	BAY	BCS

Plant Protection Product - EXP11120A

Location in Dossier	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Data protect. claimed	Owner
Doc K AIIIb 9.2.1	Kley, C; Ellerich, C.	2007	Predicted environmental concentrations in groundwater (PECgw) for fluopicolide and its metabolites calculated with FOCUS PEARL and FOCUS PELMO, Use in potatoes in Europe Bayer CropScience Report no. MEF-07/165 Edition No. M-287355-01-1 Date: 2007-04-25 no GLP, unpublished	BAY	BCS

B.9 ECOTOXICOLOGY

This addendum addresses ecotoxicological issues raised during the EU peer review of the Draft Assessment Report (DAR) prepared by the RMS, UK, for EU consideration of inclusion of the fungicide new active substance (NAS), fluopicolide, in Annex I of EU Directive 91/414/EEC on plant protection products.

Ecotoxicological issues to be addressed were identified in Section 5 of the Evaluation Table which were derived from EU peer review comments and responses compiled in Section 5 of the Reporting Table [Reporting Table, fluopicolide, rev.1 (26.01.2007)].

For ease of reference the proposed EU uses of fluopicolide are re-presented in Table B.9.0.1.

Table B.9.0.1 Summary of intended EU uses of fluopicolide

Crop (formulation)	Maximum individual fluopicolide application rate (kg a.s./ha)	Maximum no. of applications	Maximum fluopicolide total dose (kg a.s./ha)	Spray water volume (L/ha)	Application timings (d - min. spray interval)	PHI (d)
Vine ('EXP 11074B')	0.133	3	0.4 kg/ha	100-1500	BBCH 53-81[1] (10)	21-35
Potato ('EXP 11120A')	0.1	4	0.4 kg/ha	200-400 (NMS) 400-1000 (SMS)	BBCH 20-91[2] (7)	7

PHI pre harvest interval
[1] inflorescences clearly visible - to beginning of ripening
[2] first basal side shoot visible- to beginning of leaf yellowing

B.9.1 Non-target vertebrates - birds and mammals (DAR B.9.1 & B.9.3)

Two issues **Evaluation Open pts. 5.1 & 5.2** were raised pertinent to the dietary risk posed to birds and mammals from proposed uses of fluopicolide in potato and vine.

Evaluation Table Open pt. 5.1

'RMS to clarify in an addendum how the MAF for different vegetation was calculated and used in the assessment of risk to birds'

5.1 RMS response:

For clarity, the Tier 1 bird and mammal risk assessment for use on potato, undertaken in accordance with SANCO 4145/2000-24/Sep/2002, is re-presented in Table B.9.1.1.

Table B.9.1.1 Tier I avian and mammalian dietary risk from proposed fluopicolide use on potato

RISK Crop / food	Indicator spp. (bw kg)	FIR /bw	a.s. app. rate kg/ha	RUD	MAF	ftwa	PT	PD	AV	ETE	Tox. end pt.	TER	Ann. VI
										mg a.s./kg bw/d			
AVIAN - ACUTE											**LD50**		
Leafy (E/L) / s. insects	insectivore (0.01)	1.04	0.100	52	n.a.	n.a.	1.00	1.00	1.00	5.408	>2250.00	**>416.05**	10
Leafy (E/L) / leaves	herbivore (0.3)	0.76	0.100	87	**1.8**[1]	n.a.	1.00	1.00	1.00	11.879	>2250.00	**>189.41**	10
AVIAN - SHORT TERM											**LC50**		
Leafy (E/L) / s. insects	insectivore (0.01)	1.04	0.100	29	n.a	n.a.	1.00	1.00	1.00	3.016	>1744.00	**>578.25**	10
Leafy (E/L) / leaves	herbivore (0.3	0.76	0.100	40	2.2	n.a.	1.00	1.00	1.00	6.772	>1744.00	**>257.52**	10
AVIAN - LONG TERM											**NOEC**		
Leafy (E/L) / s. insects	insectivore (0.01)	1.04	0.100	29	n.a.	n.a.	1.00	1.00	1.00	3.016	88.90	**29.48**	5
Leafy (E/L) / leaves	herbivore (0.3)	0.76	0.100	40	2.2	0.53	1.00	1.00	1.00	3.567	88.90	**24.92**	5
MAMMALIAN - ACUTE											**LD50**		
Leafy (E/L) / leaves	herbivore (3.0)	0.28	0.100	87	**1.8**[1]	n.a.	1.00	1.00	1.00	4.377	>5000.00	**>1142.46**	10
Leafy (E/L) / l. insects	Insectivore[2] (0.01)	0.63	0.100	14	n.a.	n.a.	1.00	1.00	1.00	0.882	>5000.00	**>5668.93**	10
MAMMALIAN - LONG TERM											**NOEC**		
Leafy (E/L) / leaves	herbivore (3.0)	0.28	0.100	40	2.2	0.53	1.00	1.00	1.00	1.314	20.00	**15.22**	5
Leafy (E/L) / l. insects	Insectivore[2] (0.01)	0.63	0.100	5	n.a.	n.a.	1.00	1.00	1.00	0.321	20.00	**62.25**	5

n.a. not applicable; [1]amended to SANCO 4145/2000 value

[2] indicator insectivorous species included as potato leaves are not grazed by herbivorous mammals

The TERs in Table B.9.1.1 are all above Annex VI thresholds indicating low risk to herbivorous and insectivorous birds and mammals following fluopicolide ('EXP 11120A') application to potato. It should also be noted that potato foliage is unattractive food for birds and mammals.

The issue (Open pt. 5.1) concerned selection of the acute MAF value in the risk assessment. The acute MAF value (1.8) used in Table B.9.1.1 was derived from Table 3 of SANCO 4145/2000 reflecting the proposed application regime (Table B.9.0.1) for potato (4 applications with a 7d spray interval). A discrepancy was noted in the acute MAF value (2.0) for a leafy crop application regime when derived from mathematical formula by the RMS. However, this had no impact on the conclusion of the risk assessment which now includes the SANCO 4145/2000 value.

Evaluation Table Open pt. 5.2

'RMS to include the corrected calculations and the refined RA in an addendum. List of endpoints has been amended. No discussion in expert meeting required unless required by MS.'

5.2 RMS response:

For clarity, the Tier 1 bird and mammal risk assessment for use on vine, undertaken in accordance with SANCO 4145/2000-24/Sep/2002, is re-presented in Table B.9.1.2.

Table B.9.1.2 Tier I avian and mammalian dietary risk from proposed fluopicolide use on vine

RISK Crop / food	Indicator spp. (bw kg)	FIR /bw	a.s. app. rate kg/ha	RUD	MAF	ftwa	PT	PD	AV	ETE mg a.s./kg bw/d	Tox. end pt. mg a.s./kg bw/d	TER	Ann. VI
AVIAN - ACUTE											**LD50**		
Vine (E/L) / s. insects	insectivore (0.01)	1.04	0.133	52	n.a.	n.a.	1.00	1.00	1.00	7.193	>2250.00	**>312.82**	10
AVIAN - SHORT TERM											**LC50**		
Vine (E/L) / s. insects	insectivore (0.01)	1.04	0.133	29	n.a.	n.a.	1.00	1.00	1.00	4.011	>1744.00	**>434.77**	10
AVIAN - LONG TERM											**NOEC**		
Vine (E/L) / s. insects	insectivore (0.01)	1.04	0.133	29	n.a.	n.a.	1.00	1.00	1.00	4.011	88.90	**22.16**	5
MAMMALIAN - ACUTE											**LD50**		
Vine (E/L) / s. insects	herbivore (0.025)	1.39	0.133	85	1.5	n.a.	1.00	1.00	1.00	23.445	>5000.00	**>213.26**	10
MAMMALIAN - LONG TERM											**NOEC**		
Vine (E/L) / s. insects	herbivore (0.025)	1.39	0.133	46	1.5	0.53	1.00	1.00	1.00	6.761	20.00	***2.96***	5

n.a. not applicable;

The TERs in Table B.9.1.2 are all above Annex VI thresholds except for the long term TER for small herbivorous mammals which indicates potential risk. Hence this risk requires further Tier II refinement (Table B.9.1.3).

Table B.9.1.3 Tier II mammalian refined dietary risk from proposed fluopicolide use on vine

RISK Crop / food	Indicator spp. (bw kg)	FIR /bw	a.s. app. rate kg/ha	RUD	MAF	ftwa	PT	PD	AV	ETE mg a.s./kg bw/d	Tox. end pt. mg a.s./kg bw/d	TER	Ann. VI
MAMMALIAN - LONG TERM											NOEC		
Vine (E/L) / s. insects	herbivore (0.025)	1.39	0.133	**23**[1]	1.5	0.53	1.00	1.00	1.00	3.38	20.00	**5.92**	5

[1] amended to reflect 70% vine canopy spray interception

In vines small herbivorous mammals consume sub-canopy (ground) vegetation, assessed as short grass, and the issue (Open pt. 5.2) addresses risk refinement via canopy spray interception. For vine fungicides, Tier 1 bird and mammal risk assessment (SANCO 4145/2000) assumes a canopy 40% spray interception. The proposed fluopicolide ('EXP 11074B') use on vine is between growth stages BBCH 53-91 (Table B.9.0.1), i.e. from inflorescence clearly visible through to start of grape ripening. These growth stages represent canopy interception from 60% (end of foliage development) through to 85% (start of ripening) and a 70% spray interception was considered an appropriate precautionary refinement. The long term risk to herbivorous mammals has been refined taking account of the higher canopy interception (Table B.9.1.3).

The TER>5 in Table B.9.1.3 indicates a low risk to small herbivorous mammals consuming sub-canopy vegetation following fluopicolide ('EXP 11074B') application to vine.

B.9.2 Aquatic organisms

Four issues **(Evaluation Table Open points 5.3, 5.4, 5.5 & 5.12)** were raised pertinent to the risk to aquatic organisms from proposed uses of fluopicolide in potato and vine crops:

Evaluation Table Open pt. 5.3

'RMS to include the information on Log Pow values for the metabolites in an addendum (only data for M02 and M03 are available in Vol.B.2.1 of the DAR). No discussion in an experts meeting is required.'

5.3 RMS response:

Log Pow values of parent and metabolites are considered in ecotoxicological risk assessment of potential bioconcentration potential.

A fish bioconcentration study was conducted (see DAR B.9.2.3.4) as fluopicolide has a logPow of 2.9, i.e. close to the logPow Annex VI threshold of >3.0 for bioconcentration assessment. The results showed that fluopicolide had bioconcentration factor (BCF) in fish of 121. Clearance of fluopicolide from fish tissues, CT50 and CT90, were 0.51 and 1.7d, respectively, and 5% of total residue remained after 18d depuration.

The overall conclusion for fluopicolide was that the low BCF and rapid clearance times of fluopicolide from fish tissues indicated a low propensity to bioaccumulate in fish. Potential for fluopicolide bioconcentration in worm- and fish-eating bird and mammals was also considered to be low (DAR B.9.15 & B.9.3.3).

Key aquatic metabolites M03, M02 and M01 (see Appendix 1) have log Pow values of 2.34, -2.0 and 0.51 (DAR Volume B.2.1), respectively, and a lower propensity for bioaccumulation than parent fluopicolide would hence be expected. M03 is also not stable in aquatic systems at environmental pH and degraded to form M02 and M01 and hence also likely to have a very low bioavailability. All other aquatic metabolites M05, M10, M11, M12, M13 and M14 are similarly structured hydroxylated and/or sulphonated derivatives of M02 (see Appendix 2). Thus such derivatives will likely have logPow values approximating to that of M02 and hence it can also be concluded that all the key aquatic fluopicolide metabolites will have little potential to bioaccumulate.

Evaluation Table Open pt. 5.4

'RMS to include the correction in a corrigendum and to update the list of endpoint. Since threshold values are different for algae and fish/invertebrates we would prefer to have TER values also for fish and invertebrates in the list of endpoints even if algae was the most sensitive organism group.'

5.4 RMS response:

List of endpoints have been updated and for purposes of clarification the corrected aquatic spray drift risk assessment for EXP 11074B and EXP11120A following respective uses on vine and potato is presented in Table B.9.2.1 below (DAR B.9.2.4.1i and B.9.2.4.2i).

Table B.9.2.1 Spray drift aquatic risk assessment for EXP 11074B and EXP11120A

Organism	Time scale	Tox. end pt.	PEC initial [1]@ 3m vine use [2]@ 1m potato use		PEC initial @ 5m vine use		Annex VI
		EXP 11074B (mg/L)	**EXP 11074B (mg /L[1])**	**TER**	**EXP 11074B (mg /L)**	**TER**	
O. mykiss	96h LC50	8.54	0.0802	106.5	-	-	100
D. magna	**21d NOEC**	**>25.0**	**0.0802**	**>311.7**	**-**	**-**	**10**
N. pelliculosa	72h EbC50 72h ErC50	0.58 0.91	0.0802 0.0802	***7.2*** 11.3	0.0362 -	16.0 -	10 10
		EXP 11120A (mg/L)	**EXP 11120A (mg /L[2])**	**TER**			
O. mykiss	96h LC50	6.57	0.0167	393.4	-	-	100
D. magna	21d NOEC	>100.0	0.0167	>5988.0	-	-	10
N. pelliculosa	72h EbC50 72h ErC50	0.40 0.63	0.0167 0.0167	24.0 37.7	- -	- -	10 10

EXP 11074B and EXP 11120A exposure was considered only likely to constitute an acute aquatic risk from spray drift following application when formulation integrity is most conserved. In Table B.9.2.1 acute TERs >Annex VI indicate that low acute aquatic risk can be expected from spraydrift following EXP 111120A on potato, however, a 5m buffer zone is required to mitigate the acute (worse case) aquatic risk to *N. pelliculosa* from EXP 11074B following use on vine.

Evaluation Table Open pts. 5.5

'RMS to include the information and argumentation regarding the ecotoxicological relevance of GW metabolites presented in column 3 in an addendum for the sake of completeness. We agree that since the TER for M05 is >18519 (vine) and >58824 (potato) for algae and this metabolite is the one of highest concentration in the FOCUS$_{gw}$ modelling, apart from M01, the risk from M10, M11, M12 and M13 to aquatic organisms can be considered to be low. The information presented is however of value for the assessment of "pesticidal activity".'

5.5 RMS response:

For aquatic ground water risk assessment of the parent, fluopicolide, the green algal diatom, *N. pelliculosa*, was by far the most sensitive species (see DAR Tables B.9.2.78 and B.9.2.85). Available aquatic data on fluopicolide metabolites also indicated *N. pelliculosa* to be most sensitive species tested, but >300x less than parent (see DAR Table B.9.2.75). The *N. pelliculosa* aquatic endpoint was used to assess the potential risk to aquatic organisms from principal leachate and groundwater average annual contaminants >0.1µg/L (Tables B.9.2.2 and B.9.2.3) (DAR B.9.2.4.1iii and B.9.2.4.2iii). It should also be noted that Env Fate (Section B.8.6.2, Addendum 1 (Nov 2007)) have recalculated PECgw based on revised modelling and the aquatic risk has been assessed in Table B.9.2.4 below.

Table B.9.2.2 Maximum fluopicolide and metabolites detected in lysimeter leachates

Leachate component[1]	µg/L	TER	Annex VI
Parent	1.69	17.15[2]	10
M01	6.69	>1495[3]	10
M02	0.10	29.0[4]	10
M03	n.d.	-	-
M05	0.90	1111[5]	10
M10	0.83	***3.5***[4]	10
M11	0.55	***5.3***[4]	10
M12	0.36	***8.1***[4]	10
M13	0.14	20.7[4]	10
M14	0.19	15.3[4]	10

n.d. not detected;
[1] see DAR B.8.2.3.3
[2] based on parent *N. pelliculosa* 72h EbC50 = 29 µg/L
[3] based on M01 *N. pelliculosa* 72h EbC50 = >10000 µg/L
[4] based on parent *N. pelliculosa* 72h EbC50 (x 0.1) = 2.9 µg/L
[5] based on M05 *N. pelliculosa* 72h EbC50 = >10000 µg/L

Table B.9.2.3 FOCUSgw PECs - *N. pelliculosa* Aquatic risk assessment

EXP 11074B use on vine (see DAR Table B.8.259)										
PEC_{gw} (80%ile)	**parent**		**M01**		**M02**		**M03**		**M05**	
Scenarios	**µg/L**	**TER[2]**	**µg/L**	**TER[3]**	**µg/L**	**TER**	**µg/L**	**TER[4]**	**µg/L**	**TER[5]**
Châteaudun	0.147	197	4.466	>2239	< 0.1	n.r.	< 0.1	n.r.	0.492	>20325
Hamburg[1]	< 0.1	n.r.	4.614	>2167	< 0.1	n.r.	**0.381**	***7.6***	0.515	>19417
Kremsmünster	< 0.1	n.r.	3.570	>2801	< 0.1	n.r.	< 0.1	n.r.	0.340	>29412
Piacenza	0.452	64	4.374	>2286	< 0.1	n.r.	< 0.1	n.r.	0.540	>18519
Porto[1]	< 0.1	n.r.	1.755	>5698	< 0.1	n.r.	< 0.1	n.r.	0.111	>90090
Sevilla	< 0.1	n.r.	3.016	>3316	< 0.1	n.r.	< 0.1	n.r.	0.168	>59524
Thiva	< 0.1	n.r.	4.131	>2421	< 0.1	n.r.	< 0.1	n.r.	0.343	>29155
	M10		**M11**		**M12**		**M13**		**M14**	
	µg/L	**TER[4]**	**µg/L**	**TER[4]**	**µg/L**	**TER[4]**	**µg/L**	**TER[4]**	**µg/L**	**TER**
Châteaudun	**0.306**	*9.5*	0.235	12.3	0.156	18.6	< 0.1	n.r.	< 0.1	n.r.
Hamburg[1]	**0.430**	***6.7***	**0.386**	***7.5***	0.258	11.2	0.160	18.1	< 0.1	n.r.
Kremsmünster	0.267	10.9	0.226	12.8	0.151	19.2	< 0.1	n.r.	< 0.1	n.r.
Piacenza	**0.316**	***9.2***	0.221	13.1	0.147	19.7	< 0.1	n.r.	< 0.1	n.r.
Porto[1]	0.125	23.2	0.187	15.5	0.124	23.4	< 0.1	n.r.	< 0.1	n.r.
Sevilla	0.148	19.6	0.221	13.1	0.147	19.7	< 0.1	n.r.	< 0.1	n.r.
Thiva	0.212	13.7	0.196	14.8	0.130	22.3	< 0.1	n.r.	< 0.1	n.r.
EXP 11120A use on potato (see DAR Table B.8.260)										
PEC_{gw} (80%ile)	**parent**		**M01**		**M02**		**M03**		**M05**	
Scenarios	**µg/L**	**TER[2]**	**µg/L**	**TER[3]**	**µg/L**	**TER**	**µg/L**	**TER[4]**	**µg/L**	**TER[5]**
Châteaudun	< 0.1	n.r.	1.223	>8177	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Hamburg[1]	< 0.1	n.r.	2.003	>4993	< 0.1	n.r.	< 0.1	n.r.	0.170	>58824
Jokioinen[1]	< 0.1	n.r.	1.331	>7513	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Kremsmünster	< 0.1	n.r.	1.224	>8170	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Okehampton[1]	< 0.1	n.r.	1.627	>6146	< 0.1	n.r.	< 0.1	n.r.	0.113	>88496
Piacenza	< 0.1	n.r.	1.526	>6553	< 0.1	n.r.	< 0.1	n.r.	0.166	>60241
Porto[1]	< 0.1	n.r.	0.303	>33003	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Sevilla	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Thiva	< 0.1	n.r.	0.559	>17889	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
	M10		**M11**		**M12**		**M13**		**M14**	
	µg/L	**TER[4]**	**µg/L**	**TER[4]**	**µg/L**	**TER[4]**	**µg/L**	**TER[4]**	**µg/L**	**TER**
Châteaudun	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Hamburg[1]	0.160	18.1	0.151	19.2	0.101	28.7	< 0.1	n.r.	< 0.1	n.r.
Jokioinen[1]	0.131	22.1	0.249	11.6	0.166	17.5	0.117	24.7	< 0.1	n.r.
Kremsmünster	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Okehampton[1]	< 0.1	n.r.	0.104	27.9	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Piacenza	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Porto[1]	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Sevilla	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Thiva	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.

n.r. not relevant; **bold / grey highlight** = scenarios TER<Annex VI threshold

[1] acidic soil modelling

[2] based on parent *N. pelliculosa* 72h EbC50 = 29 µg/L

[3] based on M01 *N. pelliculosa* 72h EbC50 = >10000 µg/L

[4] based on parent *N. pelliculosa* 72h EbC50 (x 0.1) = 2.9 µg/L

[5] based on M05 *N. pelliculosa* 72h EbC50 = >10000 µg/L

Table B.9.2.4 FOCUSgw PECs (see Addendum Section B.8.6.2)- *N. pelliculosa* Aquatic risk assessment

VINE	Highest 80th percentile concentrations (µg/l, *scenario*)		TER Based on *N. pelliculosa*	
	PEARL	**PELMO**	**PEARL**	**PELMO**
Parent[1]	0.147 (*P*)	0.519 (*P*)	197.2	55.9
M-01[2]	5.879 (*H*)	6.265 (*H*)	1701.0	1596.2
M-02	0.019 (*H*)	0.038 (*P*)	n.r.	n.r.
M-03[3]	**0.423 (*H*)**	**0.525** (*H*)	***6.9***	***5.5***
M-05[4]	0.672 (*H*)	0.715 (*H*)	14881.0	13986.0
M-10[3]	**0.444 (*H*)**	**0.586 (*H*)**	***6.5***	*4.9*
M-11[3]	**0.371 (*H*)**	**0.516 (*H*)**	***7.8***	***5.6***
M-12[3]	0.247 (*H*)	**0.344 (*H*)**	11.7	***8.4***
M-13[3]	0.181 (*H*)	0.216 (*H*)	16.0	13.4
M-14	0.032 (*H*)	0.033 (*H*)	n.r.	n.r.

POTATO	Highest 80th percentile concentrations (PEARL; µg/l, *scenario*)			TER Based on *N. pelliculosa*		
Application	**1 p.a.**	**1 in 2y**	**1 in 3y**	**1 p.a.**	**1 in 2y**	**1 in 3y**
Parent[1]	0.104 (*P*)	0.036 (*P*)	0.024 (*P*)	278.8	n.r.	n.r.
M-01[2]	6.628 (*H*)	3.153 (*H*)	2.10 (*H*)	1508.8	3171.6	4761.9
M-02	0.02 (*N*)	0.009 (*H*)	0.005 (*H/N*)	n.r.	n.r.	n.r.
M-03[3]	**0.386 (*H*)**	0.201 (*H*)	0.118 (*N*)	***7.5***	14.4	24.6
M-05[4]	0.697 (*H*)	0.315 (*H*)	0.210 (*H*)	14347.2	31746.0	47619.0
M-10[3]	**0.525 (*J*)**	0.242 (*H/J*)	0.158 (*H*)	***5.5***	12.0	18.4
M-11[3]	**0.669 (*J*)**	**0.333 (*J*)**	0.206 (*J*)	***4.3***	***8.7***	14.1
M-12[3]	**0.446 (*J*)**	0.222 (*J*)	0.137 (*J*)	***6.5***	13.1	21.2
M-13[3]	**0.312 (*J*)**	0.142 (*J*)	0.091 (*J*)	***9.3***	20.4	n.r.
M-14	0.033 (*H*)	0.015 (*H*)	0.01 (*H*)	n.r.	n.r.	n.r.

POTATO	Highest 80th percentile concentrations (PELMO; µg/l, *scenario*)			TER Based on *N. pelliculosa*		
Application	**1 p.a.**	**1 in 2y**	**1 in 3y**	**1 p.a.**	**1 in 2y**	**1 in 3y**
Parent[1]	0.212 (*P*)	0.076 (*P*)	0.041 (*P*)	136.8	n.r.	n.r.
M-01[2]	6.733 (*H*)	3.152 (*H*)	2.003 (*H*)	1485.2	3172.6	4992.5
M-02	0.018 (*P*)	0.006 (*H*)	0.004 (*H*)	n.r.	n.r.	n.r.

VINE	Highest 80th percentile concentrations (µg/l, *scenario*)			TER Based on *N. pelliculosa*		
M-03[3]	0.275 (*H*)	0.119 (*H*)	0.079 (*H*)	10.5	24.4	36.7
M-05[4]	0.592 (*H*)	0.271 (*H*)	0.170 (*H*)	16891.9	36900.4	58823.5
M-10[3]	**0.534 (*J*)**	0.243 (*H*)	0.160 (*H*)	***5.4***	11.9	18.1
M-11[3]	**0.813** (*J*)	**0.371 (*J*)**	0.249 (*J*)	***3.6***	***7.8***	11.6
M-12[3]	**0.542** (*J*)	0.247 (*J*)	0.166 (*J*)	***5.4***	11.7	17.5
M-13[3]	**0.369** (*J*)	0.177 (*J*)	0.117 (*J*)	***7.9***	16.4	24.8
M-14	0.027 (*H*)	0.012 (*H/P*)	0.008 (*H/P*)	n.r.	n.r.	n.r.

P = Piacenza, H= Hamburg, J= Jokioinen, N= Okehampton

n.r. not relevant; **bold / grey highlight** = scenarios TER<Annex VI threshold

[1] based on parent *N. pelliculosa* 72h EbC50 = 29 µg/L

[2] based on M01 *N. pelliculosa* 72h EbC50 = >10000 µg/L

[3] based on parent *N. pelliculosa* 72h EbC50 (x 0.1) = 2.9 µg/L

[4] based on M05 *N. pelliculosa* 72h EbC50 = >10000 µg/L

From TERs (Table B.9.2.2) based on available parent and metabolite *N. pelliculosa* toxicological end points (see DAR Table B.9.2.75) and, in the absence of data, the parent *N. pelliculosa* endpoint with a 10x safety factor (SANCO 3268/2001 rev. 4), a low aquatic risk was indicated for parent and metabolites, M01, M02, M05, M13 and M014, detected in lysimeter leachate. However, metabolites, M10, M11 and M12, gave TERs < Annex VI threshold using the surrogate parent endpoint, requiring further consideration.

It is considered that M10, M11 and M12 are not structurally related to fluopicolide and do not contain the biological toxophore (see pt. 5.12 below). They are derived from M02, and are structurally related to, M05 and for these metabolites *N. pelliculosa* is >100x less sensitive than fluopicolide, therefore the toxicity profile of M10, M11 and M12 will likely be closer to M02/M05 than parent and a risk assessment based these endpoints (with a 10x safety factor) would indicate low risk. Furthermore, theoretical FOCUS estimations predict lower GW contamination than that detected in lysimeter leachate by these metabolites in all scenarios (see below).

The GW risk assessment (Table B.9.2.3) was conducted using PECs derived from FOCUS groundwater modelling (DAR Tables B.8.259 & B.8.260) following proposed respective uses of EXP11074B and EXP 11120A on vine and potato at the proposed EU GAPs. A further aquatic risk assessment was undertaken (Table B.9.2.4) using refined PECgws derived from further environmental modelling (see Addendum 1, Section B.8.6.2).

Following EXP11074B use on vine (Tables B.9.2.3-4), PECgws for M02 and M14 from all modelled scenarios were <0.1µg/L and hence were not considered further. For parent, metabolites, M01, M05, M12 (PEARL) and M13, in scenarios where the PECgw >0.1µg/L, all TERs were > Annex VI threshold indicating low risk to aquatic organisms. In two scenarios M10 was the only metabolite with TERs (9.5 & 9.2) < Annex VI threshold (10) and in one scenario (Hamburg) metabolites M03, M10, M11 and M12 (PELMO) had respective TERs (7.6, 6.7, 7.5 & 8.4) < Annex VI threshold.

Following EXP11120A use on potato (Table B.9.2.3), PECgws for parent (fluopicolide), M02, M03 and M14 from all scenarios were <0.1µg/L and hence were not considered further. For metabolites, M01, M05, M10-M13, in scenarios where the PECgw >0.1µg/L, all TERs were > Annex VI threshold indicating low risk to aquatic organisms. However, further PECgw modelling refinement (Table B.9.2.4) gave TERs <10 for M03 (PEARL - Hamburg), M10, M11, M12 & M13 in PEARL and PELMO GW modelling in Hamburg and Jokioinen scenarios based on one treatment regime per annum. From biennial treatment only TERs for M11 (PEARL and PELMO - Jokioinen) were < Annex VI threshold and following triennial treatment no TERs were < Annex VI threshold.

However, it should be noted that the risk is assessed presuming aquatic organisms will be exposed at the groundwater PECs, whereas it is reasonable to assume that at least a 10x dilution would likely occur (SANCO 3268/2001). For vine application a correction for 60% canopy interception would also have further reduced potential exposure (see DAR B.8.2.3.3). It should also be noted that M10 and M11 are chemically structurally related to M05 and M11 is purported to be an isomer of M12

(Appendix 2), hence these metabolites would likely exhibit similar aquatic toxicity, i.e. approximately 300x less toxicity than parent. M03 is structurally closely related to parent (Appendix 2) and was not seen in lysimeter leachate and only detected in two PECgw scenarios; it is unstable in water at most environmental pH and therefore negligible exposure via groundwater is expected.

Therefore, overall the weight of evidence indicates a low risk to aquatic organisms from predicted exposure to fluopicolide and principal metabolites occurring in groundwater following proposed uses of EXP 11074B and EXP 11120A on vine and potato.

Evaluation Table Open pt. 5.12

'RMS to present the complete assessment for the relevance of ground water metabolites in and addendum. Special attention should be paid to the fact that at this stage for metabolites M01, M05 and M10 the threshold of 0.75 μg/L is also exceeded either in the lysimeter or the FOCUS modelling.'

(See also Section B.6.1.4.1, Addendum 1 (Nov 2007) for full Relevance Assessment of Groundwater Metabolites).

5.12 RMS response:
Environmental relevance of GW metabolites

Formation of metabolites

Fluopicolide is a pyridinyl-benzamide fungicide (see Appendix 2). In soil the proposed fluopicolide degradation is initiated via cleavage at the amide bridge to a pyridinyl (M02) and a benzyl (M01) derivative after formation of transient hydroxylated fluopicolide intermediate (M03). M01 is relatively stable before undergoing mineralization but M02 undergoes further transformation. M02 can be sulphated by substitution of the chlorine group at the 3' position on the pyridine ring forming M05 and M10. Further ring hydroxylation of M02, M05 and M10 can also occur forming M13, M11/M12 (isomers) and M14 derivatives (Appendix 2). Parent and metabolites, M01, M02, M05, M10, M11, M12, M13 and M14, were identified in lysimeter leachate at an annual average >0.1μg/L (Table B.9.2.2) whereas parent and metabolites, M01, M03 (2 scenarios only), M05, M10, M11, M12 and M13, were predicted to occur at an annual average >0.1μg/L in some groundwater scenarios by FOCUS modelling (Table B.9.2.3); hence consideration of overall environmental relevance is required (SANCO 221/2000 rev.10).

Biological activity

Initial efficacy active substance screening and numerous tests on vegetative vigour and seedling emergence indicated that fluopicolide has no significant herbicidal activity (see DAR B.9.9.1.1). In laboratory screening (see DAR B.9.9.4) fluopicolide also did not exhibit insecticidal activity. Furthermore, in screens on 5 soil fungal species of different classes fluopicolide fungicidal sensitivity was specific to only one species, Phytophora (oomycetes) [Lechelt-Kunze, 2003e-m]. In tests on fluopicolide-sensitive fungi, grape downy mildew (*Plasmopara viticola*) and potato late blight (*Phytophthora*

infestans), fluopicolide metabolites M01, M02, M05, M10, M14 and M15 were all shown to be <<50% active compared with parent [Lechelt-Kunze, 2003e-m, Latorse & Flahout, 2004]. The fact that M01and M02, benzyl and pyridinyl derivatives formed from fluopicolide cleavage at the amide bridge (and their derivatives M05, M10 and M14) all retain no fungicidal activity is strongly indicative that the fluopicolide biological activity toxophore comprises of the intact pyridinyl-benzamide molecule.

Untested GW pyridinyl metabolites M11 and M12 (isomers), tentatively identified as hydroxylated derivatives of M10, and M13, a hydroxylated derivative of M02, are structurally similar and hence do not contain the toxophore and will not retain biological activity. M03 is a structurally-related transient hydroxylated-derivative of fluopicolide and is an unstable intermediate prior to cleavage of fluopicolide to M01 and M02. It is very unstable in water and at environmental pH will rapidly degrade to M01 and M02 and the RMS considers it inconceivable that significant exposure to M03 will occur via GW. Thus the RMS concludes that all metabolites theoretically occurring in GW >0.1µg/L will not retain or express biological activity of the parent, fluopicolide.

Other GW metabolite ecotoxicological testing

All GW metabolites were considered to be irrelevant in terms of mammalian risk (see DAR B.6.1.4 and B.6.80, Addendum 1, B.6.1.4.1) and M01 was considered of low ecotoxicological risk to mammals (B.9.1, DAR B.9.3). M01 was formed in the hen metabolism study (DAR B.7.2.2) indicating that fluopicolide avian toxicity test encompass M01 effects, and, on a molar basis, M01 was not more acutely toxic to birds and low avian risk from M01 was also indicated (DAR B.9.1). In aquatic tests M01, M02 and M05 were at least 10x < toxic than fluopicolide which included the most sensitive species, *N. Pelliculosa* (DAR Table B.9.2.75). M10, M11, M12 and M13 are GW metabolites not tested on aquatic species, are structurally similar to M02 and M05, which were significantly less toxic than fluopicolide when tested on most sensitive fish and algae species (DAR Table B.9.2.75). Low aquatic risk was concluded for all fluopicolide GW metabolites (B.9.2.1.2, DAR B.9.2). Furthermore, M01, M02 and M03 were not more toxic to worms than fluopicolide and constituted less overall risk (DAR B.9.6). Folsomia, soil microbes, soil fungi and litter decomposition, non-target plants were not more sensitive to M01 than fluopicolide and hence low ecotoxicological terrestrial risk was indicated (DAR B.9.7-9). None of the GW metabolites is predicted to have bioconcentration/bioaccumulation potential. Thus overall fluopicolide metabolites were considered unlikely to express significant ecotoxicological activity and the RMS considers that the weight of evidence suggests that GW metabolites can be regarded as not ecotoxicologically relevant.

Conclusion

From an ecotoxicological viewpoint, sufficient evidence is considered available to support the contention that all metabolites identified in groundwater at an average annual concentration >0.1µg/L can be considered environmentally 'non-relevant'.

B.9.3 Non-target vertebrates - mammals (see B.9.1 above)

B.9.4 Bees - no Open pts. to address.

B.9.5 Non Target Arthropods

One issue **(Evaluation Table Open point 5.6)** was raised pertinent to the risk to NTAs from proposed uses of fluopicolide in potato and vine crops:

Evaluation table Open pt. 5.6
RMS to correct the list of endpoint with exact %-age effect on fecundity instead of <50%. Note that highest conc. with effects <50% for A. rhopalosiphi was 2 L/ha.

5.6 RMS response
For clarity the results from NTA fluopicolide data are re-presented in Table B.9.5.1 below.

Table B.9.5.1 Summary of results from fluopicolide testing on NTAs.

SPECIES	TEST	MORTALITY LR50	FECUNDITY	DAR SECTION
Fluopicolide - applied as 'AE C638206 SC 480 A2' (487 g fluopicolide/L)				
		mL product/ha [CL 95%] (fluopicolide g/ha)	**mL product/ha (fluopicolide g/ha) [% control]**	
Aphidius rhopalosiphi	Laboratory (glass plate)	>861 [n.c.] (>419)	861 (419) [-15.7]	B.9.5.1.1 i)
Typhlodromus pyri	Laboratory (glass plate)	642 [591 - 698] (312)	574 (279) [-3.5]	B9.5.1.1 ii)
'EXP 11074B'containing fluopicolide (45.1g/kg) + fosetyl Al (671g/kg)				
		Kg product/ha [CL 95%] (fluopicolide g/ha)	**Kg product/ha (fluopicolide g/ha) [% control]**	
Aphidius rhopalosiphi	Laboratory (glass plate)	8.23 [7.81 - 8.67] (371) in/off-field HQ = 0.84/0.06	4.6 (207) [-44.1] 6.9 (311) [-66.4]	B.9.5.1.2a i)
Typhlodromus pyri	Laboratory (glass plate)	7.13 [6.62 - 7.67] (322) in/off-field HQ = 0.97/0.07	4.6 (207) [-23.9] 6.9 (311) [-19.9]	B.9.5.1.2a ii)
'EXP 11120A' containing fluopicolide (64.7 g/L) + propamocarb HCl (634 g/L)				
		L product/ha [CL 95%] (fluopicolide g/ha)	**L product/ha (fluopicolide g/ha) [% control]**	
Aphidius rhopalosiphi	Laboratory (glass plate)	2.48 [1.76 - 3.76] (161) in/off-field HQ = 1.74/0.03	0.43 (27.8) [-46.8] 0.81 (52.4) [-72.7] 2.92 (188.9)[-89.6]	B.9.5.1.2b i)
Typhlodromus pyri	Laboratory (glass plate)	3.24 [2.69 - 4.14] (210) in/off-field HQ = 1.33/0.03	0.4 (25.9) [-7.7] 0.72 (46.6) [-19.7] 1.29 (83.5) [-41.0] 2.32 (150.1) [-49.6] 4.17 (269.8) [-86.3]	B.9.5.1.2b iii)
Aphidius rhopalosiphi	Ext. lab. (leaf)	>8.0 [n.c.] (>518)	1.0 (64.7) [-7.6] 2.0 (129.4) [-20.3] 4.0 (258.8) [-50.0] 8.0 (517.6) [-98.7]	B.9.5.1.2b ii)
Typhlodromus pyri	Ext. lab. (leaf)	>4.17 [n.c.] (>270)	0.4 (25.9) [-12.9] 0.72 (46.6) [-17.9] 1.29 (83.5) [-27.6] 2.32 (150.1) [-29.8] 4.17 (269.8) [-34.3]	B.9.5.1.2b iv)
Chrysoperla carnea	Laboratory (glass plate)	>6.4 [n.c.] (>414)	6.4 (414.1) [-2.7]	B.9.5.1.2b v)

n.c. not calculable

Under extended laboratory (leaf) conditions *Aphidius rhopalosiphi* fecundity was most sensitive with 50 and 20% inhibition seen at 4.0 and 2.0L EXP 11120A/ha dosing, respectively, thus a <50% fecundity inhibition would be expected at >2x the proposed maximum individual application rate. However, all in-field and off-field HQ values, which take account of multiple applications, for both potato and vine uses (DAR 9.5.2) were below Annex VI thresholds indicating low NTA risk.

B.9.6-8 Effects on soil organisms

Four issues **(Evaluation Table Open points 5.7, 5.8, 5.9, 5.10)** were raised pertinent to the risk to soil organisms from proposed uses of fluopicolide in potato and vine crops.

B.9.6 Earthworm

Evaluation Table Open pt. 5.7

'RMS to update the list of endpoints for earthworms. It is still not clear if the values for the formulation are based on a.s. or formulation concentrations. Furthermore, values should be given as mg/kg DS.'

5.7 RMS response:

For clarity, the revised earthworm endpoints and risk assessment are presented in Table B.9.6.1 (DAR B.9.6.3.1 and B.9.6.3.2).

Table B.9.6.1 Summary of acute and chronic toxicity end points, PEC_{soil} values and TERs for earthworms from EXP 11074B use on vine

APPLICATION Test Substance	Toxic end point mg/kg DS (corrected)	Max. PEC_{soil} (mg/kg DS)	Toxicity exposure ratio	Annex VI threshold
EXP 11074B on vine				
Acute	**14dLC50**		**TER_a**	
Fluopicolide (log Pow = 2.9)	>1000 (>500[2])	0.268[1]	>1866	10
M-01 (log Pow = 0.51)	750	0.043[1]	17442	10
M-02 (logPow = -2.0)	>1000	0.026[1]	>19230	10
M-03 (logPow = 2.34)	>1000 (>500[2])	0.017[1]	>29412	10
EXP 11074B (fluopicolide)	>43.5 (>21.75[2])	0.268[1]	>81.1	
Chronic	**28/56dNOEC**		**TER_{lt}**	
Fluopicolide	62.5[3,5]	0.268[1]	233	5
M-01	250[4]	0.046[1]	5435	5
EXP 11074B (fluopicolide)	2.435[5]	0.268[1]	9.1	5
EXP 11120A on potato				
Acute	**14dLC50**		**TER_a**	
Fluopicolide (log Pow = 2.9)	>1000 (>500[2])	0.2016[1]	>2480	10
M-01 (log Pow = 0.51)	750	0.0174[1]	43103	10
M-02 (logPow = -2.0)	>1000	0.019[1]	>26316	10
M-03 (logPow = 2.34)	>1000 (>500[2])	0.013[1]	>38462	10
EXP 11120A (fluopicolide)	>57.3 (>28.65[2])	0.2016[1]	>142	10
Chronic	**28/56dNOEC**		**TER_{lt}**	
Fluopicolide	62.5[3,5]	0.2016[1]	233	5
M-01	250[4]	0.0174[1]	5435	5
EXP 11120A (fluopicolide)	2.587[5]	0.2016[1]	12.8	5

DS = dry soil
[1] peak accumulated 5cm depth (see DAR Table B.8.198)
[2] value reduced by a factor of 2 (logKow>2/10% soil OM)
[3] based on growth (28d)
[4] based on reproduction
[5] conducted in 5% soil OM (correction not required)

For Table B.9.6.1 correction (x0.5) was required for fluopicolide, M03 and product acute endpoints as log Pow > 2 and 10% soil OM was used, in chronic studies 5% soil OM was used and no correction was required. Revised TERs indicate low acute and chronic risk to earthworms from fluopicolide and principal soil metabolites, M01, M02 and M03, following application of EXP 11074B and EXP 11120A to vine and potato.

B.9.7 Other soil non-target macro-organisms

Evaluation Table Open pts. 5.8 & 5.9

'Pending on the discussion on the PECsoil in the section on Fate and behaviour, a revision of the risk assessment for soil organisms might be necessary.'

(Reporting Table comment at 5(45) and 5(47)

5.8 & 5.9 RMS response:

RMS will address, as appropriate, if evaluation/discussion of Applicant's response by Environmental Fate results in PECsoil amendment.

B.9.8 Non-target soil microorganisms

Evaluation Table Open pt. 5.10

'RMS to include the argumentation for why no studies with soil micro-organisms are required with M 03 in an addendum for the sake of completeness. No discussion in an expert meeting is required.'

5.10 RMS response:

OECD 216/217 guidance for soil microbial activity recommends tests to be undertaken at soil pH 5.5 - 7.5. At these pHs M03 has a DT50 <1.0d and in acidic soils pH5.0 - 5.5 M03 has a DT50 of <5d (DAR B.8.1.8). Therefore rapid decay would be expected in these soils and any resulting toxicity mostly expressed via M01 and M02 derivatives of M03. Furthermore, it is likely that soil microorganisms could be exposed transiently to M03 in fluopicolide and product soil microorganism studies which were all conducted at soil pH5.4 - 5.9 over 28d where no effects were reported at up to 10x proposed application rate (DAR B.9.8.1). M03 has a very similar chemical structure to fluopicolide (Appendix 2) and significantly increased toxicity would not be anticipated. Moreover, no effects of M03 on earthworm at 1000 mg/kg DS (pH 5.7-6.0) over 14d were reported and TERs for acute (14d) and long term fluopicolide effects > Annex VI (soil pH 6 -7) over 56d, where some transient M03 formation may be expected. Fluopicolide also did not affect straw litter bag decomposition in soil (pH 6.72) over 184d again where some transient exposure to M03 might be assumed. Where tested M01 and M02, both M03 soil degradation products, also had no significant impact on soil organisms and processes. Therefore overall the RMS considered that there was sufficient weight of evidence to suggest that M03 would not have a significant effect on soil organisms and processes in the absence of a specific soil microbial M03 study.

B.9.9 Non-target flora

One issue **(Evaluation Table Open point 5.11)** was raised pertinent to the risk to off-field non-target plants from proposed uses of fluopicolide in potato and vine crops:

Evaluation Table Open pt. 5.11

'RMS to include the argumentation regarding risk to non-target plants from exposure to M 01 in an addendum for the sake of completeness. No discussion in an expert meeting is required.'

5.11 RMS response:

For non-target plants off-field risk is only considered and contamination will result primarily from spray drift. M01 is a soil metabolite and not present in spray applications. Hence, pre-emergent effects on non-target plants following M01 formation in off-field soil contaminated with fluopicolide by spray drift are most relevant.

The pre-emergent M01 non-target plant study revealed no effects >50% on seedling germination and growth at rates ≤ 0.0121 mg/kg soil and an ER50 of >0.0121 mg M01/kg DS (5 cm) was established. From theoretical in-field PECsoils (Table B.8.198) for fluopicolide and M01 and spray drift values (6.9% for vine; 1.9% for potato), max. M01 off-field PECsoils of 0.00196 and 0.00039 mg/kg (5cm) can be derived for vine and potato use, respectively. Thus TERs of >6 and >31 can be established for M01 off-field pre-emergent effects on non-target plants indicating low risk. This is considered to be a worse case scenario as the ER50 is > highest dose tested and no off-field interception of spray drift deposition is assumed.

B.9.13 Additional References Relied On:

Annex Point/ Location in Dossier	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Data protect. claimed	Owner
Doc Kb Position Papers	Radix,P; Payraudeau, V.	2006	Fluopicolide (AE C638206) - Position paper regarding the endpoint used for the ecotoxicological long term risk assessment for mammals Bayer CropScience AG, Edition No.: M-268483-01-1, Date: 2006-03-23 Non GLP, unpublished	Yes	BCS

B.9 ECOTOXICOLOGY - CORRIGENDUM

Please note the following correction to the original DAR:

i) Tables B.9.2.60 and B.9.2.63 need amendment (2nd a.s. is propamocarbHCl not fosetyl-Al as reported)

ii) Tables B.9.5.1-4; B.9.9.3-4. 'SC 40' should be 'SC480'

iii) B.9.2.2.1 S phrases (and Vol 1) should be amended to 'S60 This material and its container must be disposed of as hazardous waste' and 'S61 Avoid release to the environment. Refer to special instructions/safety data sheets'
Justification 'Recommended for substances that may cause effects in the environment'.

iv) Tables B.9.5.10, 9.5.12 'kg/ha' should be 'L/ha'

v) B.9.5.1.2b iii) 2nd para '2.04-10.35 kg/ha' should be '0.4-4.7 L/ha)

vi) B.9.7.3.2/9.8.3.2 '10cm' should be '5 and 10cm'

vii) B.9.7.3.1/9.8.3.1 '10cm' should be '5cm'

viii) Table B.9.9.15 M01 '0.046' should be '0.043'.

ix) Table B.9.2.77 Heading 'AE F05361606 WG71 A1' should be 'EXP 11074B'

APPENDIX 1

Summary of the significant metabolites of fluopicolide identified in studies in animals, plants and the environment

M-Code number (Company code number)	Other identifiers	Structure	Formula	Presence in metabolism studies
AE C638206	Fluopicolide (parent)	F_3C, Cl, Cl, NH, N, O, Cl	2,6-dichloro-N-{[3-chloro-5-(trifluoromethyl)-2-pyridinyl]methyl}benzamide $C_{14}H_8Cl_3F_3N_2O$ MW = 383.59	
M-01 (AEC653711)	BAM	Cl, H_2N, O, Cl	2,6-dichlorobenzamide $C_7H_5Cl_2NO$ MW = 190.0	rat liver, laying hen, crop, soil, lysimeter leachate, rotational crop
M-02 (AEC657188)	PCA UMET/2	F_3C, Cl, N, OH, O	3-chloro-5-trifluoromethyl-pyridine-2-carboxylic acid $C_7H_3ClF_3NO_2$ MW = 225.6	rat, crop, rotational crop, soil, water
M-05 (AE 1344122)	P1x RPA433497	F_3C, $SOCH_3$, N, COOH	3-methylsulfinyl-5-trifluoro-methylpyridine-2-carboxylic acid $C_8H_6F_3NO_3S$ MW = 253	rotational crop, lysimeter leachate,
M-04 (AEC657378)	3-hydroxy BAM	Cl, H_2N, OH, O, Cl	2,6-dichloro-3-hydroxy-benzamide $C_7H_5Cl_2NO_2$ MW = 206	rotational crop rat (BAM ADME study)

List of metabolites continued

Company code number	Other identifiers	Structure	Formula	Presence in metabolism studies
M-06 (AEC643890)	3-OH 206 MET IV MET.F/16 FMET/38 UMET/51 FMET/8 UMET/44 UMET/53 FMET/33		2,6-dichloro-N-[(3-chloro-5-trifluoromethylpyridin-2-yl) methyl]-3-hydroxybenzamide $C_{14}H_8Cl_3F_3N_2O_2$ MW = 399	laying hen, lactating cow crop, confined rotational crop, rat
M-07 (AE 0712556)	4-OH 206 UMET/54 UMET/26		2,6-dichloro-N-[(3-chloro-5-trifluoromethylpyridin-2-yl) methyl]-4-hydroxybenzamide $C_{14}H_8Cl_3F_3N_2O_2$ MW = 399	laying hen, lactating cow rat
M-08 (AEC653598)			3-chloro-5-trifluoromethyl pyridine-2-carboxamide $C_7H_4ClF_3N_2O$ MW = 224.57	confined rotational crop
M-09 (AE B102859)			3-chloro-2-hydroxy-5-trifluoromethylpyridine $C_6H_3ClF_3NO$ MW = 197.54	confined rotational crop
M-03 (AE060800)	RPA427967		4-N-[3-chloro-5-trifluoro-methylpyridin-2-yl) (hydroxyl)methyl]-2,6-dichlorobenzamide $C_{14}H_8Cl_3F_3N_2O_2$ MW = 399.58	soil
M-10 (AE 1344123)	P4 RPA433965		3-sulfo-5-trifluoromethyl pyridine-2-carboxylic acid $C_7H_4F_3NO_5S$ MW = 271.17	lysimeter leachate, soil (PCA soil degradation study)

List of metabolites continued

Company code number	Other identifiers	Structure	Formula	Presence in metabolism studies
M-11 M-12	P2 Mixture of 2 isomers (P2a and P2b)	F_3C, SO_3H, HO, N, COOH	isomers x-hydroxy -y-sulfo-5-trifluoromethylpyridine-2-carboxylic acid $C_7H_4F_3NO_6S$ MW = 287.17	lysimeter leachate, soil (PCA soil degradation study)
M-13	P3	F_3C, Cl, HO, N, COOH proposal not confirmed	$C_7H_3ClF_3NO_3$ MW = 241.3	lysimeter leachate,
M-14 (AE 1388273)	P7 RPA433986	F_3C, SO_2CH_3, N, OH	3-mesyl-5-(trifluoromethyl) pyridin-2-ol $C_7H_6F_3NO_3S$ MW = 241.19	lysimeter leachate, soil (PCA soil degradation study)
M-15 (AE 1413903)	P8	F_3C, Cl, Cl, SO_3H, H, N, N, O, Cl	3,5-dichloro-4-[3-chloro-5-trifluoromethylpyridine-2-yl-methyl) carbamoyl]benzene sulfonic acid $C_{14}H_8Cl_3F_3N_2O_4S$ MW = 463.65	lysimeter leachate,
M-16	P9 UMET/40 FMET/23	F_3C, Cl, Cl, H, N, N, OH, O, SO_3H	3-chloro-2-[({3-chloro-5-trifluoromethylpyridine-2-ylmethyl}amino) carbonyl]-6-hydroxybenzene sulfonic acid $C_{14}H_9Cl_2F_3N_2O_5S$ MW = 444	lysimeter leachate, rat
M-17	Metabolite 1	F_3, Cl, Cl, H, N, N, O, Cl, S, O, O, CH_3	2,6-dichloro-N-{[3-chloro-5-(trifluoromethyl)pyridin-2-yl]methyl}-3-(methylsulfonyl)benzamide $C_{15}H_{10}Cl_3F_3N_2O_3S$ MW = 462	laying hen

List of metabolites continued

Company code number	Other identifiers	Structure	Formula	Presence in metabolism studies
M-18	HS (hydroxy sulphate of fluopicolide) UMET/45 UMET/47	or	2,4-dichloro-3-[({[3-chloro-5-(trifluoromethyl)pyridin-2-yl]methyl}amino)carbonyl] phenyl hydrogen sulfate or 3,5-dichloro-4-[({[3-chloro-5-(trifluoromethyl)pyridin-2-yl]methyl}amino)carbonyl] phenyl hydrogen sulfate $C_{14}H_7Cl_3F_3N_2O_5S$ MW = 477	laying hen lactating cow rat
M-19	DHS (dihydroxy sulphate of fluopicolide) UMET/23 UMET/39 UMET/46 UMET/49		3,5-dichloro-4-[({[3-chloro-5-(trifluoromethyl)pyridin-2-yl]methyl}amino)carbonyl] hydroxyphenyl hydrogen sulfate $C_{14}H_7Cl_3F_3N_2O_6S$ MW = 493	laying hen lactating cow rat

APPENDIX 2

Fluopicolide soil degradation pathway proposed by Applicant

fluopicolide MOO

MO3

MO2

MO1

M13

M1O

MO5

M11/M12

M14

APPENDIX 3

[NB. Section B.10 is the UK Efficacy assessment which is not included in the EU DAR. The following section relates to the biologically activity assessment and is presented for completeness in response to the Reporting Table point 2(25). It has not been updated, therefore for a full assessment of the relevance of groundwater metabolites - please see Section B.6.1.4.1, Addendum 1 (November 2007)].

B.10.7.5 Effects of Metabolites in Ground Water

The applicant identified the potential metabolites in groundwater as M-01, M-02, M05, M10, M14 and M-15. Evidence had been provided from the initial screening data to indicate that fluopicolide has no significant insecticidal or herbicidal activity (B10.7.1). The applicant therefore tested these metabolites for fungicidal activity only. Reference was made to fate and behaviour metabolite studies submitted under Annex II.

(Latorse & Flahaut 2004)

In vitro tests for powdery mildew and late blight showed no activity for any of these metabolites tested at 100 mg/l (equivalent to 100 g/ha). This included a range of doses for the major metabolite, AE C653711. These results were summarised further in a position paper on the non-relevance of metabolites found in lysimeter leachate and field leaching studies. The paper argued that the data from the biological screens indicated that both the pyridine and phenyl rings of the molecule are required for fungicidal activity. Any metabolites without both these rings would be predicted to have no fungicidal activity. It was also noted that functional groups, especially polar ones to the phenyl ring causes loss of fungicidal activity.

(Leake & Payraudeau 2004a)

The position paper also summarised further studies with M-01 against five species of fungi. These showed M-01 gave no inhibition of growth at rates between 0.3 and 30 mg/kg dry soil.

(Lechelt –Kunze 2003k, 2003f, 2003m)

Further supporting evidence of the lack of activity of AE C653711 was referenced from a Tier II seedling emergence and vigour study showing no effects on ten different non-target plants.

(Pallett & Gosch, 2004)

Further evidence of lack of insecticidal activity was provided in the Ecotoxicology studies with no effect against Collembola.

(Klein & Luhrs 2003a)

Assessment

Various biological screening data confirmed that neither fluopicolide nor its metabolites have herbicidal or insecticidal activity. Metabolites were therefore investigated for any fungicidal effects, and the studies indicate that none of those tested had any significant biological activity. The rapporteur differed in their assessment of which metabolites have the potential to occur in groundwater above the 0.1 µg/L level, specifying M-01, M-05 and M-10 to M-14 (see B8.6.2). Of these only M11 and M12 (mixture of 2 isomers) and M13 were not tested for fungicidal activity. These three are all single pyridine ring structures and are unlikely to have any significant fungicidal activity.

APPENDIX 4

Bayer CropScience
Regulatory Toxicology

Subject: FLUOPICOLIDE

CONTENTS

Evaluation of the oral bioavailability of Fluopicolide in the rat.

Author:

Philip Fisher, B.Sc., Ph.D.
Human Risk Assessment
Regulatory Toxicology Department
Sophia Antipolis Research Centre
Bayer CropScience
BP 153
F-06903 Sophia Antipolis Cedex - FRANCE

Date: 10th April 2007

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Evaluation of the oral bioavailability of Fluopicolide in the rat.

Introduction

Toxicokinetic studies on the absorption, distribution, metabolism and excretion of Fluopicolide (AE C638206) have been investigated in the Sprague Dawley CD rat. These studies were performed using two different ^{14}C radiolabel sites as shown below:

* = pyridyl label sites, § = uniformly labelled phenyl ring.

During the preparation of the DAR the Rapporteur Member State determined that the Admissible Operator Exposure Level (**AOEL**) for fluopicolide was **0.05 mg/kg bw/day** being derived from the modified NOAEL of 8.4 mg/kg bw/day from the 90-day dietary study in rats and allows for a 100-fold safety margin to account for interspecies and intraspecies differences. The RMS stated that:

"*A correction factor of 0.62 was allowed to account for the extent of oral absorption which is based on that determined for the pyridyl radiolabel in the biliary excretion study. The basis for lower oral absorption estimate using the pyridyl radiolabel (62%), rather than the phenyl radiolabel (80%) is unclear and hence the more conservative estimate has been relied upon for the derivation of the AOEL.*"

In the reporting table comments for comment N°s 2(8) and 2(15) referring to Volume 3 sections B.6.10 and B.6.10.3 respectively, the notifier supported the value initially proposed in the tier 2 summary of 74%.

The RMS replied with the following explanation:

"*The main route of elimination of radiolabel is in faeces. The critical point is the difference in biliary excretion levels between pyridyl and phenyl radiolabel and the biological reasons for such a difference. For the biliary studies, recovery of radiolabel was excellent, approximately 100% so justification for attempting to use another study in which biliary study is unknown is necessary……. There is also no basis to find an average of pyridyl and phenyl radiolabel unless there is biological justification.*

This position paper presents a case that suggests that the oral bioavailability of fluopicolide in the rat is higher than 62% and uses the data obtained from the toxicokinetic database generated by the notifier, including ADE, PK and metabolism end points, to support this proposition and present a biological justification.

Results

The results presented in Tables 1 and 3 have been normalised to 100% (the corresponding Tables with the actual experimental values are presented in Appendix 1 in the same order). The determination of the oral absorption level is generally obtained from ADE studies using bile duct cannulated rats. This model permits the determination of the proportion of the dose that was absorbed and subsequently eliminated *via* the bile. The proportion of the administered radioactivity that is found in the bile, urine (plus cage rinse) and tissues (excluding intestinal contents) is summed to provide the oral absorption value in terms of percentage of dose administered. For fluopicolide two such studies (refs 1 & 2) were performed using either the [Phenyl-U-^{14}C] or the [Pyridyl-2,6-^{14}C] radiolabels. Table 1 presents the results from the single oral low dose groups from these studies.

Table 1: Recovery of Radioactivity from Bile Duct Cannulated Rats following Single Oral Administration of [phenyl-U-^{14}C]-fluopicolide and [Pyridyl-2,6-^{14}C]-fluopicolide at the rate of 10 mg/kg bw

Normalised results expressed in terms of % of administered dose.

Sample	Males				Females			
	Pyridyl label		Phenyl label		Pyridyl label		Phenyl label	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Urine	5.84	2.41	4.58	4.24	10.11	4.94	6.61	1.96
Faeces	40.56	6.78	21.62	7.04	38.15	6.16	18.90	6.88
Bile	52.09	9.52	71.12	8.63	49.72	10.11	72.26	6.95
Cage wash	0.70	0.37	0.76	0.78	1.53	1.88	0.89	0.58
Tissues[a]	0.80	0.38	1.93	0.34	0.50	0.13	1.35	0.12
Total Absorbed[b]	59.44	6.78	78.38	7.04	61.85	6.16	81.10	6.88
Total	100.00		100.00		100.00		100.00	

SD = standard deviation (n = 4)
[a] : excluding intestinal and stomach content
[b] : sum of radioactivity in urine, cage rinse, bile and tissues.

Comparison of these recovery normalised results from these studies reveals that the principal differences between the two radiolabels appear in the levels observed in the bile and the faeces with the urinary levels being similar. Overall the mean total absorbed for the pyridyl radiolabel was 61 ± 6% and that for the phenyl radiolabel was 80 ± 7%.

Another measure of systemic exposure can be obtained from the blood and plasma pharmacokinetic data. Studies were, again, performed using both radiolabels following single oral low doses of 10 mg/kg bw (ref 3). A summary of these parameters is provided in Table 2.

Table 2: Summary of the Calculated Pharmacokinetic Parameters following Single Oral Administration of either [Pyridyl-2,6-^{14}C]-fluopicolide or [Phenyl-U-^{14}C]- fluopicolide at the rate of 10 mg/kg b/w.

WHOLE BLOOD		C_{max} (µg equiv./g)	T_{max} (hours)	$t_{0.5}$ (hours)	$AUC_{(0-168h)}$ (µg.h/g)	$AUC_{(0-inf)}$ (µg.h/g)
Phenyl	Males	1.50 ± 0.24	7.5 ±1	56.63 ±1.61	48.04 ± 8.35	51.65 ± 8.66
	Females	1.19 ± 0.44	5.5 ± 2.52	120.67 ± 26.18	52.87 ± 8.16	73.54 ± 12.62
Pyridyl	Males	1.49 ± 0.51	7.0 ± 1.16	80.34 ± 14.32	40.59 ± 5.18	45.37 ± 15.16
	Females	1.18 ± 0.26	6.0 ± 1.63	140.32 ± 25.38	45.22 ± 4.78	67.72 ± 12.92
PLASMA		C_{max} (µg equiv./g)	T_{max} (hours)	$t_{0.5}$ (hours)	$AUC_{(0-168h)}$ (µg.h/g)	$AUC_{(0-inf)}$ (µg.h/g)
Phenyl	Males	2.20 ± 0.39	8.0 ± 0	18.85 ± 1.49	54.24 ± 10.85	55.22 ± 11.36
	Females	1.61 ± 0.67	6.5 ± 3	19.72 ± 6.21	38.88 ± 9.84	40.28 ± 8.97
Pyridyl	Males	2.14 ± 0.62	7.0 ± 1.16	14.44 ± 2.62	48.39 ± 20.34	48.93 ± 20.32
	Females	1.59 ± 0.23	6.5 ± 1.92	12.67 ± 2.76	30.61 ± 5.11	30.96 ± 5.17

C_{max} = maximal concentration, T_{max} = time of maximal concentration, $t_{0.5}$ = terminal elimination half-life, AUC = area under curve.

The estimation of the area under the curve from the whole blood results ($AUC_{(0-168h)}$) indicated a similar level of systemic exposure for the males (48.04 ± 8.35 µg.hour/g for the phenyl label, and 40.59 ± 5.18 µg.hour/g for the pyridyl label) and females (52.87 ± 8.16 µg.hour/g for the phenyl label, and 45.22 ± 4.78 µg.hour/g for the pyridyl label) with no significant differences between the labels.

The estimation of the area under the curve from the plasma results indicated a similar systemic exposure for the males (54.24 ± 10.85 µg.hour/g and 48.39 ± 20.34 µg.hour/g for phenyl and pyridyl label respectively) and females (38.88 ± 9.84 µg.hour/g and 30.61 ± 5.11 µg.hour/g for phenyl and pyridyl label respectively).

As stated in the DAR, these data demonstrate that, following a single oral dose using either [phenyl-U-^{14}C]-fluopicolide or [pyridyl-2,6-^{14}C]-fluopicolide the general pharmacokinetic profiles were similar between both the radiolabels and the sexes. Radiolabelled fluopicolide was absorbed moderately rapidly with mean maximal concentrations being achieved between 7 and 10 hours post dose followed by a moderately rapid elimination such that the majority was eliminated by 48 hours post dose followed by a slower terminal elimination phase with a mean half-life of ca 103 hours.

These results are in apparent contradiction to the bile excretion study results.

Additionally there are ADE data (refs 4 & 5) from non bile duct cannulated rats that can be compared. Table 3 presents the dose normalised results from these experiments.

Table 3: Normalised recovery of radioactivity in excreta and tissues following a Single Oral Administration of [Pyridyl-2,6-^{14}C]-fluopicolide or [Phenyl-U-^{14}C]- fluopicolide at the rate of 10 mg/kg bw.

Results expressed in terms of percentage of administered dose (normalised)

Sample	Males				Females			
	Pyridyl		Phenyl		Pyridyl		Phenyl	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Urine	20.05	1.24	10.83	3.88	22.33	5.04	13.28	2.93
Faeces	77.11	1.63	86.85	4.31	71.71	4.19	83.82	2.70
Cage Wash	2.13	0.99	1.01	0.48	5.48	1.22	1.89	0.60
Tissues	0.71	0.42	1.31	0.08	0.48	0.03	1.01	0.04
Total	100	0	100	0	100	0	100	0

SD = standard deviation

There was a tendency towards a higher urinary excretion level for the pyridyl radiolabel (22.2 % in males and 27.8 % in females) compared to the phenyl radiolabel (11.8 % in males and 15.2 % in females). This was mirrored by a tendency towards a lower faecal elimination level for the pyridyl radiolabel (77.1 % in males and 71.7 % in females) compared to the phenyl radiolabel (86.9 % in males and 83.8 % in females). There was also a tendency towards lower tissue levels for the pyridyl radiolabel (0.7 % in males and 0.5 % in females) compared to the phenyl radiolabel (1.3 % in males and 1.0 % in females). This suggests that a proportion of the metabolites that were formed differed between the two radiolabels to the extent that the elimination profile was altered

The investigations into the metabolism of fluopicolide in the rat (refs 6, 7 & 8) demonstrated that it was capable of being extensively metabolised by the rat. Generally the biotransformations observed included aromatic ring hydroxylation, hydrolysis, dealkylation, acetylation, oxidative N-dealkylation and conjugation with glucuronic acid, sulphate and glutathione. The glutathione conjugates were seen to be further metabolised by loss of glycine and glutamic acid to leave cysteine conjugates. The cysteine conjugates were seen to be further metabolised by acetylation to form the mercapturic acids or to be dealkylated and S-methylated to form S-methyl metabolites. The S-methyl metabolites were seen to be oxidised to both sulphones and sulphoxides. These studies demonstrated that the molecule was subject to a cleavage leading to the formation of PCA (M-02) and BAM (M-01) to a small (up to approximately 10% of the administered dose) but appreciable extent.

The proposed metabolic profile is presented in Appendix 2. The structural identification results from the single oral low dose (10 mg/kg bw) study using the pyridyl radiolabel indicate that metabolites that had arisen from, or following, cleavage of the fluopicolide molecule to form PCA accounted for 10% and 2% of the administered dose in males and females respectively and were found uniquely in the urine (taken from non-normalised report tables). In total 92.9% of the radioactivity eliminated in the urine by the male rats was assigned a proposed structure that accounted for 16.2% of the administered dose. For the females a total of 82.6% of the radioactivity

eliminated in the urine by the female rats was assigned a proposed structure which accounted for 17.1% of the administered dose. The presence of PCA (M-02) in the faeces was suggested by comparison of the metabolite fraction HPLC retention time with that of PCA standard but this was not confirmed by structural analysis. This would have added a further 2.3% (males) and 0.6% (females). By summing the components that were assigned structures in the urine and the faeces, a total of 87.2% of the administered radioactivity were identified and assigned a structure for the males and 90.8% of the administered radioactivity for the females.

The structural identification results from the single oral low dose (10 mg/kg bw) study using the phenyl radiolabel indicate that metabolites that had arisen from, or following, cleavage of the fluopicolide molecule to form BAM (M-01) accounted for 0.8% and 0.7% of the administered dose in males and females respectively and were found in both the urine and the faeces (taken from non-normalised report tables). The components that were assigned structures in the urine and the faeces constituted a total of 77.2% of the administered radioactivity for the males and 75% of the administered radioactivity for the females.

Discussion

Comparison of the recovery normalised results from the bile excretion studies reveals that the principal differences between the two radiolabels appear in the levels observed in the bile and the faeces with the urinary levels being similar. Overall the calculated mean total absorbed for the pyridyl radiolabel was 61 ± 6% and that for the phenyl radiolabel was 80 ± 7%.

The term bioavailability is used to describe the fraction of an administered dose that reaches the systemic circulation. The area under the curve (AUC) is the area under the curve in a plot of concentration of xenobiotic in the plasma against time and the AUC (from zero to infinity) represents the total amount of xenobiotic absorbed by the body. It is therefore a measure of the systemic exposure of the organism to the administered compound. Comparison of these values indicates the relative systemic exposure.

The data provided by the blood/plamsa pharmacokinetic study demonstrate that, following a single oral dose using either [phenyl-U-^{14}C]-fluopicolide or [pyridyl-2,6-^{14}C]-fluopicolide the general pharmacokinetic profiles were similar between both the radiolabels and the sexes. The estimation of the area under the curve from both the whole blood and the plasma results ($AUC_{(0-168h)}$) indicated a similar level of systemic exposure with no significant differences between the labels.

The kinetic data therefore appear to contradict the bile excretion data.

A comparison of the recovery data from the bile excretion studies and the equivalent studies performed with non bile duct cannulated rats is provided in Table 4.

Table 4: Normalised mean recovery of radioactivity in excreta and tissues following a Single Oral Administration of [Pyridyl-2,6-^{14}C]-fluopicolide or [Phenyl-U-^{14}C]- fluopicolide at the rate of 10 mg/kg bw.

Results expressed in terms of percentage of administered dose (normalised)

Sample	Males				Females			
	Cannulated		Non-cannulated		Cannulated		Non-cannulated	
	Pyridyl	Phenyl	Pyridyl	Phenyl	Pyridyl	Phenyl	Pyridyl	Phenyl
Urine +cage wash	6.5	5.3	22.2	11.8	11.6	7.5	27.8	15.2
Faeces +bile	92.7	92.7	77.1	86.9	87.9	91.2	71.7	83.8
Tissues	0.8	1.9	0.7	1.3	0.5	1.4	0.5	1.0
Total	100	100	100	100	100	100	100	100

Actual values are presented in Tables 1a and 3a in Appenix 1.

The data from the non cannulated rats indicate that the higher levels of radioactivity found in the urine following administration of the pyridyl radiolabel are mirrored by higher levels in the faeces following administration of the phenyl radiolabel (*ca* 10% difference for the males and *ca* 12% difference for the females). The inference from this data, when taken in conjunction with the blood/plasma kinetic data, is that the degree of absorption/elimination was similar between the two radiolabels with a preference for the urinary route for the pyridyl label and the biliary route for the phenyl radiolabel.

Following bile duct cannulation the difference in urinary elimination between the radiolabels was reduced from *ca* 10-12% dose to *ca* 1-4% dose. This change was mirrored in the sum of both faecal and bile radioactivity levels that now only represented *ca* 0-3% dose. There was however a much larger difference in biliary elimination between the radiolabels that represented *ca* 19-23% dose (higher levels for the phenyl radiolabel).

A key observation is the decrease in urinary elimination following bile cannulation which implies that there is an active entero-hepatic circulation in operation in the non-bile duct cannulated rats. By removing the entry of the bile into the intestine the amount of material being reabsorbed was eliminated. The pyridyl radiolabel appears to have been more susceptible to this effect than the phenyl radiolabel.

Comparison of the urinary elimination data for the cannulated and non-cannulated rats for the phenyl radiolabel reveals that the reduction in urinary elimination following cannulation was 6.5% dose for the males and 7.7% for the females. Making the same comparison for the faeces results for the non-cannulated rats and the sum of the faecal and biliary elimination for the cannulated rats reveals that the levels of radioactivity were 5.9% higher for the cannulated males and 7.3% higher for the cannulated female rats. Such a close match indicates that it is highly likely that this six to

seven percent of the administered dose represented the amount of radioactivity that would have been reabsorbed and eliminated *via* the urine in non-cannulated rats.

Performing the same calculations for the pyridyl radiolabel indicates a reduction in urinary elimination of 15.6% dose for the males and 16.2% dose for the females for the cannulated rats i.e. same direction aas the phenyl radiolabel but involving approximately double the administered dose. Once again this decrease in urinary elimination is found in a corresponding increase in the levels of radioactivity found in the bile & faeces of 15.5% dose for the males and 16.2% dose for the females. It would appear that the entero-hepatic recirculation of pyridyl radiolabelled metabolites was greater than that of the phenyl radiolabelled metabolites.

Biologically this difference between the two radiolabels must be related to a difference in metabolic fate i.e. the fluopicolide molecule would need to have been cleaved. As seen in the results section the metabolism studies performed with radiolabelled fluopicolide indicated that up to at least *ca* 10% of the administered dose could be subject to cleavage based upon the detection and identification of cleavage products (PCA & BAM and/or their metabolites).

Studies performed with the administration of single oral low doses of either PCA or BAM (refs 9 & 10) demonstrated that their ADME profiles differed. Following adminstration of PCA at the rate of 10 mg/kg bw the majority (>86%) of the radioactivity was eliminated *via* the urine with approximately 7% dose found in the faeces. The PCA parent molecule accounted for between 98% and 99% of the radioactivity in the urine and 97% to 98% of the radioactivity found in the faeces indicating that it underwent very little biotransformation and was very well absorbed from the intestine. Following adminstration of BAM at the same rate of 10 mg/kg bw the majority (>81%) of the radioactivity was eliminated *via* the urine with approximately 13% dose found in the faeces. The BAM parent molecule accounted for approximately 17% of the radioactivity in the urine and 84% to 89% of the radioactivity found in the faeces indicating that BAM was subject to a greater degree of biotransformation than PCA including aromatic ring hydroxylation, hydrolysis, dealkylation, acetylation, and conjugation with glucuronic acid, sulphate and glutathione (similar to that observed for fluopicolide) and was eliminated to a greater extent *via* the bile.

Thus the difference between the two radiolabels could be due to the formation of cleaved metabolites, PCA and BAM, whose fates differed in that a much greater proportion of the PCA derived metabolites would be reabsorbed and eliminated *via* the urine than the BAM derived metabolites.

It is therefore reasonable to calculate the bioavailabilty of fluopicolide based upon the results of both the cannualted and non-cannulated rats by using the urinary data from the non-cannulated rats to take into account the material undergoing entero-hepatic recirculation and the biliary elimination data from the cannulated rats (tissue data was taken from the non-cannulated rats). This yields mean percentages for the pyridyl radiolabel of 75% dose and 78% dose for the males and females respectively. The mean percentage bioavailability derived from the phenyl radiolabel was 84% and 88% for the males and females respectively.

Conclusion

This position paper demonstrates that the oral bioavailability of fluopicolide in the rat is higher than 62% and provides a biological justification for the difference in the values obtained in the bile excretion study for the two radiolabels.

The bile excretion results alone provide an underestimation of the oral bioavailability of fluopicolide as a significant level of entero-hepatic recirculation of fluopicolide and/or its metabolites that would normally occur could not be measured. Additionally the fate of the metabolites formed following cleavage of the fluopicolide molecule differed in that PCA and its related metaoblites were more likely to be reabsorbed and eliminated *via* the urine than BAM and its related metabolites, which would be more likely to be reabsorbed and eliminated *via* the bile. It is for this reason that the blood kinetc data suggest that the systemic exposure was the same for the two radiolabels (functioning entero-hepatic recirculation) whereas the bile excretion study data suggest that there was a difference.

When the entero-hepatic recirculation is taken into account the oral bioavailability of the two radiolabels was found to be closer than originally presumed with an overall mean range between 75% dose to 88% dose.

Therefore the bioavailabilty value of 74% proposed by the notifier in the tier 2 summary was already a conservative estimate.

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APPENDIX 1

Table 1a: Recovery of Radioactivity from Bile Duct Cannulated Rats following Single Oral Administration of [phenyl-U-^{14}C]-fluopicolide and [Pyridyl-2,6-^{14}C]-fluopicolide at the rate of 10 mg/kg bw

Results expressed in terms of % of administered dose.

Sample	Males				Females			
	Pyridyl label		Phenyl label		Pyridyl label		Phenyl label	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Urine	5.83	2.48	4.55	4.34	10.42	5.04	6.71	1.82
Faeces	40.27	6.81	21.48	7.47	39.16	4.81	19.28	7.03
Bile	51.69	9.33	70.02	6.79	51.74	12.77	73.88	8.03
Cage wash	0.70	0.37	0.77	0.79	1.50	1.77	0.91	0.60
Tissues[a]	2.11	1.12	2.03	0.40	0.80	0.35	1.48	0.16
Total Absorbed[b]	59.00	6.92	77.24	5.31	64.05	10.67	82.89	7.72
Total	100.59	2.80	98.85	3.45	103.63	7.87	102.26	2.33

SD = standard deviation (n = 4)
[a] : excluding intestinal and stomach content
[b]: sum of radioactivity in urine, cage rinse, bile and tissues.

Table 3a: Recovery of radioactivity in excreta and tissues following a Single Oral Administration of [Pyridyl-2,6-^{14}C]-fluopicolide or [Phenyl-U-^{14}C]- fluopicolide at the rate of 10 mg/kg bw.

Results expressed in terms of percentage of administered dose

Sample	Males				Females			
	Pyridyl		Phenyl		Pyridyl		Phenyl	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Urine	18.81	1.48	10.03	3.73	21.37	4.64	13.09	3.34
Faeces	72.37	5.12	82.58	3.83	68.78	4.70	82.09	2.68
Cage Wash	2.04	1.12	0.96	0.46	5.27	1.24	2.03	0.78
Tissues	0.66	0.37	1.25	0.07	0.46	0.03	0.99	0.07
Total	93.87	6.78	95.09	0.53	95.88	1.33	98.2	4.52

SD = standard deviation

APPENDIX 2

PROPOSED METABOLIC PATHWAY FOR FLUOPICOLIDE IN THE RAT

Figure 1 (1/3): A Proposed Metabolic Pathway for AE C638206 in the Rat

Figure 1 (2/3): A Proposed Metabolic Pathway for Fluopicolide in the Rat

Proposed metabolic pathway part A
Continued fromFigure 5.1.1

Urine & Faeces

Urine

Urine

Urine

Urine

Urine

Urine

Urine

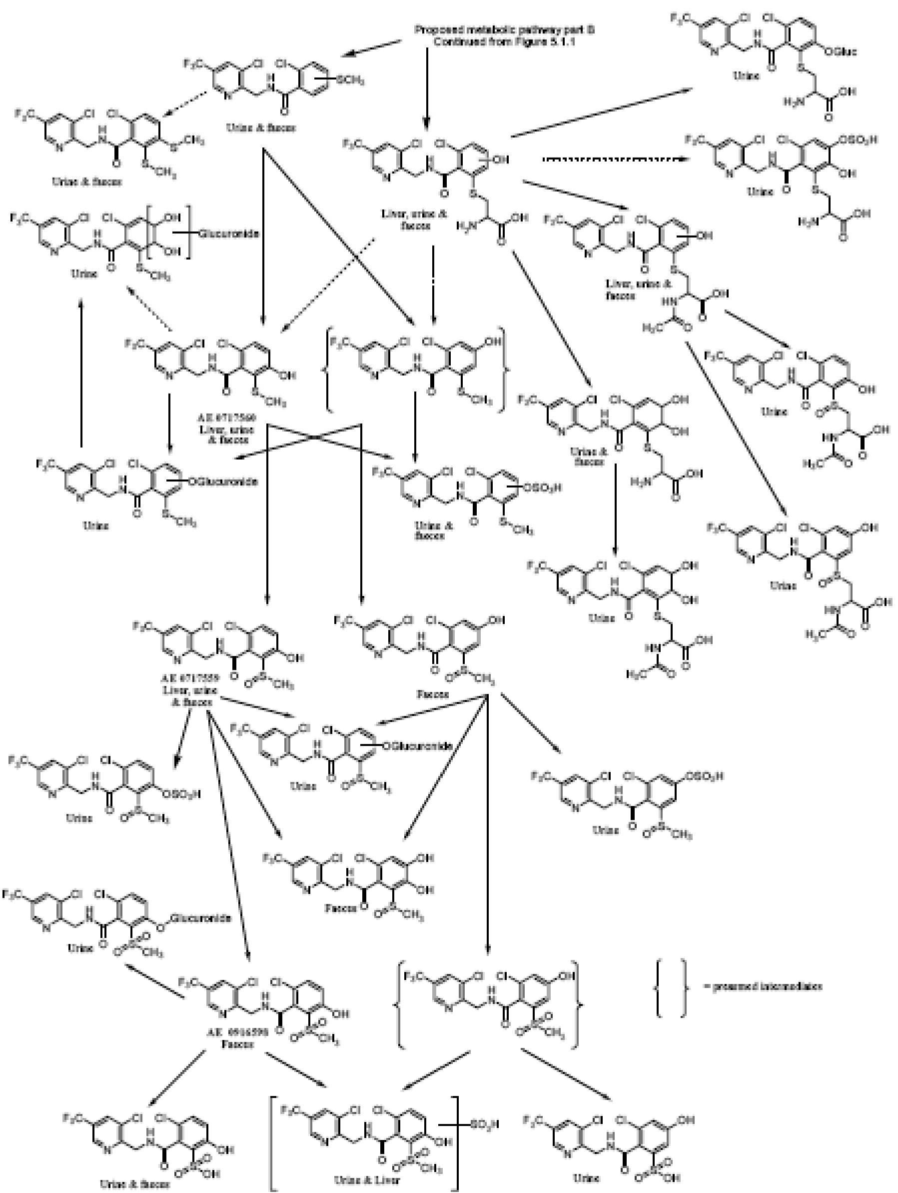
Liver, urine & faeces

Urine

Urine

{ } = presumed intermediate

Figure 1 (3/3): A Proposed Metabolic Pathway for Fluopicolide in the Rat



APPENDIX 5

REGULATORY TOXICOLOGY

POSITION PAPER

Subject :
AEC638206 (fluopicolide)

CONTENTS :
Waiver for an Acute Reference Dose (ARfD) setting

Author :

Dr. Virginie Payraudeau
Regulatory Toxicologist

Bayer CropScience
BP 153
06903 Sophia Antipolis Cedex
FRANCE

Date : 07th March 2006

BUSINESS CONFIDENTIAL PROPERTY OF BAYER CROPSCIENCE

1. SUMMARY

In the Draft Assessment Report, the Rapporteur Member State, UK PSD (Pesticide Safety Directorate) proposed the NOAEL from the 28-day rat toxicity study to establish an Acute Reference Dose (ARfD). The notifier Bayer CropScience supports that in view of the overall toxicological profile available for fluopicolide, the establishment of an ARfD is not appropriate.

2. INTRODUCTION AND BACKGROUND

The Rapporteur Member State, UK PSD has proposed an acute reference dose (ARfD) of 0.18 mg/kg based on a 18 mg/kg/day NOAEL in the 28-day toxicity study performed in rats. This study was selected for ARfD setting on the basis of liver and kidneys (identified as target organs) assessments after fluopicolide repeated administration.

Bayer CropScience acknowledges that the acute neurotoxicity study originally proposed, while being of an appropriate exposure duration and in an appropriate species, lacks specific detection of effects in the liver and kidney. However, in the opinion of Bayer CropScience the histological effects seen in liver and kidneys upon subacute/subchronic exposures were either considered of adaptative and/or the result of multiple dosing, and thus not relevant for ARfD setting.

Following the "Guidance on setting of acute reference dose (ARfD) for pesticides" (Solecki et al, 2005, EU 2001), the evaluation of the total data base of fluopicolide showed no appropriate endpoints for setting an ARfD.

3. TOXICOLOGICAL PROFILE OF FLUOPICOLIDE

Acute toxicity

Fluopicolide is of very low acute oral, dermal and inhalation toxicity (C008135, C008136, C008140). Fluopicolide is not irritating to rabbit skin or eye (C008137, C008138). Fluopicolide is not a skin sensitizer (C008139). In accordance with Commission Directive 2001/59/EEC, no classification is required for the acute toxicity of fluopicolide.

Short-term toxicity

Short term toxicity studies on fluopicolide have been performed in rats, mice and dogs.

In rats, the target organs of toxicity were the liver (centrilobular hepatocellular hypertrophy) and kidney (accumulation of hyaline droplets). The NOAEL in the 28-day dietary study was 200 ppm (equivalent to 17.7 mg/kg bw/day, C009846), and the NOAEL in the 90-day dietary study was 100 ppm (7.4 mg/kg bw/day, C008603).

In mice, the main target organ was the liver (centrilobular hepatocellular hypertrophy). The NOAEL in the 28-day dietary study was 64 ppm, (equivalent to 10.4 mg/kg bw/day, C008274). The NOAEL in the 90-day dietary study was 50 ppm (equivalent 10.4 mg/kg bw/day, C018138).

In dogs, increased absolute and relative liver weight, and increased cholesterol levels were observed suggesting slight liver function impairment. The NOAEL in the 28-day oral toxicity study was 100 mg/kg bw/day (C008283). The NOAEL in the 90-day dietary study was 70 mg/kg bw/day (C010655). In the one year oral toxicity study, the NOAEL was 300 mg/kg bw/day (C029194).

Genotoxicity

The genotoxic potential of fluopicolide was investigated in a range of *in vitro* and *in vivo* studies.

The overall weight of evidence suggests that fluopicolide is devoid of any mutagenic activity in both prokaryotic and eukaryotic cells (C012566, C026130). Fluopicolide had weak clastogenic properties *in vitro* (C008174, C011815) but very limited clastogenic potential *in vivo* (C008175, C035885, C037459, C008141) at toxic dose levels and is considered unlikely to present a genotoxic hazard to humans.

Long-term toxicity and carcinogenicity

Carcinogenicity studies on fluopicolide have been performed in rats and mice.

The administration of fluopicolide to rats at dietary concentrations of up to 2500 ppm for 104 weeks did not provide any evidence of oncogenic potential (C038733). The liver (centrilobular hepatocellular hypertrophy and foci of alterations) and kidneys (degenerative and proliferative changes) were identified as the primary target organs at high dose levels. The NOAEL in the 2-year chronic toxicity and carcinogenicity study in rats was 200 ppm (equivalent to 8.4 mg/kg bw/day in males).

Fluopicolide caused an increase in hepatocellular adenomas in male and female mice at 3200 ppm a dose level at which the maximum tolerated dose has been attained (severe body weight gain reduction) by a mechanism considered to be not relevant to humans (C038732). A mechanistic study showed that fluopicolide is a phenobarbital-like product. The NOAEL in the 78-week oncogenicity dietary study in mice was 50 ppm (corresponding to 7.9 mg/kg bw/day in males).

Reproduction toxicity

Dietary administration of fluopicolide at concentrations of 100, 500 or 2000 ppm in the multigeneration study was generally well tolerated by the F0 and subsequent F1 parental animals and their respective progeny (C033054). Fertility and reproductive performance of the F0 and F1 parental animals were unaffected by treatment and litter parameters at birth of the F1 and F2 progeny and their survival to weaning showed no adverse effects of treatment. The liver (hepatocellular hypertrophy) and kidneys (accumulation of hyaline droplets, mineralization, inflammation, tubular basophilia) were found as target organs in adults at 500 ppm and/or 2000 ppm in both generations. Detailed histopathological examination of reproductive tissues and macroscopic abnormalities did not reveal any treatment-related findings at 2000 ppm.
The NOAEL for reproductive toxicity in the multigeneration study in rats was 2000 ppm, (equivalent to 103.4 mg/kg bw/day for F0 males and 127.3 mg/kg bw/day for F0 females for the period before pairing). The overall NOAEL for pups and developing offspring was 500 ppm (equivalent to 25.5 mg/kg bw/day for the males and 32.9 mg/kg bw/day for the females).

Developmental toxicity

The NOAEL for maternal toxicity and foetotoxicity in rats was 60 mg/kg bw/day based on decreased body weight in dams and reduction in mean foetal body weights and crown-rump lengths in foetuses at 700 mg/kg bw/day, the highest test dose (C016312). Further evidence of foetotoxicity at the highest test dose were increased incidences of minor defects at the thoracic vertebrae, sternebrae and ribs as well as retardations (delayed ossification) which is noted to be a maternally toxic dose level. Fluopicolide was not teratogenic in the developmental toxicity study in rats.

The NOAEL for maternal toxicity and foetotoxicity in rabbits was 20 mg/kg bw/day

(C016495). At 60 mg/kg bw/day, mortality, high incidence of premature delivery, reduction in body weight gain and food consumption were observed in dams, while reduction in body weights and crown-rump lengths were observed in foetuses. Fluopicolide was not teratogenic in the developmental toxicity study in rabbits.

Neurotoxicity

Acute oral neurotoxicity studies (C021425, C019695) as well as a 13-week neurotoxicity study (C019700) were performed in rats. Neurobehavioural screening, macroscopic and histopathological examination of the associated tissues (including anatomical measurements of the brain) revealed no treatment-related findings. In addition, no unusual signs or patterns of behaviour were observed at all routine observations.
The NOAEL for acute neurotoxicity was 100 mg/kg bw/day and the NOAEL for subchronic neurotoxicity was >10000 ppm (equivalent to 781 mg/kg bw/day in males and 866 mg/kg bw/day in females). There was no evidence of neurotoxicity following the continuous administration of fluopicolide to rats for 13 weeks at dietary concentrations up to 10000 ppm.

Fluopicolide is of very low acute toxicity. In subchronic and chronic oral toxicity studies the major target organ was the liver in rats and mice. Fluopicolide is not mutagenic *in vivo* and is not expected to produce any reproductive, developmental, carcinogenic or neurological hazards.

3.2 Liver as a relevant target organ in rodent studies of fluopicolide

The liver was identified as a target organ during short term and long term exposures with fluopicolide (see table 1). Nevertheless, the effects were limited to reversible increased liver weights, hepatocellular hypertrophy/hyperplasia and increased metabolising hepatic enzyme activities which are mostly considered to be adaptative responses rather than adverse effects.
In addition, these effects were shown to be subsequent to increased liver P450 induction with a Phenobarbital-like profile. As discussed in the dossier, an explanatory study in mice (C040806) was carried out to determine if the hepatic effects seen in rats and mice were of a type thought to be relevant to humans at low doses. There is a high degree of similarity between fluopicolide's CYP450 induction profile, cell proliferation, and induction of transient centrilobular hypertrophy followed by hyperplasia, all being characteristic of phenobarbital-type liver induction.
The relevance of these effects to human health at low doses was recently reviewed in a Guidance Document written by Dr David Andrew from UK PSD (May 2005).

In addition, the guidance document for ARfD setting clearly stated that "such responses of the liver following exposure to xenobiotics are considered not to be relevant effects for the setting of an ARfD" (Solecki et al., 2005, EU 2001).

3.3 Kidney as a relevant target organ in rodent studies of fluopicolide

The kidney was also identified as a target organ following repeated exposures to fluopicolide (table 1). However, the main histopathological findings observed in the kidneys were considered to be specific to male rats (accumulation of hyaline droplets associated with cortical basophilia observed in high dose animals within the sub-chronic and chronic administration of fluopicolide) and thus of no relevance to humans. Additional renal histopathological findings (mineralization, interstitial inflammation, hyperplasia observed within the two-generation reproduction and carcinogenicity studies) were considered to be subsequent to repeated exposures and of no relevance for a single day exposure scenario.
According to the guidance document, an appropriate toxicological endpoint for ARfD setting should be observed during a single day exposure. All these findings were therefore of no relevance for ARfD setting.

4. CONCLUSION

Flupicolide is devoid of any acute oral toxicity with an oral LD_{50} higher than 5000 mg/kg. In subacute and subchronic repeat dose studies, no relevant endpoints (of acute alerts) for ARfD setting were observed. In particular, no signs of hematotoxicity, immunotoxicity or neurotoxicity were evidenced.
No endocrine or developmental effects were observed. The liver and the kidneys were identified as target organs during short term and long term exposures with fluopicolide. Nevertheless, the liver and kidneys effects were clearly considered to be adaptative, or specific to male rats, or only observed after prolonged exposures. These findings are of no relevance for ARfD setting (Solecki et al, 2005, EU 2001).

In conclusion, BCS proposes that setting an ARfD is not appropriate for fluopicolide.

Table 1: Summary of the different NOAELs and main findings observed in toxicity studies performed with fluopicolide

Study	Species	NOAEL	Main findings
28-day oral	rat	200 ppm (17.7 mg:kg/day)	Centrilobular hepatocellular hypertrophy, accumulation of hyaline droplets in kidneys
28-day oral	mouse	64 ppm (10.4 mg:kg/day)	Centrilobular hepatocellular hypertrophy
28-day oral	dog	1000 mg/kg/day	Increased liver weights and cholesterol levels
28-day dermal	rat	1000 mg/kg/day	-
90-day oral	rat	100 ppm (7.4 mg/kg/day)	Centrilobular hepatocellular hypertrophy, accumulation of hyaline droplets in kidneys
90-day oral	mouse	50 ppm (10.4 mg/kg/day)	Centrilobular hepatocellular hypertrophy
90-day oral	dog	1000 mg/kg/day	Increased liver weights and cholesterol levels
52-week oral	dog	300 mg/kg/day	Lower body weight gain and increased cholesterol levels
104-week - chronic	rat	200 ppm (8.4 mg/kg/day)	Centrilobular hepatocellular hypertrophy, degenerative and proliferative changes in kidneys
78-week - chronic	mouse	50 ppm (7.9 mg/kg/day)	Increased incidence of hepatocellular adenoma (Phenobarbital-like mechanism)
2-generation reproduction	rat	500 ppm (25.5 mg/kg/day)	Centrilobular hepatocellular hypertrophy, degenerative and proliferative changes in kidneys
Oral teratology	rat	Maternal & developmental: 60 mg/kg/day	Reduced body weights in dams and fetuses
Oral teratology	rabbit	Maternal & developmental: 20 mg/kg/day	Mortality, reduced body weight gain
90-day, neurotoxicity	rat	10000 ppm (781 mg/kg/day)	-

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APPENDIX 6

REGULATORY TOXICOLOGY

POSITION PAPER

Subject :
AE C638206 (fluopicolide)

CONTENTS :
Assessment of hepatocellular proliferation and lack of carcinogenicity potential

Author :

Dr. Virginie Payraudeau
Regulatory Toxicologist

Bayer CropScience
BP 153
06903 Sophia Antipolis Cedex
FRANCE

Date : 02[sd] August 2006

M-275342-01-1

Introduction

Fluopicolide was assessed for carcinogenicity in rats (Cooper, S., 2003) and mice (Chevalier, G., 2003). In mice, a statistically significantly increased incidence of hepatocellular adenoma (HCA) was observed in animals at 3200 ppm, when compared to controls (11/49 vs 5/50 in males and 16/46 vs 1/50 in females). These benign tumours were only observed in high dose animals exceeding the Maximum Tolerated Dose (MTD) where significantly more than a 10% reduction in body weight gain was recorded. Since no treatment-related HCA were reported at the lower dose levels, this effect was thus devoid of any dose-relationship. In addition, no hepatocellular carcinoma was noted in either sex. Given the lack of genotoxicity potential, the absence of carcinoma following a prolonged exposure to fluopicolide, and the absence of any dose-relationship, this increased incidence of HCA in high dose mice was therefore considered to be subsequent to a threshold mechanism with a probable Phenobarbital-like mechanism of action: hepatocellular hypertrophy associated with transient liver cell proliferation followed by a steady state (Grasso, P. *et al.*, 1991, Hildebrand, B. *et al.*, 1991, Jirtle, R.I., *et al.*, 1991). To address this mechanism of action, the liver enzyme induction potential as well as the liver cell proliferation potential (BrdU assessment) were investigated for fluopicolide and Phenobarbital in 28-day explanatory toxicity studies. An additional Proliferating Cell Nuclear Antigen (PCNA) assessment in the liver was also conducted in the 90-day regulatory toxicity study performed in mice with fluopicolide (Wason, S., 2001a, b).

1. Fluopicolide: 28-day explanatory toxicity study in mice – BrdU assessment

To identify whether fluopicolide was a phenobarbital-like compound, a 28-day explanatory toxicity study was performed in C57BL/6 mice (Langrand-Lerche, C., 2004). In such a toxicity study, 15 female mice received fluopicolide at dose levels of 0 or 3200 ppm (high dose level from the 78-week oncogenicity study) in the diet for 28 days. Satellite subgroups of 20 female mice were added to each group for interim sacrifice after 7 days of treatment. Bromodeoxyuridine (BrdU) was administered in drinking water for 7 days before scheduled sacrifices (interim and final) for liver cell proliferation assessment. In addition, at interim sacrifice after 7 days, hepatic cytochrome P-450 isoenzymes were assessed.

At interim sacrifice on Day 7, fluopicolide produced lower mean bodyweights (by 9%) as well as higher relative liver weights to body weights (by 37%) in females at 3200 ppm, when compared to controls (table 1). Histopathological examination of the liver showed diffuse perilobular to panlobular hepatocellular hypertrophy in all females at 3200 ppm. Fluopicolide induced a 2-fold increase in total cytochrome P-450 as well as 19-fold and 12.4-fold higher benzoxyresofurin O-debenzylation (BROD) and pentoxyresofurin O-depentylation (PROD) activities, respectively (table 1). The mean BrdU labeling index was 6.5-fold higher in females at 3200 ppm when compared to controls showing a marked hepatocellular proliferation in high dose female mice on day 7 (table 1).

At final sacrifice on Day 28, lower bodyweights (by 9%) as well as higher relative liver weights to body weights (by 56%) were seen in females at 3200 ppm, when compared to controls (table 1). Histopathological examination of the liver showed a diffuse perilobular to panlobular hepatocellular hypertrophy in all females at 3200 ppm. The mean BrdU labeling index in female mice at 3200 ppm was similar to controls showing the absence of hepatocellular proliferation

on day 28 (table 1).

Table 1: Body weight and liver changes in controls and 3200 ppm fluopicolide female mice following a 7-day and 28-day treatment period

	Interim sacrifice (Day 7)		Final sacrifice (Day 28)	
Doses (ppm)	0	3200	0	3200
BW (g)	20.1	**18.3****	21.0	**19.3****
Liver weight (g)	4.504	**6.157***	4.410	**6.892***
Hepatocellular hypertrophy	0/20	**20/20**	0/15	**15/15**
Total P-450 (nmol/min/mg protein)	1.11	**2.19b**	ND	ND
BROD (pmol/min/mg protein)	57.3	**1079.8a**	ND	ND
PROD (pmol/min/mg protein)	18.3	**227.4a**	ND	ND
BrdU labeling Index	23.55	**152.95b**	29.62	17.00

* $p < 0.05$; ** $p < 0.01$ statistically different from controls using T test
a $p < 0.05$; b $p < 0.01$ statistically different from controls using the Mann-Whitney exact test
ND: not determined

These findings clearly indicate that fluopicolide is a strong inducer of total cytochrome P-450 and BROD and PROD activities associated with hepatocellular hypertrophy. In addition, fluopicolide produced a marked transient liver cell proliferation on day 7 which returned to control levels on day 28.

2. **Phenobarbital: 28-day explanatory toxicity study in mice – BrdU assessment**

In a 28-day explanatory toxicity study performed in C57BL/6 mice, Phenobarbital was orally administered by gavage to 15 male and 15 female mice at dose levels of 0 or 80 mg/kg/d for 28 days (Langrand-Lerche, C., 2004). Satellite subgroups of 20 male and 20 female mice were added to each group for interim sacrifice after 7 days of treatment. Bromodeoxyuridine (BrdU) was administered in drinking water for 7 days before scheduled sacrifices (interim and final) for liver cell proliferation assessment. In addition, at interim sacrifice after 7 days, hepatic cytochrome P-450 isoenzymes were assessed.

At both interim and final sacrifices, mean bodyweights were slightly lower (by around 4%) in both males and females and relative liver weights to body weights were higher (by around 15%) in treated animals, when compared to controls (table 2). Histopathological examination of the liver showed a diffuse centrilobular to midzonal hepatocellular hypertrophy in almost all treated animals. Phenobarbital induced a 1.8-fold (combined sexes) increase in total cytochrome P-450 as well as 40-fold and 15-fold (combined sexes) higher benzoxyresofurin O-debenzylation (BROD) and pentoxyresofurin O-depentylation (PROD) activities, respectively.

At the interim sacrifice on Day 7, the mean BrdU labeling index was around 10-and 8.5-fold higher respectively in males and females treated with Phenobarbital, when compared to controls showing a marked hepatocellular proliferation (table 2). At final sacrifice on Day 28, the mean BrdU labeling index was only 1.4-fold higher in males while no changes were observed in females, when compared to controls.

Table 2: Body weight and liver changes in controls and 80 mg/kg/d Phenobarbital male and female mice following a 7-day and 28-day treatment period

	Males				Females			
	Interim sacrifice (D7)		Final sacrifice (D28)		Interim sacrifice (D7)		Final sacrifice (D28)	
Doses (mg/kg/d)	0	80	0	80	0	80	0	80
BW (g)	24.1	**23.1****	25.3	**24.6***	20.0	**19.3****	21.6	20.9
Liver weight (g)	4.378	**4.834****	4.262	**4.931****	4.645	**5.508****	4.820	**5.487****
Hepatocellular hypertrophy	0/20	**20/20**	0/15	**15/15**	0/20	**18/20**	0/15	**15/15**
Total P-450 (nmol/min/mg protein)	1.16	**2.26b**	ND	ND	1.25	**2.21b**	ND	ND
BROD (pmol/min/mg protein)	11.9	**764.7 a**	ND	ND	63.1	**1005.8 a**	ND	ND
PROD (pmol/min/mg protein)	6.1	**123.2 a**	ND	ND	16.7	**153.5a**	ND	ND
BrdU labeling Index	9.1	**92.1b**	16.9	**23.7***	16.4	**138.7b**	29.4	28.7

* $p < 0.05$; ** $p < 0.01$ statistically different from controls using T test
a $p < 0.05$; b $p < 0.01$ statistically different from controls using the Mann-Whitney exact test
ND: not determined

These findings clearly indicate that Phenobarbital is a strong inducer of total cytochrome P-450 and BROD and PROD activities associated with hepatocellular hypertrophy. In addition, Phenobarbital produced a marked transient liver cell proliferation on day 7 which remained to control levels on day 28 (steady state level, Schulte-Hermann, R., 1974, 1979). These data show that these Phenobarbital-induced liver changes are similar to those observed with fluopicolide suggesting that fluopicolide produced liver cell hypertrophy and transient liver cell proliferation with a phenobarbital-like mechanism of action.

3. Fluopicolide: 90-day toxicity study in mice – PCNA assessment

In a regulatory 90-day toxicity study (Wason, S., 2001a), fluopicolide was administered via the diet to 10 male and 10 female C57BL/6 mice at dose levels of 0, 50, 200, 800 and 3200 ppm for 90 days. Routine clinical, biochemical and pathological examinations were performed to determine the potential toxic effects of fluopicolide in mice following a 90-day treatment period.

Fluopicolide produced lower mean bodyweights (by around 10%) in both males and females at 3200 ppm (table 3). Increased relative liver weights to body weights (by around 32%) were observed in both sexes at 3200 ppm. Histopathological examination of the liver showed a diffuse centrilobular hepatocellular hypertrophy in all high dose animals at 3200 ppm (table 3).

At the request of the Japanese Food Safety Commission (in 2006), additional Proliferating Cell Nuclear Antigen (PCNA) staining on selected liver slides was conducted (Wason, S. 2001b). The determination of labeling index was performed on liver slides from all surviving animals from the control and high dose (3200 ppm) groups) at the terminal sacrifice (on Day 90). Fluopicolide did not produce any changes in the number of PCNA-positive hepatocytes when compared to controls on day 90 (table 3).

Table 3: Body weight and liver changes in controls and 3200 ppm fluopicolide male and female mice following a 90-day treatment period

	Males		Females	
Doses (ppm)	0	3200	0	3200
BW on Day 90 (g)	26.1	25.3	21.2	20.5
Liver weight (g)	4.3	**5.6****	4.4	**5.9****
Hepatocellular hypertrophy	0/8	**8/8**	0/9	**10/10**
PCNA labeling Index	1.86	1.93	2.23	2.22

** $p < 0.01$ statistically different from controls using T test

These data show that fluopicolide produced higher liver weights associated with hepatocellular hypertrophy in high dose animals following a 90-day treatment period. Fluopicolide did not produce hepatocellular proliferation on day 90. These data are in line with the data observed from the 28-day explanatory toxicity study where fluopicolide showed to induce a transient cell proliferation on day 7 which returned to control levels on day 28 with a Phenobarbital-like mechanism of action.

4. Conclusion

The dietary administration of fluopicolide produced higher incidence of hepatocellular adenoma (HCA) in high dose male and female mice following a 78-week treatment period. Given that these HCA were not observed at lower dose levels in mice, not observed in rats following a 2-year treatment period and

taken into account the lack of genotoxicity potential of fluopicolide, the higher incidence of HCA was thus considered to be subsequent to a threshold mechanism with a Phenobarbital-like mechanism of action (hepatocellular hypertrophy and transient cell proliferation) which is a well known mechanism of action specific to the mouse and of no relevance to humans (Anderson *et al.*, 1992, Grasso, P. *et al.* 1991).

In a 28-day explanatory toxicity study, fluopicolide was shown to be a strong inducer of total cytochrome P450 and BROD and PROD associated activities. In addition, fluopicolide produced a marked transient liver cell proliferation on day 7 which returned to control levels on day 28. These findings were similar to those observed with Phenobarbital showing that fluopicolide is a phenobarbirtal-like compound. Moreover, the PCNA assessment on liver tissue from animals at 3200 ppm showed that fluopicolide did not produced hepatocellular proliferation on day 90. This is completely consistent with the lack of cell proliferation observed on day 28 with the BrdU assessment. These findings emphasize that the transient liver cell proliferation followed by a returned to control levels (steady state) is necessary to the development of HCA following a long term exposure period to Phenobarbital-like product in mice (Schulte-Hermann, R., 1974, 1979, Grasso, P. *et al.*, 1991, Hildebrand, B., *et al.*, 1991). This mechanism of action is clearly specific to the mouse and of no relevance to humans.

In conclusion, fluopicolide by producing a marked transient liver cell proliferation in high dose mice would allow the development of HCA following a prolonged exposure period. Therefore, in the opinion of BCS, the higher incidence of HCA observed in high dose male and female mice following a 78-week treatment period with fluopicolide are of no relevance to humans. Fluopicolide is thus devoid of any carcinogenicity potential.

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APPENDIX 6

REGULATORY TOXICOLOGY

POSITION PAPER

RE-ASSESSMENT OF LIVER LESIONS/TUMORS FROM STUDY PDR/49

BAM: DIETARY ADMINISTRATION TO RATS FOR 2 YEARS

COMPLEMENTARY STATISTICAL ANALYSIS
OF HEPATOCELLULAR TUMORS IN FEMALE RATS

AUTHOR:

Dr Catherine PALLEN, PhD
Global Expert in Statistics & Epidemiology

Bayer CropScience
BP 153
06903 Sophia Antipolis Cedex
FRANCE

DATE: November 20 , 2007

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INTRODUCTION

Only the data for the females is analyzed here.

A rat study of 2 years duration was conducted at Huntingdon Life Sciences between November 1968 and November 1970 and reported in 1971. Five groups of 35 male and female rats received 0, 60, 100, 180 and 500 ppm of BAM.

Female rats were allocated to groups on the study as follow:

Group	Treatment levels (ppm)	Animal numbers Females
1	Control (0)	176-210
2	60	211-245
3	100	246-280
4	180	281-315
5	500	316-350

In 1996, a new study was conducted to re-evaluate the pathological findings in slides produced from liver sections taken on the rat study of 2-years duration. Due to the age of the study and the procedures in use in 1970, not all the archived liver sections were available to be read, interpreted and reported.

Group	Treatment levels (ppm)	Animal numbers Females
1	Control (0)	176, 188,190,191,196,197,199,200,202, 206-208,210
2	60	211-213,215-229,231,233-237,240-242,244
3	100	246,248-263,268-272,274-277,279,280
4	180	281-300,302-305,307-313,315
5	500	316-350

The slides for animals 205 (0 ppm), 214 (60 ppm) and 251 (100 ppm), which were originally assessed, were either no longer available or no longer readable.

Re-assessment of hepatocelullar neoplastic findings showed the following results:

	Females				
Dosage Levels (ppm)	**0**	**60**	**100**	**180**	**500**
Hepatocellular adenoma	0	1	0	0	5
Hepatocellular carcinoma	0	0	0	0	0

STATISTICAL PROCEDURES

Survival adjusted analyses, considering any possible incurrent mortality differences due to the competing toxicity among the treated groups, were performed on lesions (Gart et al., 1986).

For non-palpable tumors, each tumor was categorized as fatal if the tumor was a factor contributing towards the death of the animal, incidental otherwise. Only animal 349 (500 ppm) had a fatal tumor. Consequently all the tumor data was analyzed using procedures for incidental tumors.

Incidental tumors were analyzed by logistic regression of tumors prevalence (Dinse and Lagakos, 1983). Logistic regression analysis is based on the assumption that the diagnosed lesions were not directly responsible for the animal's death. Treated and control group lesion rates were compared using the corrected score test.
The reported results reflect 1-sided testing.

Statistical significances were evaluated at the 5% and 1% levels of significance.

Statistical analyses were conducted using SAS programs (Version 8.2) and LOPRAN.EXE program.

STATISTICAL REFERENCES

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SAS Software Release 8.2, SAS Institute Inc., Cary, NC, USA.

LOPRAN.EXE program: program to perform logistic regression analysis of tumor prevalence (of incidental tumors).

RESULTS

In the complementary statistical analysis, the data analyzed is summarized in the following table:

	Females				
Dosage Levels (ppm)	**0**	**60**	**100**	**180**	**500**
Hepatocellular adenoma	0	1	0	0	5
Total of animal livers examined	24	28	27	32	35
Logistic Regression tests [1]	/	p=0.4569 NS	/	/	p=0.1399 NS

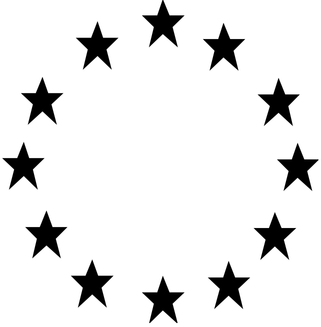
[1] Beneath each treated group is the p-value corresponding to the pairwise comparisons between the treated and control groups. All statistical tests conducted were one-sided tests.

NS : Not Significant

CONCLUSION

Comparison of liver/adenoma finding showed that all the treated groups were not significantly different from the control group in females.

Council Directive 91/414/EEC



Fluopicolide (AE C638206)

ADDENDUM 1 (FATE & ECOTOX ONLY)
TO THE DRAFT ASSESSMENT REPORT PREPARED BY THE UNITED KINGDOM

Draft: November 2007

PESTICIDES SAFETY DIRECTORATE
Mallard House, Kings Pool,
3 Peasholme Green,
York YO1 7PX, UK
Website: www.pesticides.gov.uk

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B.8 ENVIRONMENTAL FATE AND BEHAVIOUR

B.8.1 Route and rate of degradation in soil

Open point 4.2

"RMS to clarify normalized laboratory DT50's values for fluopicolide and metabolites, i.e., for fluopicolide in LoEP the range is 194 – 333 d when for example in Allan 2003 c study degradation in one soil results in a normalized DT_{50} = 373 d (or for another example 664 d for Lamberton soil in Allan 2003e). Please do it in an addendum or in an updated list of end points following the updated template where the origin of the different end points and normalization procedures may be easily tracked.

See reporting table 4(10)"

To clarify the values given in the list of endpoints, reference is made to DAR Volume 3, Section B.8.1.8, p. 715, Table B.8.142.

The values given in the LoEP for laboratory degradation rate of fluopicolide are values from the Applicants calculations, rather than from the RMS calculations. This the reason why the range given in the endpoints differs from the specific values stated in the Open Point 4.2 of 373 and 664 days which are from the RMS calculations. The differences between the outcome of the Applicant and RMS calculations can in part be explained by the fact that the Applicant used all data points, whereas the RMS only used data points from within the first 120 days of the study, given that this is the length of study specified by SETAC guidelines and that it is known that microbial viability of soils can become compromised over longer study duration periods. In addition, the DT50 of 664 days is extrapolated well beyond study duration and is associated with an r^2 value of only 0.583, i.e. below the 0.85 value specified in the 'Persistence Guidance Document' for acceptability for use in comparison with Directive persistence triggers, and below the 0.7 value specified for used in exposure modelling. If the 664 day DT50 is excluded due to low r^2 and results from 2 different radiolabels for the same soil in individual studies are geometrically meaned, the subsequent geometric mean of RMS calculated DT50 values is 260.5 days, comparable with the overall geometric mean of 271 days for the applicant's calculations.

The values quoted for the metabolites are also from the Applicant calculations.

The LoEP has been updated according to the latest template.

In relation to the point made above relating to the 'Persistence Guidance Document', the RMS recognises that whilst the kinetics and associated statistics for this evaluation were not derived in strict accordance with the FOCUS Degradation Kinetics guidance, the evaluation was conducted significantly before agreement/adoption of the guidance document. We are not convinced that applying the principles of the guidance document would significantly influence the endpoints selected for exposure assessment.

Route and rate of degradation in soil

Open point 4.5

"MS experts to discuss potential influence of the different extraction method employed on the respective results of the laboratory and field studies.

Applicant provided an explanatory note in the "Comments to the reporting table". To be considered by MSs experts in their discussion.

See reporting table 4(26)."

As a reminder to MS experts, lab studies used 3-4 extractions with acetonitrile/water at ambient temperature followed by an acetonitrile Soxhlet extraction. Field studies used 2 extractions of acetonitrile/water/formic acid under ambient conditions.

RMS notes the Applicants statement, however, the RMS has further investigated extraction in the lab studies. Considering the representative chromatograms presented in studies, (in the Allen, 2003c study), Soxhlet extractions at 369 DAT accounted for 14.2 – 23.3% AR, with fluopicolide accounting for 9.7 – 17.6% AR in the Soxhlet extracts. In the Allen, 2003b study, at 98 DAT Soxhlet extractions accounted for a further 5.4 – 6.1% AR as fluopicolide. Information relating to the amount of fluopicolide extracted with each successive ambient extraction in lab studies is not available.

RMS considers that in light of this information, there is still some uncertainty over the suitability of the extraction methods for the field dissipation studies and that this should discussed by MS experts with a view to obtaining an appropriate resolution.

B.8.1.3. Route and rate of degradation in soil - photolysis

Data Requirement 4.1

"Notifier to provide an estimation of soil photolysis half lives at other latitudes (i.e. 40 ºN and 45 ºN). Applicant indicated to submit a position paper (Report MEF-06/495) by April 2007.

See reporting table 4(14)."

Background:
Soil photolysis was performed by simulating irradiation in Scotland, (latitude 55°N). As fluopicolide is also intended for use in Southern EU Member States, further estimates were requested of the contribution of photolysis to soil degradation at latitudes around 40°N-45°N.

In the field dissipation studies (DAR B.8.1.8), fluopicolide was sprayed to bare soil surface. This also prompted discussion over the influence of photolysis on the results of field dissipation studies, compared to under normal conditions of use in the field and

the possible relevance of photolysis to the biphasic degradation observed, with faster degradation occurring in the initial period. (See reporting table point 4(42)).

RMS evaluation of new data:
Two photolysis studies were conducted using thin soil layers (sandy loam, *ca*. 3 mm depth, treated with [pyridyl-2,6-^{14}C]-labelled fluopicolide (Keirs and Lowrie, 2001) and [phenyl ring-U-^{14}C]-labelled fluopicolide (Mackie, 1999) and exposed to artificial sunlight for up to 15 days, at 20°C. These studies were assessed in the DAR, B.8.1.8.

Irradiated samples were exposed to continuous illumination (24 hours per day) under artificial sunlight. The level of irradiance was intended to be equivalent to the total radiation received in one summers day (5470 W*h/m^2*d) at East Lothian, Scotland (55°N). Assuming 12 hours of light per day (5470 W*h/m^2*d / 12 d), this gives an intended hourly irradiation value of 456 W/m^2 (or W*h/m^2*h).

Actual irradiance in the studies was measured using a Radialux meter, fitted with a global sensor to measure light intensity in the wavelength region 290-800 nm, at the start and end of the study.

Table 8.1 Study irradiance measurements (290-800 nm) (Keirs & Lowrie, 2001, Mackie, 1999).

Time (days)	[Pyridyl-2,6-^{14}C] labelled experiment (Keirs and Lowrie, 2001)		[Phenyl ring-U-^{14}C] labelled experiment (Mackie, 1999)	
	Irradiance (W/m^2)		Irradiance (W/m^2)	
	Pre-incubation	Termination	Pre-incubation	Termination
3 days	461	467	418	418
5 days	459	464	420	426
7 days	452	457	460	480
10 days	406	412	480	480
15 days	419	420	460	480
Median	455		460	

Hourly levels of irradiance during the study have been described by the applicant using the median values of 455 and 460 W/m^2 from Table 8.1 (pyridyl and phenyl labelled experiment, respectively). This irradiance or light intensity (W/m^2) measured between 290 - 800 nm only represents part of total light intensity (280 - 3000 nm). The applicant provided global radiation data (from CIE publication no. 20, 1972)[10] which gave a breakdown of percentage of total radiation for each wavelength range. For the wavelength bands 200-400 nm and 400-800 nm, the percentage of total radiation was 6.1% and 51.8%, respectively. Based on this the applicant assumed that (6.1+51.8%=) 57.9% of total light intensity falls in the wavelength range 200-800nm and that the

[10] CIE (1972): Empfehlung fur die Gesamtbestrahlungsstarke und die spektrale Verteilung kunstlicher Sonnenstrahlung fur Prufzwecke. Publication CIE, No. 20 (TC-2.2),

filtered light intensity measured in the study represents 57.9% of total light intensity, recalculated as below[11].

Total irradiance (W/m^2, 280-3000 nm) = measured irradiance (W/m^2, 290-800 nm)/ 0.579
Total irradiance = 455 or 460 W/m^2 / 0.579
Total irradiance = 785 or 794 W/m^2 (pyridyl or phenyl label, respectively)

The hourly or instantaneous solar radiation (W/m^2) was then converted into an energy yield of the solar radiation per day (kJ/m^2*d) by:

instantaneous total irradiance (W/m^2, 280 - 3000 nm) * 86400 (seconds/day) /1000
(note: 1 hour = 3600 seconds x 24 hour = 86400 seconds/day)

Assuming total irradiance of 785 W/m^2 or 794 W/m^2, in the above equation gives an energy yield of solar radiation of 67.82 and 68.64 MJ/m^2 per day, respectively for the [pyridyl-2,6-^{14}C] and [phenyl ring-U-^{14}C]-labelled experiments.

The applicant has recalculated the photodegradation rate of fluopicolide, based on these studies, assuming single exponential first-order kinetics using Excel Solver to obtain the best 'least squares fit'. Due to the variability of the recovery data, the soil residue data (%AR) were normalised for total recovery at each time point, with no correction for dark control residues, (since no significant decline was observed in the dark).

[11] using Chemtec, (1995): Solar radiation data - Handbook of Material Weathering, 2nd edition. Chemtec Publishing, Ontario, Canada.

Table 8.2 Decline of [pyridyl-2-6-^{14}C]-fluopicolide (normalised for total recovery) in soil photolysis.

Irradiated Samples

Time (days)	Total Recovery	Measured Fluopicolide	Normalised Fluopicolide
	% of applied radioactivity		
0	100.56	98.44	97.89
3	98.14	90.87	92.59
5	95.76	87.97	91.87
7	96.09	87.33	90.88
10	95.83	85.98	89.72
15	96.32	86.68	89.99

Non-Irradiated Samples

Time (days)	Total Recovery	Measured Fluopicolide	Normalised Fluopicolide
	% of applied radioactivity		
0	100.56	98.44	97.89
3	99.90	96.65	96.75
5	95.59	92.99	97.28
7	94.97	91.65	96.50
10	96.58	94.35	97.69
15	95.86	92.96	96.97

Table 8.3 Decline of [phenyl-2-6-^{14}C]-fluopicolide (normalised for total recovery) in soil photolysis.

Irradiated Samples

Time (days)	Total Recovery	Measured Fluopicolide	Normalised Fluopicolide
	% of applied radioactivity		
0	109.20	102.10	93.50
3	94.14	81.35	86.41
5	91.73	79.51	86.68
7	98.99	82.84	83.69
10	100.00	80.92	80.92
15	90.81	71.60	78.85

Non-Irradiated Samples

Time (days)	Total Recovery	Measured Fluopicolide	Normalised Fluopicolide
	% of applied radioactivity		
0	109.20	102.10	93.50
3	96.07	91.61	95.36
5	96.54	89.61	92.82
7	100.1	94.61	94.52
10	102.1	96.96	94.97
15	100.2	95.13	94.94

Table 8.4 Laboratory photolysis DT50 values and conditions for fluopicolide.

Radiolabel	Rate constant	DT_{50}	Fitting criteria			Instant. irradiance (290 - 800 nm)	Instant. total irradiance (280 - 3000 nm)	Solar energy yield
	(d^{-1})	(d)	(error of χ^2 in %)	(r^2)	(*B* value)	(W/m^2)	(W/m^2)	(kJ/m^2*d)
Pyridyl	0.0052	134.13	1.3	0.688	0.9997	454.5	785	67822
Phenyl	0.0111	**62.55**	3.1	0.909	0.9997	460	794	68643

The photodegradation DT50 values above have been independently verified by the RMS, with non-linear regression analysis in MS Excel Solver (SFO, no reps with fit). The DT50 of 62.55 days (i.e. the faster of the two photodegradation rates calculated, representing most photodegradation) was used in further modelling, this is worst case in the context of the applicant trying to demonstrate the impact of photolysis. Note that the calculated DT50 values are extrapolated well beyond study duration which may account, at least in part, for the apparently large difference in DT50 between the two radiolabelling positions.

To assess the influence of photodegradation in the overall degradation of fluopicolide under field conditions, the applicant ran simulations for fluopicolide in the FOCUS PEARL model with and without taking into account photodegradation. As FOCUS PEARL does not take into account photodegradation, a soil surface layer of 2 mm in which photochemical transformations may occur was implemented in the model. A comparison of the residues with depth and time was made for 2 FOCUS groundwater scenarios, Kremsmünster (48.03°N) and Sevilla (37.22°N) and one field dissipation trial, Philippsburg (49.14°N), (latter evaluated at DAR, B.8.1.5. (g) and B.8.1.7.(c)). No justification was provided for this particular selection of scenarios/ sites. However, the RMS presumes that the reason was that the 2 groundwater scenarios were relevant to the intended crops and that the Philippsburg site was chosen as it was a 5 year trial, with soil hydrology data being available.

A 2 mm soil surface layer was implemented in the FOCUS PEARL model to simulate photodegradation by increasing the biodegradation factor (f_r), which is usually set to 1 at the soil surface.

Biodegradation factor $f_r = (k_{soil} + k_{photo}) / k_{soil}$.

where k_{soil} is microbial degradation and k_{photo} is photodegradation, combined to represent total degradation in the top soil.

Therefore, photodegradation is considered as part of the total degradation in the top soil layer and is also connected to the moisture and temperature dependency used for the total degradation rate. The RMS is not convinced that photodegradation processes are influenced by soil temperature and moisture to the same extent as microbial degradation. The RMS considers that in this case, selection of a 2mm soil layer in which photolytic processes occur will have a relatively small influence to overall degradation. This is likely to be case for soil photolysis in practice. As photolytic rate could not be corrected for daily solar radiation values in the PEARL model, site

specific photolytic DT50 values were recalculated, taking into account mean solar radiation for approximately 4 months after application of fluopicolide.

The photodegradation DT50 of 62.55 days from the phenyl-label study, (laboratory solar energy yield 68643 kJ/m^2*d) was recalculated for site specific radiation using the formula below, (verified by the RMS):

DT50 actual = DT50 laboratory * solar energy yield per day of laboratory study / solar energy yield per day of specific site (e.g. in season of interest, in kJ/m^2*d)

e.g. for Kremsmünster
DT50 = 62.55d x 68643 kJ/m^2*d/ 16285 kJ/m^2*d = 263.65 solar days

Table 8.5 Site and season specific photodegradation DT50 values for fluopicolide based on laboratory photodegradation DT50 of 62.55 days at 68643 kJ/m^2*d.

Site	Period	Mean solar energy yield	Site specific photodegradation DT_{50}
		kJ/m^2*d	d
Kremsmünster	June – Sept.	16285 [F]	263.7
Sevilla	May – August	24907 [F]	172.4
Philippsburg	June – Sept.	13979 [K]	307.2

[F] daily mean values from standard FOCUS gw scenarios (Focus, 2000)

[K] daily mean values as used for kinetic field evaluation (Kley, 2003a)

The RMS notes that the time periods selected would represent greatest exposure to sunlight. The solar energy yield values given are referenced as from FOCUS 2000, presumably the MARS database and have been accepted as quoted. They appear comparable with values provided by the applicant for a range of 9 other locations (in the UK, EU, USA at latitudes from 36.80°N to 56.26°N) of 16000-27000 kJ/m^2*d, derived from the Solar Radiation Handbook of Material Weathering 2nd edition, Chemtec Publishing 1995.

Table 8.6 Irradiation in the laboratory* in relation to summer days.

Location	Latitude (°N)	Mean solar energy yield (KJ/m^2*d)	DT50 (solar days) Phenyl-label	DT50 (solar days) Pyridyl- label
Philippsburg	49.14	13979	307.15	650.76
Kremsmünster	48.03	16285	263.65	558.61
Sevilla	37.22	24907	172.38	365.24
Dundee (UK)	56.26	17,000	252.56	535.11
London (UK)	51.31	16,000	268.35	568.56
Vienna (Austria)	48.14	19,000	225.98	478.79
Zurich (Switzerland)	47.23	18,000	238.53	505.39
Portland (USA)	43.39	19,000	225.98	478.79
Boston (USA)	42.22	21,000	204.46	433.19
Philadelphia (USA)	39.53	21,000	204.46	433.19
Athens (Greece)	38.03	20,000	214.68	454.85
Tunis (Tunisia)	36.80	27,000	159.02	336.92

*(For phenyl-label suntest irradiation 1501.2 h equated to lab DT50 of 62.55 d. For pyridyl label suntest irradiation 3219.12 h equated to lab DT50 of 134.13 d).

The same standard soil degradation rates, equilibrium sorption coefficients and application schemes were assumed for running the FOCUS scenarios Kremsmünster and Sevilla, as were used in the groundwater assessment for fluopicolide (DAR, B.8.6.2). For the Philippsburg field dissipation site, the soil degradation rates (inverse evaluated (Kley, 2003a)), equilibrium sorption coefficients and application schemes specific for this soil and site were taken from the original kinetic evaluation of field dissipation studies (Kley, 2003a DAR, B.8.1.5.1). The applicant acknowledged that the $DT50_{field}$ values used could potentially have included an element of photodegradation as well as microbial degradation. However, they considered that any effect of the slow photodegradation on the $DT50_{field}$ rates of fluopicolide, occurring at the soil surface only, would be apparent in this evaluation, if significant.

Table 8.7 Parameters input for FOCUS PEARL simulations.

	Kremsmünster	**Sevilla**	**Philippsburg**
Crop	Vines	Vines	Bare soil
Application rate (g/ha)	3 x 133	3 x 133	400
Application dates	5 + 15+ 25 June	5 + 16+ 26 May	20 June 2000
Crop interception (%)	70, 70, 85	70, 70, 85	-
Soil $DT50_{field}$ (d)	138.8*	138.8*	108.56#
Site specific photolysis DT50 (solar days)[a]	263.7	172.4	307.2
Biodegradation factor (F_r) for upper 2 mm	1.526	1.805	1.353
Koc (Kom), mean	321.1 l/kg (186.2 l/kg)	321.1 l/kg (186.2 l/kg)	248.3 l/kg [b](soil specific)
1/n, mean	0.9028	0.9028	0.841 [b] (soil specific)

* (mean $DT50_{field}$, bare field). # (site specific $DT50_{field}$, from inverse DT50 evaluation, Kley, 2003a).
[a] worst case (faster $DT50_{lab}$) derived from data from study using phenyl-labelled fluopicolide.
[b] reported in DAR, Table B.8.152.

Model parameters:
Dispersion length (λ) was 5 cm and photolysis layer 2mm. FOCUS-PEARL 3.3.3 was run for Kremsmünster and Sevilla, and FOCUS-PEARL 1.1.1 was run for the field site, Philippsburg (soil hydrology manually calibrated).

The applicant presented depth profiles for individual time points (days 14, 60, 180, 240, 450 and 720) over 2 years for each of the FOCUS GW scenarios, with and without the 2 mm soil layer for photodegradation for comparison. Concentrations of fluopicolide in 50 cm soil depth over approximately 10-12 years were also reported. Dissipation curves for fluopicolide over 30 cm soil depth were also presented with and without photodegradation, for the field dissipation site, Philippsburg. The applicant concluded that there were no significant differences, with or without the additional photodegradation rate, observed for any of the scenarios over time.

Figure 8.1 Depth profiles of fluopicolide residues for Kremsmünster, (applicant calculated).

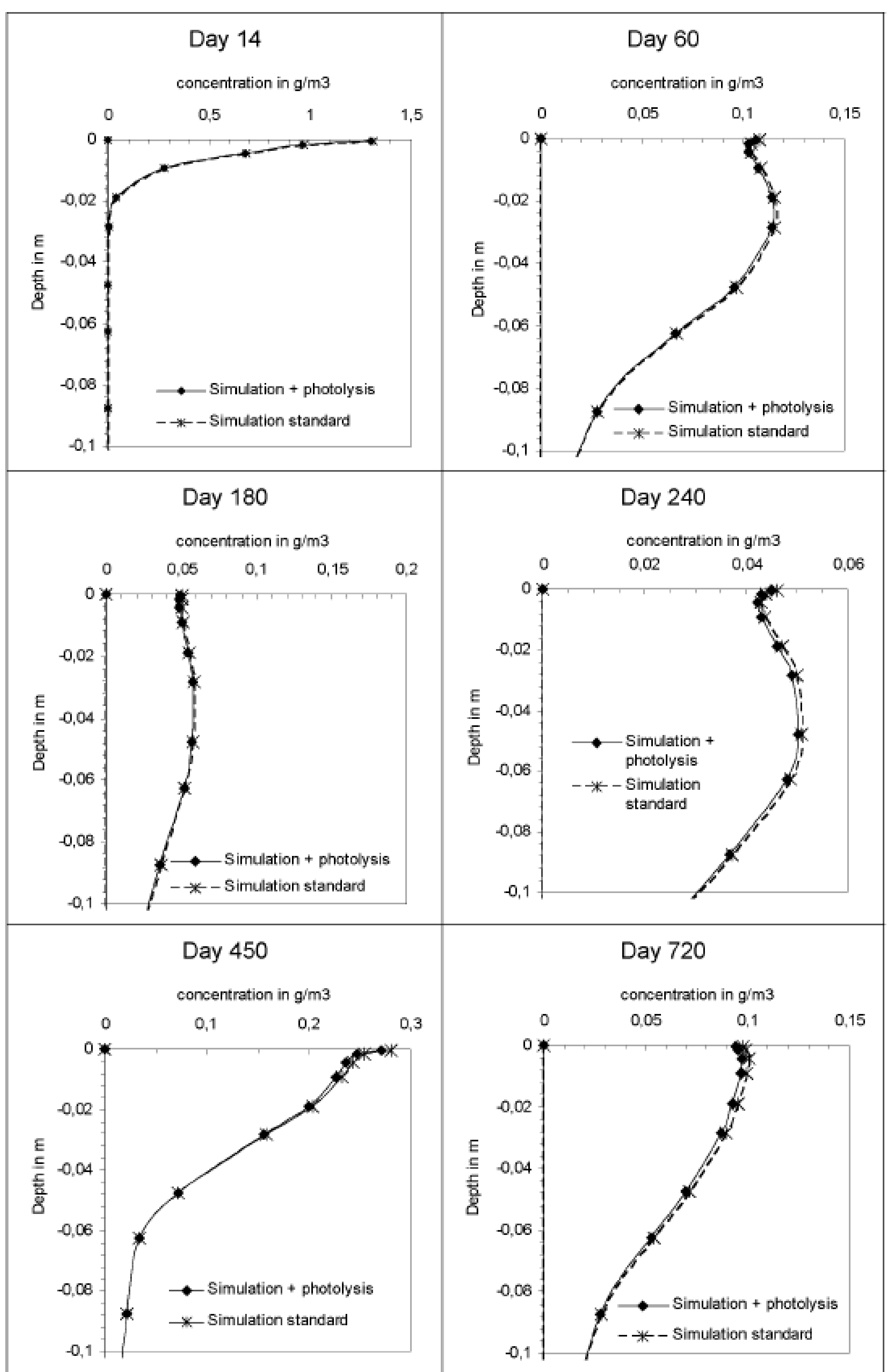


Figure 8.2 Depth profiles of fluopicolide residues for Sevilla (applicant calculated).

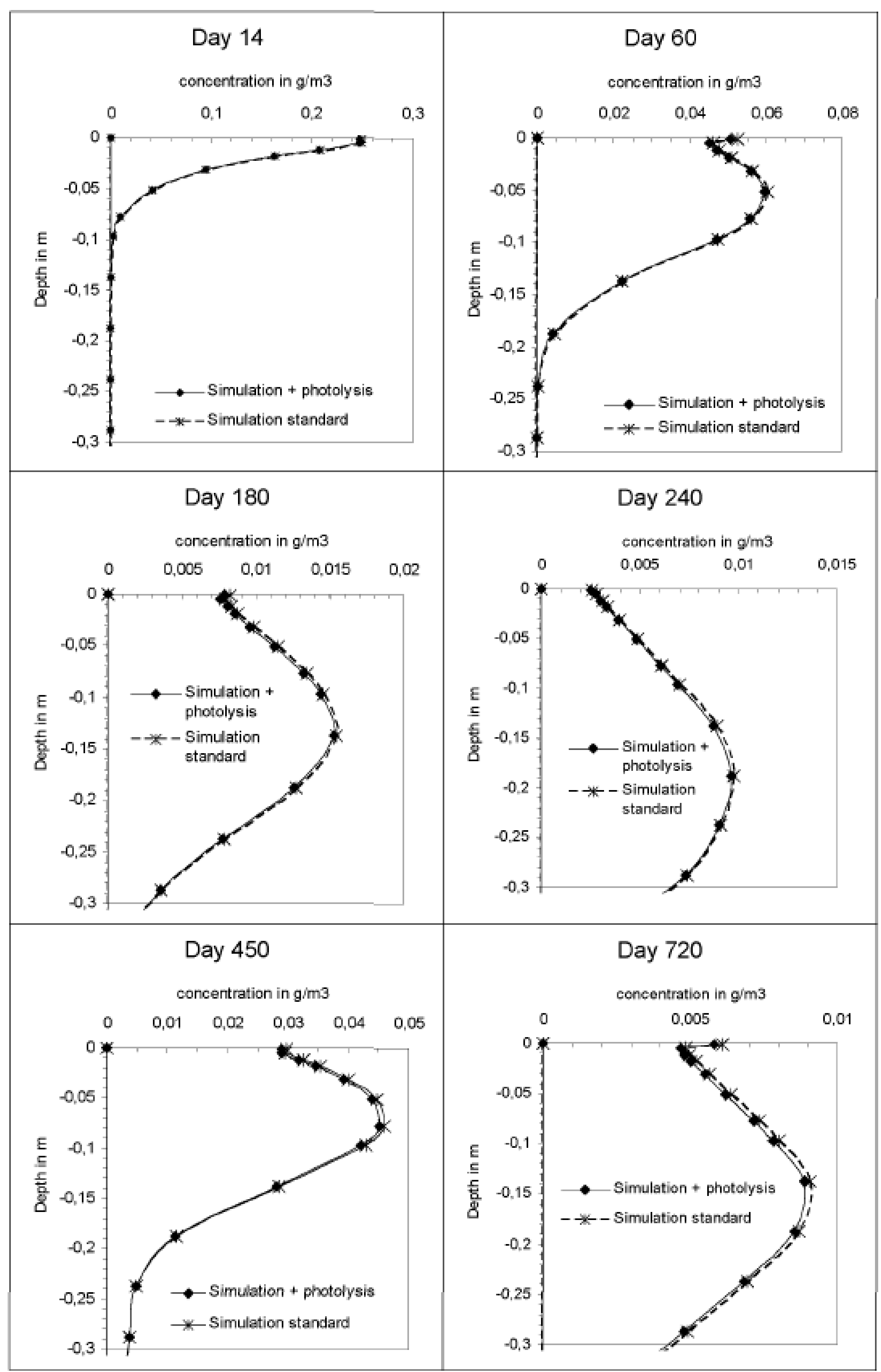


Figure 8.3 Concentration of fluopicolide residues in upper soil layers (50cm) for Kremsmünster scenario.

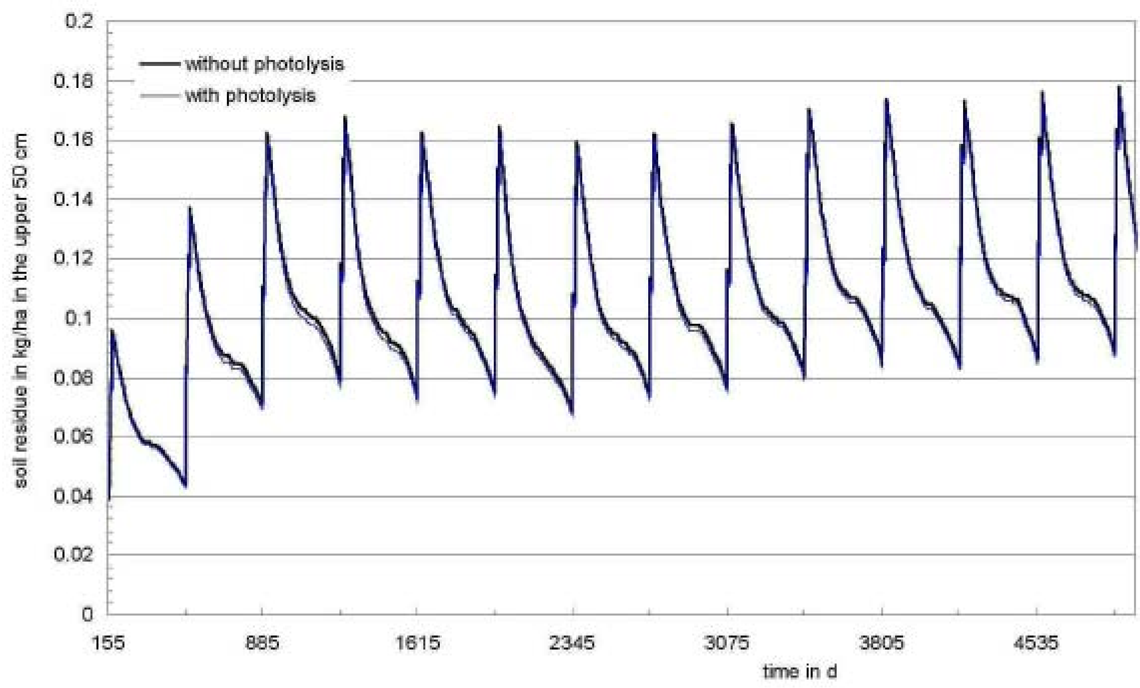


Figure 8.4 Concentration of fluopicolide residues in upper soil layers (50cm) for Sevilla scenario.

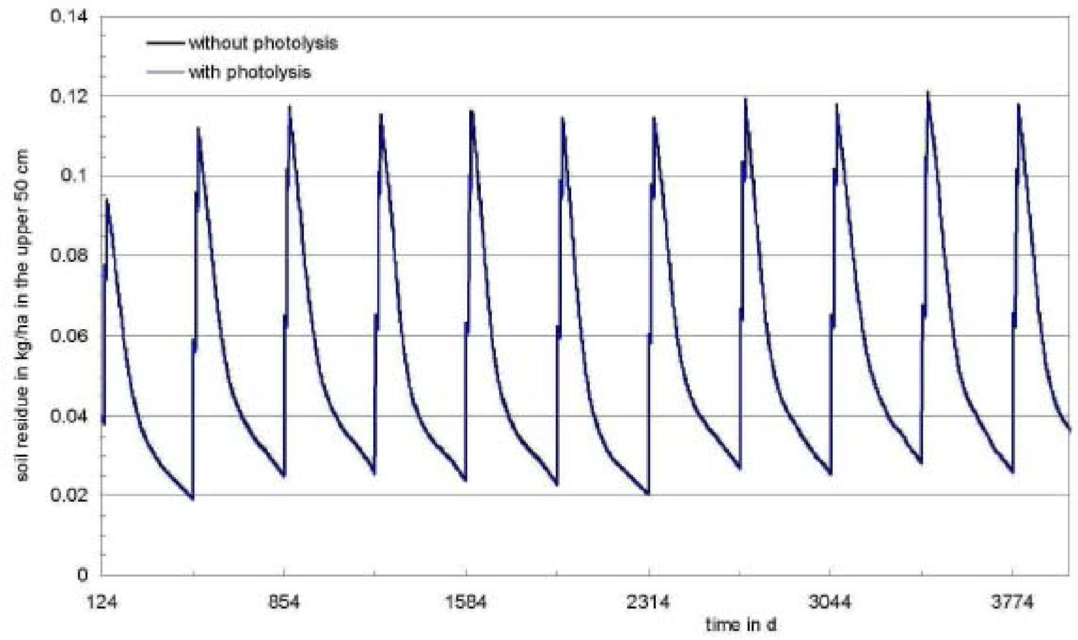
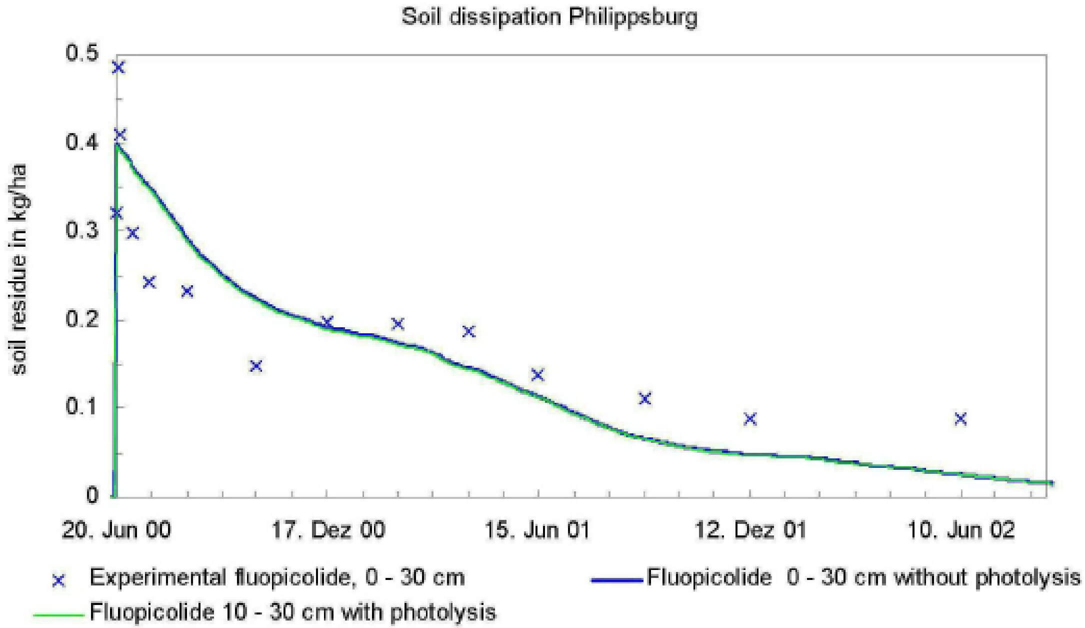


Figure 8.5 Concentration of fluopicolide residues in upper soil layers (30cm) for Philippsburg field dissipation trial.



The applicant concluded that photodegradation did not appear to contribute to the biphasic dissipation pattern seen in the field. Possible alternative explanations for the biphasic dissipation behaviour of fluopicolide in the field were proposed by the applicant, such as experimental artefacts, seasonal climatic changes, or the effect of time dependent sorption.

Experimental artefacts may be due to insufficient soil homogenisation or compression of the soil due to cultivation methods, which could cause artificially high residues at the start of the study. However, the applicant considered that if this explanation was likely it would be expected that biphasic degradation would be seen in only some trials, with other trials appearing to follow SFO kinetics.

The applicant considered that climatic changes were a possible explanation, as the degradation of fluopicolide in the field was moisture and temperature dependent. Degradation occurred more rapidly initially in spring/summer, then slowed over colder winter months.

The effect of time dependent sorption contributing to biphasic dissipation was also investigated by the applicant. The full assessment of this investigation is described later in this Addendum, at B.8.6.2 in the context of the groundwater assessment.

To summarise, sorption of pesticides on soil is described with a Freundlich-type equation. It is assumed in the FOCUS PEARL model that degradation and sorption in soil may be described by both instantaneous (equilibrium sorption) and long-term or gradual sorption processes (non-equilibrium sorption). Transformation of active substance is assumed to only occur in the equilibrium domain, with slow release of compound from the non-equilibrium domain.

The applicant provided additional reports (Kley 2004, MEF-04/346 and MEF - 04/347) describing their approach to calculating a degradation rate constant specific to the equilibrium domain (k_t) and kinetic sorption parameters for use in the PEARL model, (kinetic-sorption rate constant, k_d and the ratio between the Freundlich coefficients in the non-equilibrium and in the equilibrium domain, f_{ne}).

In these reports the applicant fitted the data from previously assessed studies to a kinetic-sorption model implemented in FOCUS-PEARL, using ACSL Optimise 1.2, (Kley 2004, MEF 04/346, Addendum B.8.X). The data used for fitting, were derived from two studies on the effect of ageing on sorption, which were reported in the DAR at B.8.2.1(c/d), (though they were not considered essential to the risk assessment in the original DAR, Allan, 2003b; Fitzmaurice, 2003). This approach was then also applied to the field dissipation data from 6 trials (reported at DAR B.8.1.5.) to derive a more realistic field degradation rate constant specific to the equilibrium domain, for use in the PEARL model, (Kley 2004, MEF 04/347, Addendum B.8.6.2)

The optimised parameters for the kinetic-sorption rate constant (k_d) and the ratio between the Freundlich coefficients in the non-equilibrium and in the equilibrium domain (f_{ne}) are shown in Table 8.8.

Table 8.8 Evaluated parameters of the kinetic-sorption model of all soils in the laboratory time dependent sorption study (Kley, 2004, MEF-04/346)

Soil	k_d (d^{-1})	f_{ne}
Philippsburg	0.0589	0.4692
Rödelsee	0.0835	0.3701
Huntlosen	0.1950	0.3657
Senas	0.1075	0.3230
Abington	0.0362	0.4485
Arithmetic mean	-	0.3953
Geometric mean	0.08211	-

A biphasic dissipation pattern may result from kinetically controlled sorption, due to the combination of degradation rate in the equilibrium domain (k_t) and the rate of transfer from the non-equilibrium to the equilibrium domain (k_d). The mean ratio between the Freundlich coefficients in the non-equilibrium and equilibrium domain (f_{ne}) calculated for fluopicolide was 0.395. The applicant claimed that this indicated that fluopicolide underwent a moderate, but measurable kinetic sorption with time, with a kinetically controlled "sorption capacity" of about 40% of the instantaneous "sorption capacity". (i.e. 60% of applied residue is available for degradation in the equilibrium domain, compared with 40% of applied residue in the non-equilibrium domain, where no degradation is assumed).

RMS Risk Assessment and Conclusions:

The RMS concludes that soil photolysis at more southerly latitudes is unlikely to significantly influence the degradation of fluopicolide. Kinetic adsorption aspects, if implemented into FOCUS modelling of environmental exposure, would be likely to result in lower peak and annual average concentrations.

Implications for Ecotoxicogical Assessment:

No change from the relevant endpoints reported in the DAR.

(Kley, C; Mackenzie, E; MEF-06/495, 2007)

B.8.1.7 Route and rate of degradation in soil – field soil accumulation

Data Requirement 4.2

"Applicant to present the position paper with their evaluation of the accumulation studies. Applicant indicated to submit a position paper assessing the field accumulation studies (Kley, C; Mackenzie, E.; Report no. M-267721-01-1) by April 2007.

See reporting table 4(41)."

Background:
The applicant has submitted a position paper presenting further evaluation of the field accumulation studies, (originally assessed at DAR B.8.1.7. and B.8.1.8), in response to the conclusion of the RMS that residues of fluopicolide and M-01 had not reached a plateau at study termination in the trial at Appilly and that results at Senas were inconclusive. The applicant has also submitted the time-points at which maximum concentrations were estimated to be reached, not previously given. (See reporting table, points 4(41), 4(51) and 4(73)).

RMS Evaluation of new data:
Field dissipation/ accumulation trials with fluopicolide were conducted over a 4 year period at sites in Philippsburg (Southern Germany), Appilly (Northern France) and at Senas (Southern France). Concentrations of fluopicolide and its metabolites, M-01, M-03 and M-02, in soil were measured following repeated annual applications of 400 or 500 g/ha p.a. to bare soil. See the assessment in the DAR at B.8.1.7. and B.8.1.8 for further details.

These data have since been evaluated further by the applicant to assess whether the plateau concentrations of fluopicolide measured in the field were reached after 4 years, or if further increases would be expected in successive years. The accumulation potential of fluopicolide and its metabolite M-01 have been evaluated at each site, using SFO kinetics. Metabolite M-01 was shown to have potential to be mobile and persistent. The metabolite M-02 was only detected at a few time points at low levels and metabolite M-03 was only detected in acidic soils, being degraded rapidly in soils with pH>7. Throughout this evaluation the applicant converted concentrations in mg/kg to g/ha for the total soil depth assuming a soil density of 1.5 g/cm^3.

PHILIPPSBURG
(S. Germany, loamy sand, pH 6.4 and 0.27% oc content)

Fluopicolide was applied annually as detailed below. The applicant measured the unused formulation remaining in the spray tank to confirm the actual amount applied ('calibrated application rate'). Three plots, each 3 m x 26 m, were treated with fluopicolide and a fourth plot left untreated as a control. The treated plots were subdivided into separate areas for the dissipation phase treated once in the first year and for the accumulation phase treated annually for up to 5 years. Details of the treatment and sampling areas were provided. Samples for the dissipation phase were taken for up to 2 years after the first application. Samples for the accumulation phase were taken

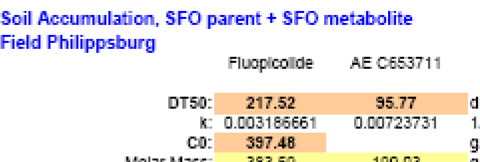
immediately after application and at 4 and 12 months after each application, with the final sample taken immediately after the 5th application.

Table 8.9 Application schedule at Philippsburg

Application Date	Days after treatment	Nominal application rate (g/ha)	Calibrated application rate (g/ha)
20 June 2000	0	400	411
24 July 2001	399	400	422
26 June 2002	736	400	398
05 June 2003	1080	400	423
06 July 2004	1478	400	418

The applicant converted the concentrations from the field (mg/kg, individual replicate values for total soil depth, including below 20 cm) into g/ha over 10 cm depth then derived the average g/ha value. The same approach was taken for metabolite M-01, but also assuming that parent compound was 100% transformed to M-01 and residues were converted to a.s. equivalents (by correction for molecular weight differences).

Figure 8.6 Philippsburg Dataset



Soil Accumulation, SFO parent + SFO metabolite
Field Philippsburg

	Fluopicolide	AE C653711	
DT50:	217.52	95.77	d
k:	0.003186661	0.00723731	1/d
C0:	397.48		g/ha
Molar Mass:	383.59	190.03	g/mol
Formation fraction:		1	

soil residues assuming BD = 1.5

	Measured field data			Simulated data		Square of differences	
Application	Time d	Fluopicolide g/ha	AE C653711 g/ha ai eq.	Fluopicolide g/ha	AE C653711 g/ha ai eq.	Fluopicolide	AE C653711
20/06/2000	0	318	0	397.48	0.00	6317	0
400 g/ha nominal	1	486	33	396.21	1.26	7972	1007
	3	400	39	393.70	3.74	40	1243
	14	294	38	380.14	16.49		
	36	235	62	354.40	37.83		
	62	225	72	326.22	57.00		
	120	147	64	271.17	82.12		
	181	191	63	223.26	91.27		
	244	190	16	182.65	90.21		
	308	186	0	148.96	83.53		
	367	135	18	123.43	75.14	123	3265
24/07/2001	399	629	99	508.94	70.27	14415	826
	525	213	141	340.63	135.35		
	762	206	75	160.06	130.83	2064	
26/06/2002	736	657	64	571.37	108.00	7333	1936
	857	300	171	388.56	163.42		
	1078	226	86	192.13	122.42	1113	1326
05/06/2003	1080	485	100	588.39	121.87	10690	478
	1203	298	274	397.60	172.77		
	1448	198	188	182.13	119.51	236	4692
07/07/2004	1478	435	152	563.00	111.08	16384	1674

$\sum(P-O)^2$ 83134

Where;
P=predicted value
O=observed value

The concentrations of fluopicolide and M-01 in soil after annual applications of fluopicolide are shown in Figures 8.7-8.9. Results for both mean and individual plots were presented for fluopicolide. The mean results of the 3 plots were also provided for M-01, though not the individual plots. The final 3 data points (461, 546, 735 DAT) from the second year of the dissipation phase of the study (September 2001-June 2002) were excluded, as they overlapped with the start of the accumulation phase (July 2001) and were not needed to assess the accumulation plateau.

The maximum concentration of fluopicolide was detected immediately after the second application (2001) and was a similar level after the third application (2002). The individual plots are presented separately. The applicant stated that the measured $C_{high\ max}$ and $C_{low\ max}$ values for fluopicolide appeared to reach a plateau and that there appeared to be a tendency for accumulation of the metabolite M-01 over the course of the study, with a plateau not being reached. The RMS notes that while the $C_{high\ max}$ value for fluopicolide seems to have reached a plateau in individual plots, the $C_{low\ max}$ decreased only at last point and slightly increased at the end for plot, T2.

Figure 8.7 Concentration of fluopicolide at Philippsburg (g/ha for total soil depth) (Mean of 3 individual treated plots T1, T2 and T3)

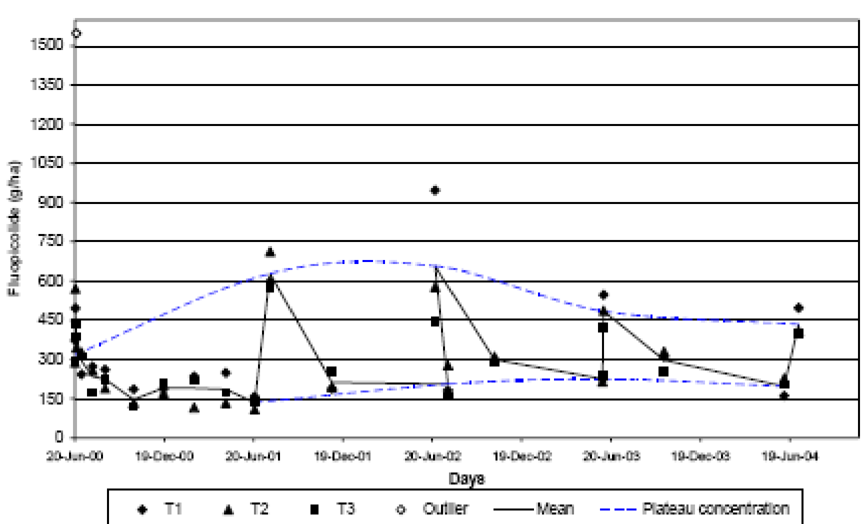


Figure 8.8 Concentration of M-01 at Philippsburg (g M-01 /ha for total soil depth) (Mean of 3 individual treated plots T1, T2 and T3)

NB Scale different.

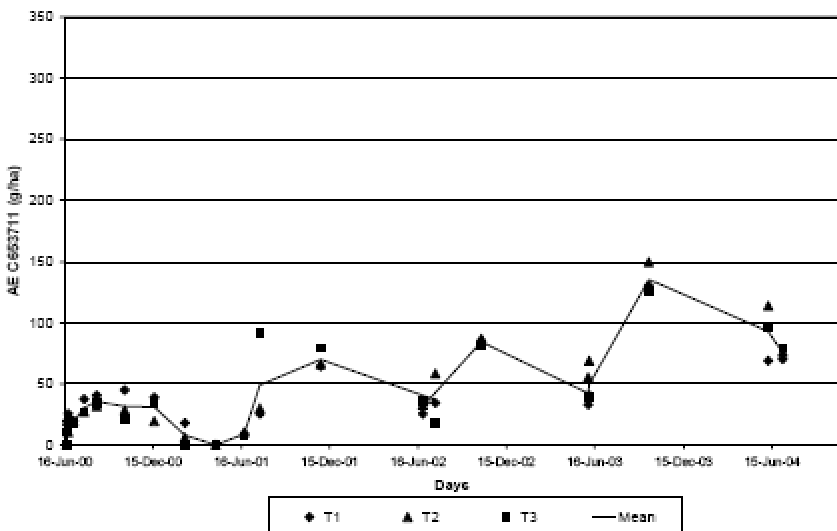
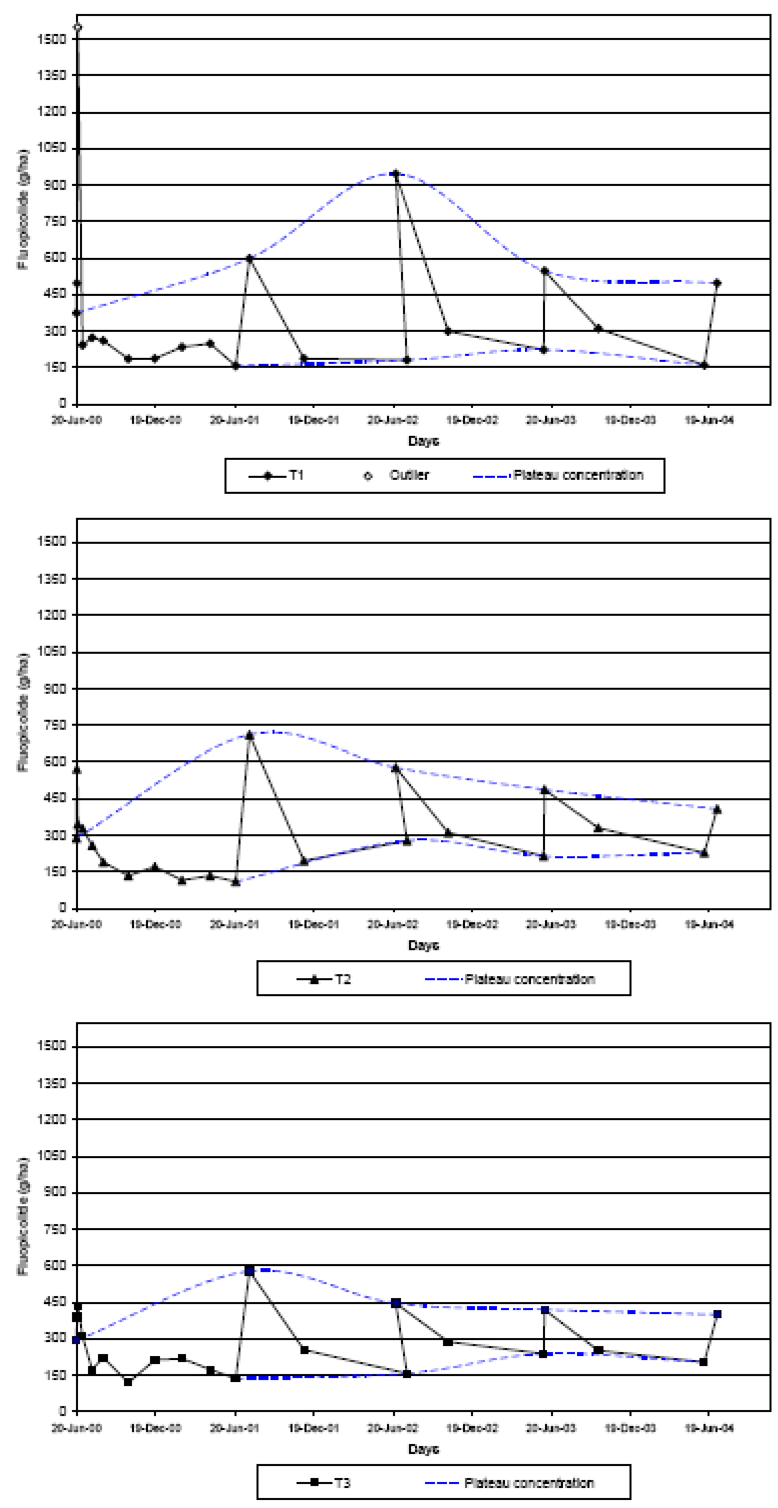


Figure 8.9 Concentration of fluopicolide at Philippsburg in 3 individual plots (T1, T2 and T3) (g/ha for total soil depth).



The plateau concentrations after 4 years were calculated by the applicant based on residues found in the 0-10 cm soil depth only, or by summing and then averaging the levels of residues found in the 0-10 cm and 10-20 cm layer.

Table 8.10 Plateau concentrations of fluopicolide at Philippsburg.

Plateau concentration	Time-point	Measured in soil increments (mg/kg)	
		0-10 cm	0-20 cm
High[1]	Day 0 2nd Application	0.341	0.191
Low[2]	Day 368 after 4th Application	0.094	0.064

[1] maximum of the high values of the "saw teeth" curve
[2] maximum of the low values of the "saw teeth" curve

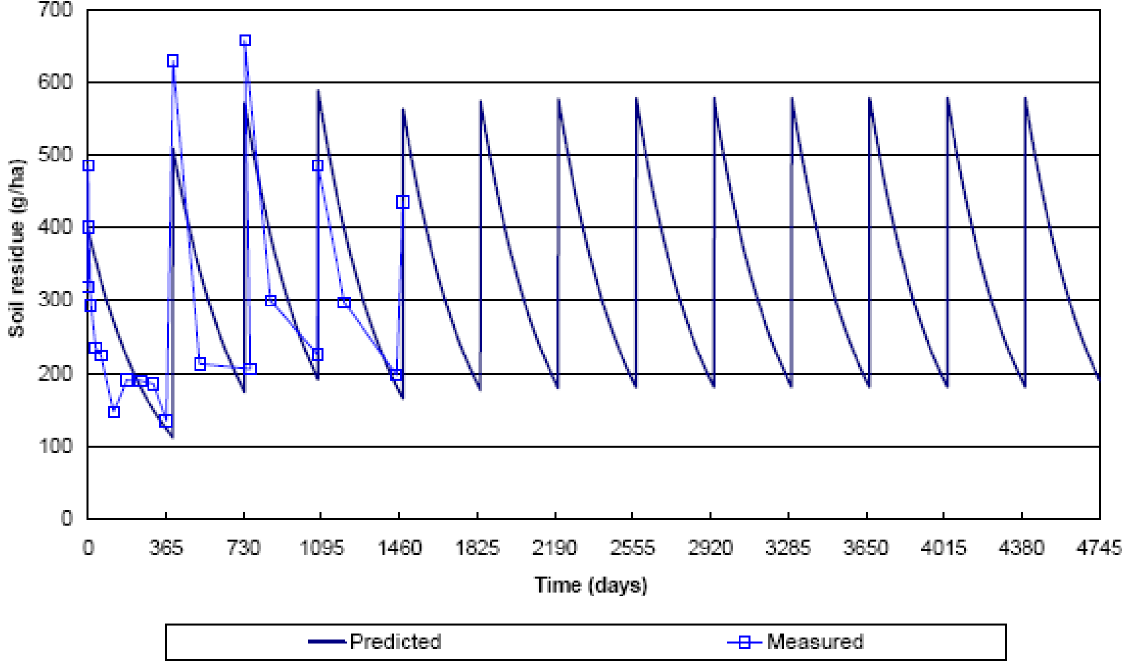
These plateau concentrations are as reported previously in the DAR. The degradation rate of fluopicolide in soil under field conditions was claimed to be moisture and temperature dependent, with faster degradation in spring and summer, followed by slower degradation in winter months. The applicant used simple first order (SFO) evaluation to describe the upper and lower concentration of the 'saw teeth' curve during the accumulation period and to calculate daily concentrations of fluopicolide in soil for each of the sites. However, it was noted by the applicant (and accepted by the RMS in the DAR) that SFO kinetics were not always the best fit for decline of fluopicolide between applications. In the DAR assessment of the field trials, biphasic (Hockey Stick) kinetics were reported as the best fit of decline at the Philippsburg and Apilly sites and SFO kinetics at the Senas site.

To simulate potential accumulation in further successive years, SFO degradation rate constants for fluopicolide (k_1) and M-01(k_2) plus the initial soil residue of fluopicolide applied annually (C_0) were optimised by the applicant using an Excel spreadsheet. The parameters derived for each dataset, k_1, k_2 and C_0, represented overall values for the 4 years. Best overall fit was reported to be derived with Excel Solver using least squares optimisation of the fluopicolide and M-01 soil concentrations measured immediately after each application ($C_{high\ max}$) and the residue remaining each year prior to application ($C_{low\ max}$). There was no detailed statistical assessment of the fit presented clearly in the study report. The optimised SFO degradation rates and annual application rate (C_0) were used in a predicted simulation of fluopicolide and M-01. Actual application dates at each site were used, with following applications at 365 day intervals; C_0 was added to the predicted soil concentration remaining immediately prior to the application date.

The predicted plateau values, $C_{high\ max}$ and $C_{low\ max}$, at each site were compared with the experimental data. At Philippsburg, the applicant reported that concentrations of fluopicolide in soil reached a plateau during the accumulation trial. SFO kinetics was claimed to give a good fit to the measured $C_{low\ max}$ values. The predicted initial concentration of 397 g/ha was close to the nominal/ calibrated rate of ca. 400 g/ha p.a. The predicted $C_{high\ max}$ values differed from the soil concentrations measured immediately after application. The RMS notes that the $C_{high\ max}$ values for year 2 and 3 appear to be under predicted, but then for year 4 and 5 are over predicted. The applicant attributed this to variations resulting from sampling and homogenisation

processes. Concentrations measured 1 and 3 days after the 1st application (2000) as well as the initial measured soil residue were included in the optimisation procedure.

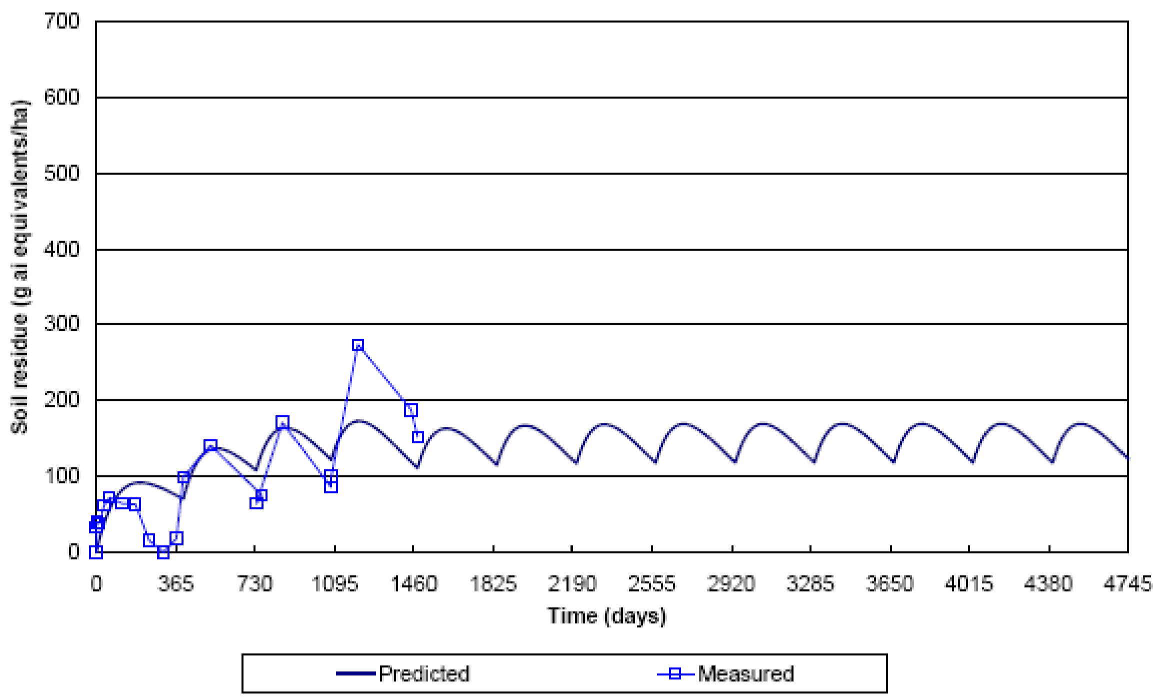
Figure 8.10 Fluopicolide residues at Philippsburg



Predicted $C_{low\ max}$ values were calculated on dates immediately prior to the application dates, which did not always occur in practice, as some measured $C_{low\ max}$ samples were taken earlier than the next application date. At the end of Year 2 (22 July 2002, 762 days) $C_{low\ max}$ samples were taken after the 3rd application (26 June 2002, 736 days), this was made to a different area of the replicate plots, so did not affect the Year 2 sampling.

The applicant concluded that fluopicolide concentrations in soil increased slightly, then reached a plateau during the accumulation study, but that repeated applications were not predicted to result in further increases in soil concentration beyond the duration of the trial. The RMS agrees that accumulation of fluopicolide in soil is not predicted beyond the duration of the study trial at Philippsburg. The predicted plateau concentration was reached by the 5th year (predicted peak plateau concentration 578 g/ha and steady state concentration of 181 g/ha, equivalent to 0.385 mg/kg and 0.121 mg/kg over 10 cm, respectively).

Figure 8.11 M-01 residues at Philippsburg



The fit to the measured concentrations of metabolite M-01 was considered reasonable by the applicant for the 2nd and 3rd years, but in the 1st and 4th years concentrations were over and under predicted, respectively. The RMS considers that the results are not sufficient to conclude that the metabolite M-01 will not accumulate in soil following repeated use of fluopicolide at this site.

Table 8.11 Results of SFO evaluation at Philippsburg

Fluopicolide	
Initial concentration (C_0)	397 g/ha
SFO rate constant (k)	0.00319 d^{-1}
DT50	217.5 days
$C_{high\ max}$	578 g/ha *(0.385 mg/kg over 10 cm)*
$C_{low\ max}$	181 g/ha *(0.121 mg/kg over 10 cm)*
M-01	
DT50	95.8 days
SFO rate constant (k)	0.00724 d^{-1}
$C_{high\ max}$	169 g as equivalents (84 g M-01/ha)
$C_{low\ max}$	118 g as equivalents (58 g M-01/ha)

APPILLY

(S. France, sandy silt, pH 7.1 and 1.51%oc content)

Fluopicolide was applied annually as detailed below.

Table 8.12 Application schedule at Apilly

Application Date	Days after treatment	Nominal application rate (g/ha)	Calibrated application rate (g/ha)
16 June 2000	384	400	397
27 Aug 2001	437	400	413
17 July 2002	761	400	410
18 June 2003	1097	400	382
30 June 2004	1475	400	400

Figure 8.12 Apilly dataset

Soil Accumulation, SFO parent + SFO metabolite
Field Appilly

	Fluopicolide	AE C653711	
DT50:	312.86	150.35	d
k:	0.002215526	0.004610365	1/d
C0:	306.20		g/ha
Molar Mass:	383.59	190.03	g/mol
Formation fraction:		1	

soil residues assuming BD = 1.5

	Measured field data			Simulated data		Square of differences	
	Time	Fluopicolide	AE C653711	Fluopicolide	AE C653711		
Application	d	g/ha	g/ha ai eq.	g/ha	g/ha ai eq.	Fluopicolide	AE C653711
16/06/2000	0	384	0	306.20	0.00	5975	0
400 g/ha	1	319	0	305.52	0.68		0
	3	317	24	304.17	2.01		
	14	338	43	296.85	9.05		
	31	397	50	285.88	18.92		
	62	230	38	266.90	34.07		
	136	147	26	226.54	58.26		
	167	129	20	202.34	67.57		
	245	140	25	177.94	73.07		
	309	141	19	154.41	74.70		
	370	108	29	134.89	73.35	734	1967
27/08/2001	437	361	38	422.49	69.80	3842	1011
	584	317	56	305.05	119.19		
	798	165	88	189.87	137.01	619	
17/07/2002	761	493	93	512.29	118.58	372	654
	889	304	150	385.80	159.95		
	1097	252	188	243.35	149.63	75	1472
18/06/2003	1097	541	107	549.55	149.63	73	1817
	1217	295	177	421.25	183.39		
	1475	240	185	237.85	157.24	5	771
30/06/2004	1475	590	174	544.05	157.24	2112	281
						$\sum(P-O)^2$	21781

Where;
P=predicted value
O=observed value

Figure 8.13 Concentration of fluopicolide at Apilly (g/ha for total soil depth) (Mean of 3 individual treated plots T1, T2 and T3)

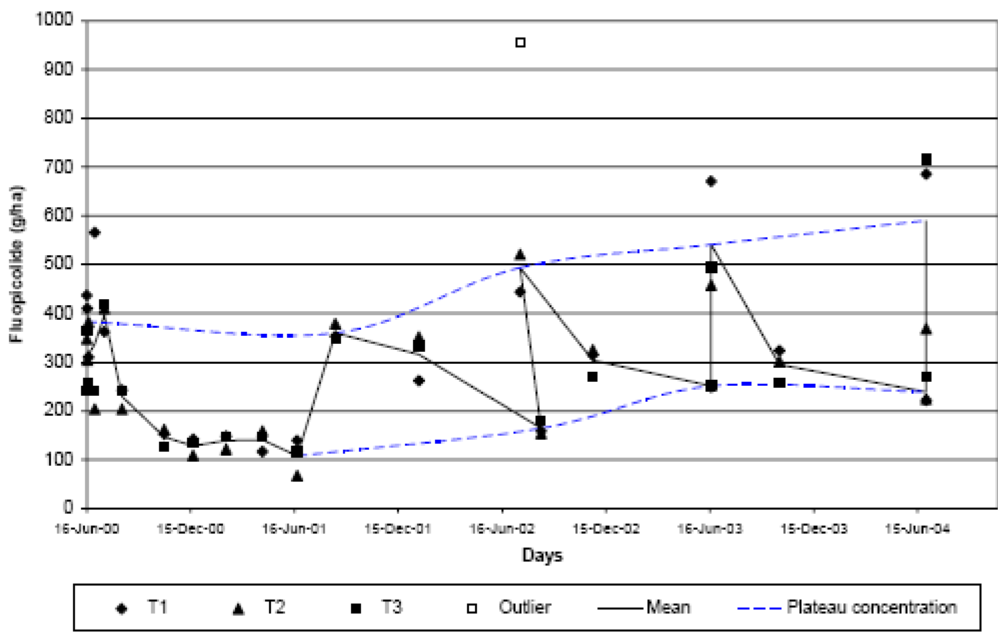
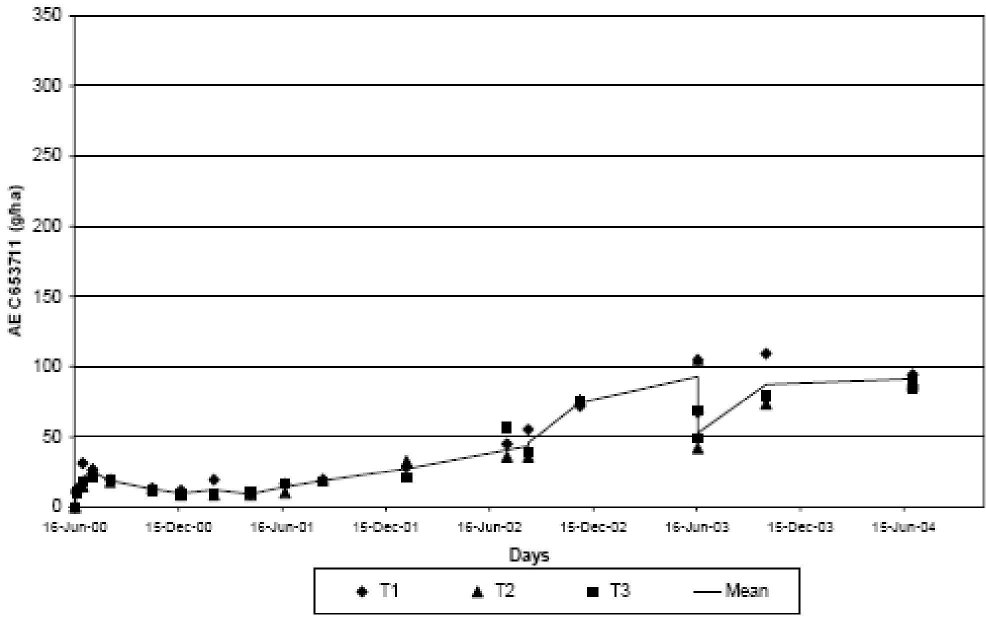


Figure 8.14 Concentration of M-01 at Apilly (g/ha for total soil depth) (Mean of 3 individual treated plots T1, T2 and T3)

NB Scale different.



The maximum mean concentration of fluopicolide detected in soil was immediately after the 5th application (2004). The applicant claimed that although the upper limit of the 'saw teeth' curve still appeared to increase, the plateau concentration at the lower limit had been reached. The applicant stated that the results for individual plots

showed good replication between the plots immediately prior to each application and that the $C_{low\ max}$ values reached a plateau by the study end. There was some variation in concentrations immediately after application ($C_{high\ max}$) between replicate plots, $C_{high\ max}$ appeared level at last point in plot T1, slightly declined in plot T2 and increased in plot T3, (in which one of the applications was excluded as an outlier). The applicant noted that this was compared to good replication at later time points and attributed the variation to the uncertainties of sampling and homogenising soil samples after application when residues were only present in the top ≤1 cm layer of soil core. The applicant concluded that measured $C_{high\ max}$ values reached a plateau in two of the three experimental plots. The RMS notes that for individual plot T3, the $C_{low\ max}$ showed a very slight increase and although for plots T1 and T2, $C_{low\ max}$ decreased by the study end, this was only at the last sample point. The RMS does not consider that there is sufficient evidence to show that a plateau concentration was reached at this site at two of the three plots.

The RMS considers there is insufficient evidence to show that the metabolite M-01 had reached a plateau concentration at this site during the study.

Figure 8.15 Concentration of fluopicolide at Apilly in 3 individual plots (g/ha for total soil depth) (T1, T2 and T3) (g/ha for total soil depth).

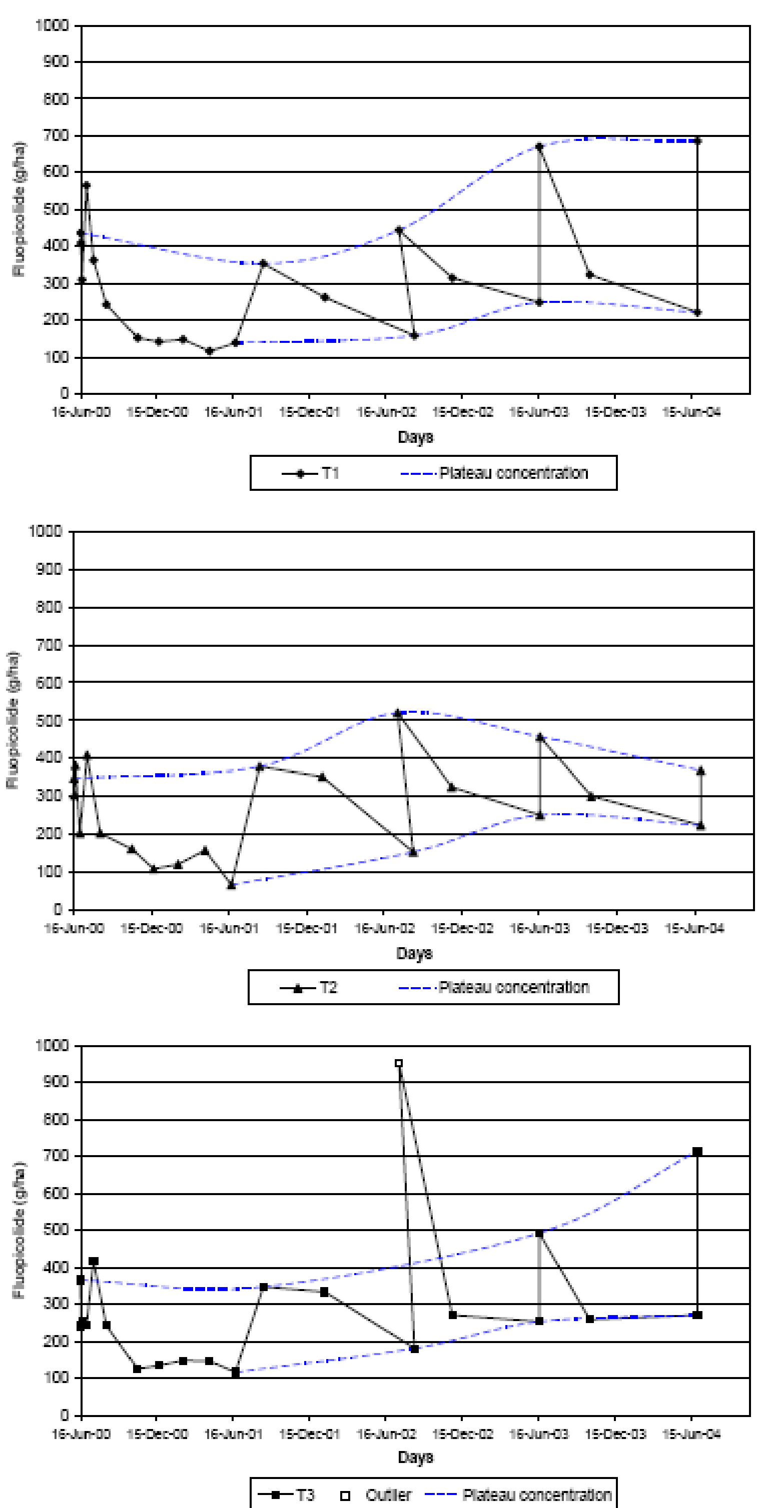


Table 8.13 Plateau concentrations of fluopicolide at Apilly

Plateau concentration	Time-point	Measured in soil increments (mg/kg)	
		0-10 cm	0-20 cm
High[1]	Day 0 5th Application	0.387	0.199
Low[2]	Day 378 after 4th Application	0.144	0.080

[1] maximum of the high values of the "saw teeth" curve
[2] maximum of the low values of the "saw teeth" curve

These plateau concentrations are the same as reported in the DAR, except for 0-20 cm (High = 0.196 mg/kg in DAR).

The applicant concluded from comparison of modelling predictions with measured values that concentrations of fluopicolide in soil reached a plateau during the accumulation trial. The predicted initial concentration at 306 g/ha was lower than the nominal and calibrated rates of *ca*. 400 g/ha p.a. SFO kinetics were reported by the applicant to give a good fit to the measured $C_{low\ max}$ values and a reasonable fit to the measured $C_{high\ max}$ values. There was no detailed statistical assessment of the fit presented clearly in the study report. The RMS notes that the last measured $C_{high\ max}$ value was under predicted.

Figure 8.16 Fluopicolide residues at Apilly

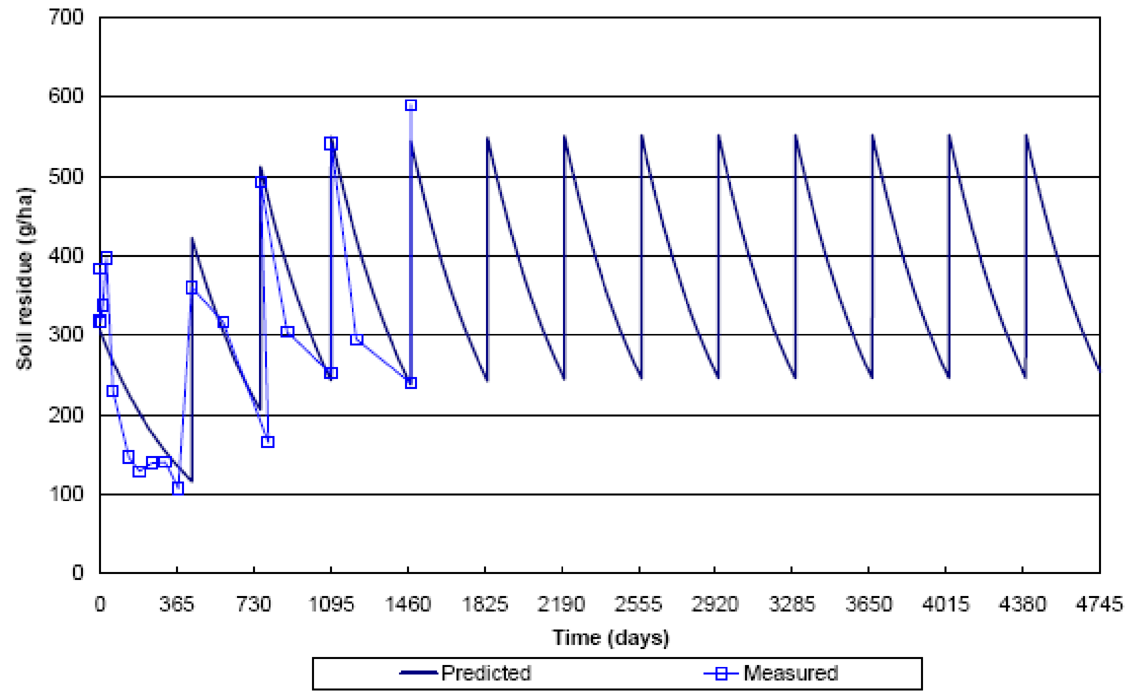
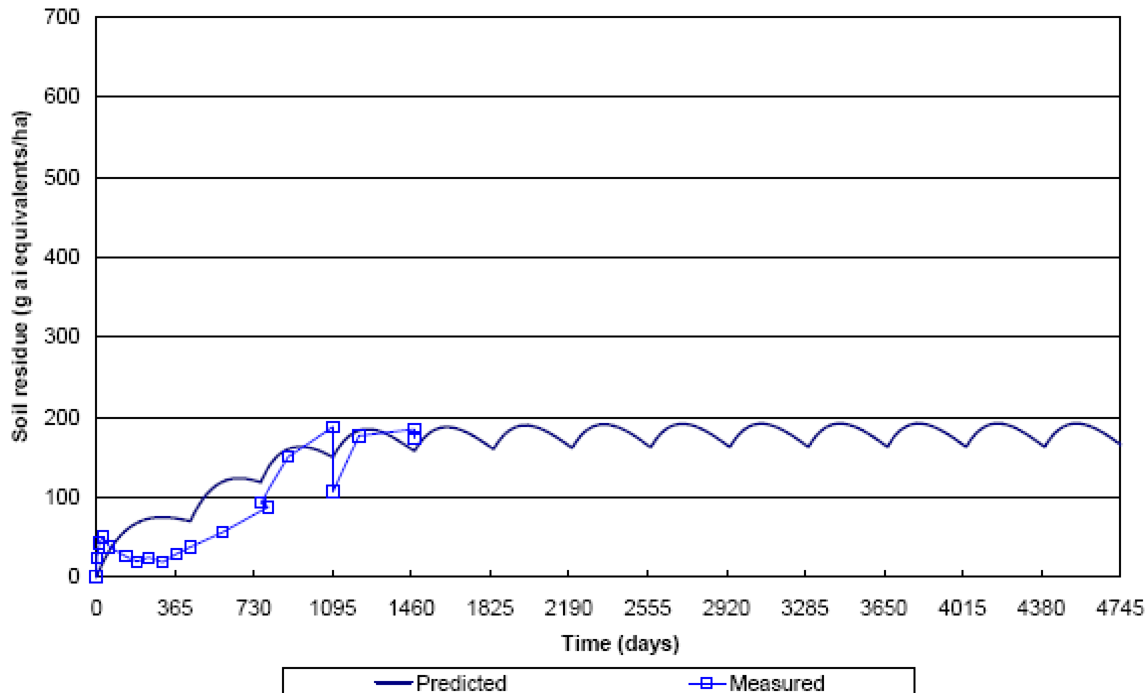


Figure 8.17 M-01 residues at Apilly



Sampling dates for the measured and predicted $C_{low\ max}$ differed, with measured values in practice being taken earlier or later than the application date. The final Year 1 sample was taken on 21 June 2001 (Day 370), before the 2nd application on 27 August 2001 (Day 437) and the final sample in Year 2 was taken on 23 August 2002 (Day 798) after the 3rd application on 17 July 2002 (Day 761). However, the plot layout allowed these samples to be unaffected by the subsequent applications.

Although accumulation of fluopicolide residues was seen, the applicant concluded that a comparison of predicted and measured concentrations confirmed that a plateau was reached during the study, and modelling did not predict further increases in successive years. The applicant's conclusion relied particularly on the $C_{low\ max}$ values. Based on the measured $C_{high\ max}$ concentrations, especially at the last time point, which was under-predicted, the RMS does not agree there is sufficient evidence that fluopicolide will not accumulate beyond the study duration at this site.

Concentrations of M-01 appeared to be over predicted by the modelling compared to the measured concentrations observed in the first 2 years, but fit the data better from day 730 – 1460. The RMS considers that the SFO evaluation is inconclusive with regards to a plateau concentration being reached for metabolite M-01 at this site.

Table 8.14 Results of SFO evaluation at Apilly

Fluopicolide	
Initial concentration (C_0)	306 g/ha
SFO rate constant (k)	0.00222 d^{-1}
DT50	312.9 days
$C_{high\ max}$	552 g/ha *(0.368 mg/kg over 10 cm)*
$C_{low\ max}$	246 g/ha *(0.164 mg/kg over 10 cm)*
M-01	
DT50	150.4 days
SFO rate constant (k)	0.00461 d^{-1}
$C_{high\ max}$	192 g as equivalents (95 g M-01/ha)
$C_{low\ max}$	163 g as equivalents (81 g M-01/ha)

SENAS
(S. France. Eyre, 2003a Report: sandy silt loam, pH 7.6 and 1.6% oc content. Pollmann, 2004 Report: loamy silt, pH 7.3 and 1.65% oc content).

The study design at the Senas site differed from at Philippsburg and Appilly. The field dissipation study was started with the first application in June 1999 and ran for 2 years (Eyre, 2003a). Additional applications were continued at the same site/ treated area from 2000-2002 (Pollmann, 2004). The RMS noted in the DAR that the application in Year 3 was made before the final sample was taken in Year 2. The applicant has since provided details of the plot and sampling layout which confirms that the final Year 2 sample would have been unaffected by the Year 3 application.

At the start of the accumulation study, the original control plot in the dissipation study, Plot C, was treated in error on 20 June 2000. Consequently Plot T2n (previously Plot 1 in Eyre, 2003) was treated later on 4 August 2000 and a new control plot, Plot Cn set up.

Fluopicolide was applied as shown below.

Table 8.15 Application schedule at Senas

Application Date	Days after treatment	Nominal application rate (g/ha)	Calibrated application rate (g/ha)
24 June 1999	0	500	500
20 June 2000 (Plots T1, T3)	362	500	524
4 Aug 2000 (Plot T2n)	407	500	500
19 June 2001	726	500	519
27 June 2002	1099	500	513

Figure 8.18 Senas Dataset

Soil Accumulation, SFO parent + SFO metabolite
Field Senas, plot T1 + T2 + T3

	Fluopicolide	AE C653711	
DT50:	166.70	105.48	d
k:	0.004158018	0.006571505	1/d
C0:	494.41		g/ha
Molar Mass:	383.59	190.03	g/mol
Formation fraction:		1	

soil residues assuming BD = 1.5

Application	Measured field data: Time (d)	Measured field data: Fluopicolide (g/ha)	Measured field data: AE C653711 (g/ha ai eq.)	Simulated data: Fluopicolide (g/ha)	Simulated data: AE C653711 (g/ha ai eq.)	Square of differences: Fluopicolide	Square of differences: AE C653711
24/06/1999	0	327	0	494.41	0.00		0
500 g/ha nominal	1	302	36	492.36	2.04		1176
	3	264	38	488.28	6.07		
	14	223	49	466.45	26.70		
	28	231	63	440.07	49.54		
	60	182	78	385.24	89.48		
	130	113	48	287.96	133.60		
	181	146	50	232.93	142.03		
	231	122	78	189.21	139.31		
	300	99	63	142.02	126.06		4030
	368	69	63	107.04	120.49	1447	
20/06/2000	362	575	65	604.15	110.15	850	2075
	363	670	107	601.64	111.93		
	365	759	145	596.66	115.42		
	376	477	197	569.99	133.09		
	390	459	218	537.75	152.18		
	421	353	221	472.72	182.84		
	483	228	196	365.30	209.12		
	543	241	197	284.64	207.09		
	606	232	145	219.04	190.12		
	664	134	132	172.10	168.60		
	725	125	164	133.55	144.41	69	364
19/06/2001	726	670	159	627.40	144.02	1772	239
	845	414	199	382.52	230.41		
	1098	131	189	133.59	148.87	7	1589
27/06/2002	1099	629	167	627.45	148.45	1	327
	1182	349	167	444.32	225.00		
	1454	138	132	143.39	156.56	29	593
						$\sum(P-O)^2$	14568

Where;
P=predicted value
O=observed value

Figure 8.19 Concentration of fluopicolide at Senas (g/ha for total soil depth) (Mean of 3 individual treated plots T1, T2 and T3)

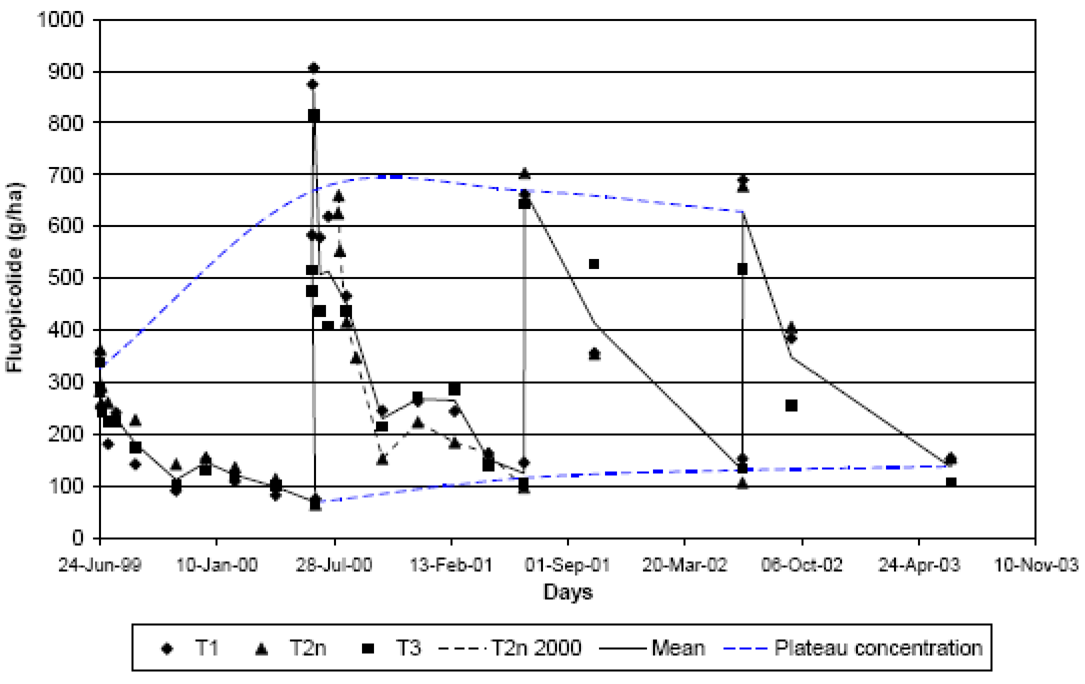
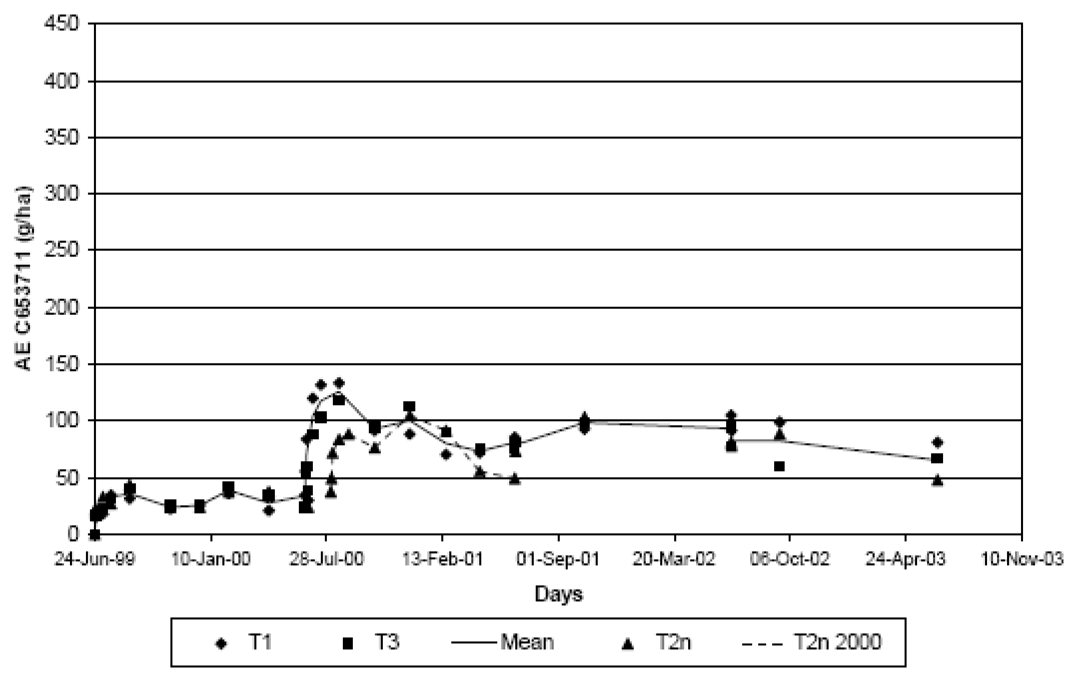


Figure 8.20 Concentration of M-01 at Senas (g/ha for total soil depth) (Mean of 3 individual treated plots T1, T2 and T3)

NB Scale different.



The maximum concentration of fluopicolide was detected immediately after the 2nd application (2000). It was stated that the rate applied to plots T1 and T3 was apparently higher than the nominal application rate of 500 g/ha. The applicant claimed no further accumulation in the upper limit of the 'saw teeth' curve was detected in subsequent applications. It was reported that the lower limit of the 'saw teeth' curve reached a plateau concentration after the 2nd application (2000) and remained relatively constant to the study end. However, the RMS observes that for plot T1 the $C_{high\ max}$ appeared to slightly increase at the last time point, while the $C_{low\ max}$ levelled off. For plot T2, the $C_{high\ max}$ values appeared to reach a plateau, though the $C_{low\ max}$ slightly increased at the end. For Plot T3, both $C_{high\ max}$ and $C_{low\ max}$ appeared to have reached a plateau.

The applicant attributed the slight increase observed in the mean $C_{low\ max}$ values from 2002 (131 g/ha) to 2003 (138 g/ha) as due to experimental variation and to not be significant, (difference between the 2 measurements equated to 0.005 mg/kg, the limit of detection). The applicant concluded that measured $C_{high\ max}$ values in the 3 experimental plots and $C_{low\ max}$ values in 2 of the 3 plots had reached a plateau at Senas.

Measured soil concentrations after the 1st and 2nd applications did not match the nominal and calibrated application rates. In the report Eyre, 2003 the apparent application rate in 1999 (at 327 g/ha) was lower than intended (500 g/ha). In the report (Pollmann, 2004) residue levels after application in 2000 were not considered appropriate as the soil concentrations of fluopicolide measured indicated the rate applied had significantly exceeded the nominal and calibrated application rate (of 500 g/ha). Therefore, only the subsequent years following application in 2001 and 2002 were considered for the evaluation of the plateau concentrations.

Figure 8.21 Concentration of fluopicolide at Senas in 3 individual plots (g/ha for total soil depth).

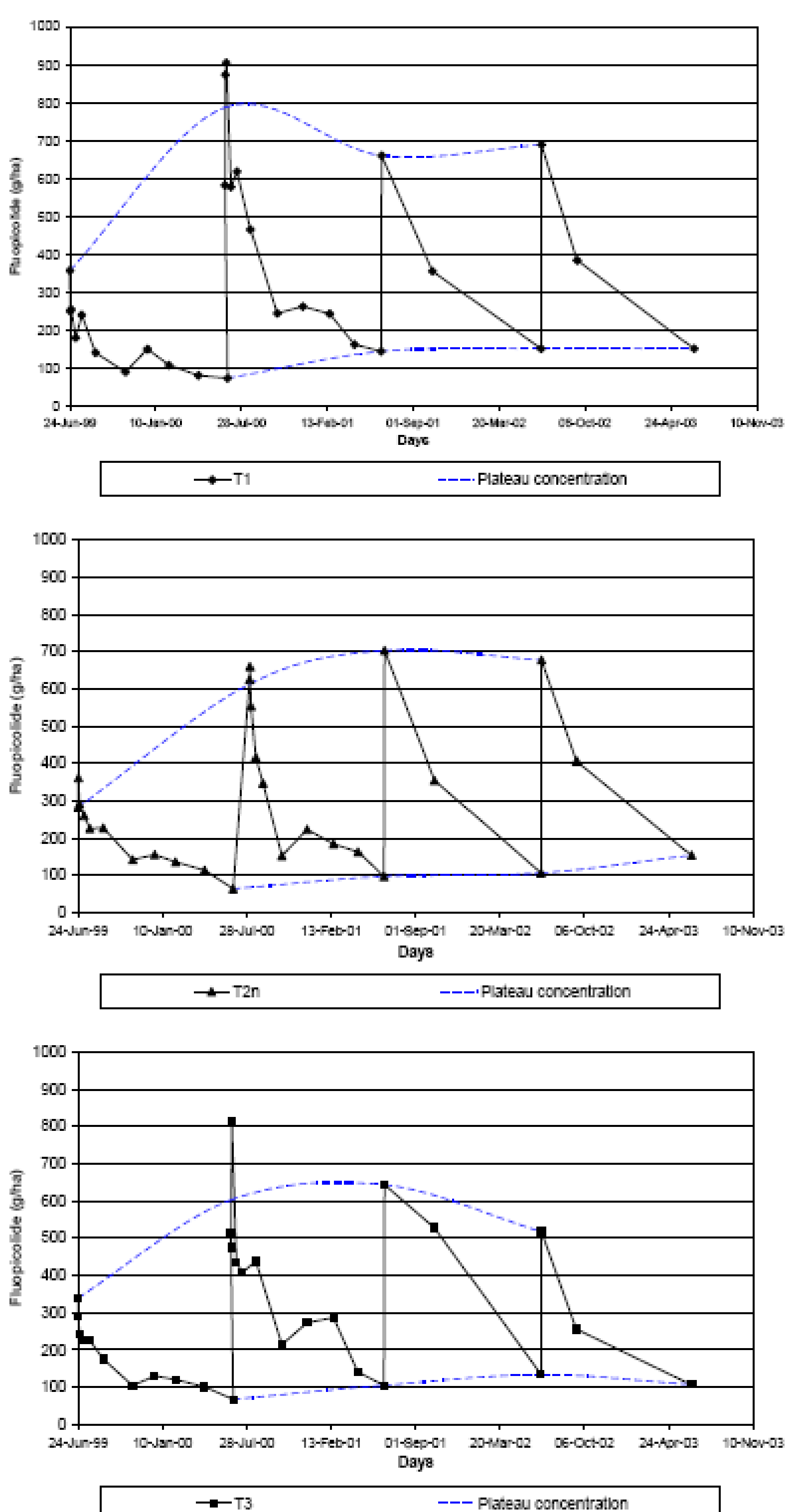


Table 8.16 Plateau concentrations of fluopicolide at Senas

Plateau concentration	Time-point	Measured in soil increments (mg/kg)	
		0-10 cm	0-20 cm
High[1]	Day 0 (4th application overall)	0.354	0.186
Low[2]	Day 372 (3rd application overall)[3]	0.082	0.044

[1] maximum of the high values of the "saw teeth" curve
[2] maximum of the low values of the "saw teeth" curve
[3] $C_{low\ max}$ (0.046 mg/kg) was measured in 0-20 cm in 2003 (Day 355 after 4th application overall)

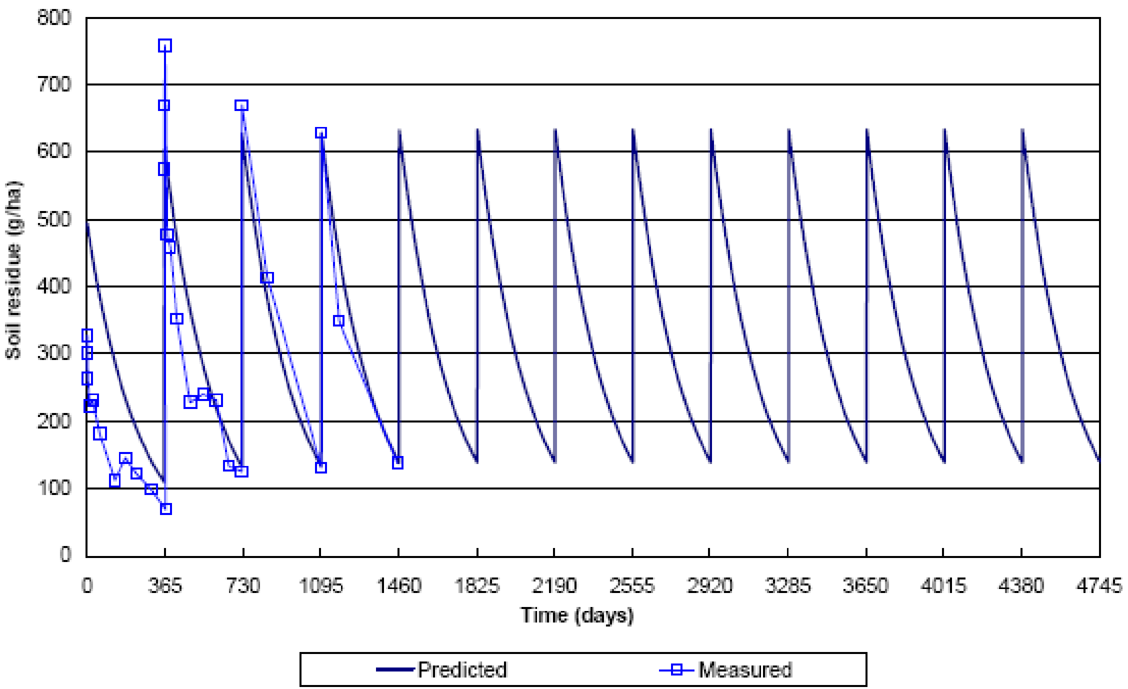
These plateau concentrations are as reported in the DAR except Low (0-10 cm/0-20 cm) was previously 0.061/0.046 mg/kg (day 355 after application 3).

The applicant concluded that based on comparison of measured and predicted concentrations of fluopicolide in soil, a plateau concentration was reached at Senas, with SFO kinetics providing a good fit to the measured $C_{low\ max}$ values. (There was no detailed statistical assessment of fit presented clearly in the study report). The predicted initial concentration (494 g/ha) was close to the nominal and calibrated application rates (*ca*. 500 g/ha). However, the predicted and measured soil concentrations immediately after the 1st and 2nd applications differed from nominal and calibrated application rates.

In the original assessment, the residue levels after application in 2000 were excluded for the assessment of the plateau concentrations, as there were indications that the rate applied had significantly exceeded 500 g/ha. Only the later years (application in 2001 and 2002) were considered. In this evaluation the initial soil residue measured in the first year (1999) was omitted from the optimisation, as the apparent application rate (327 g/ha) was lower than that achieved in later years, but all other years were considered.

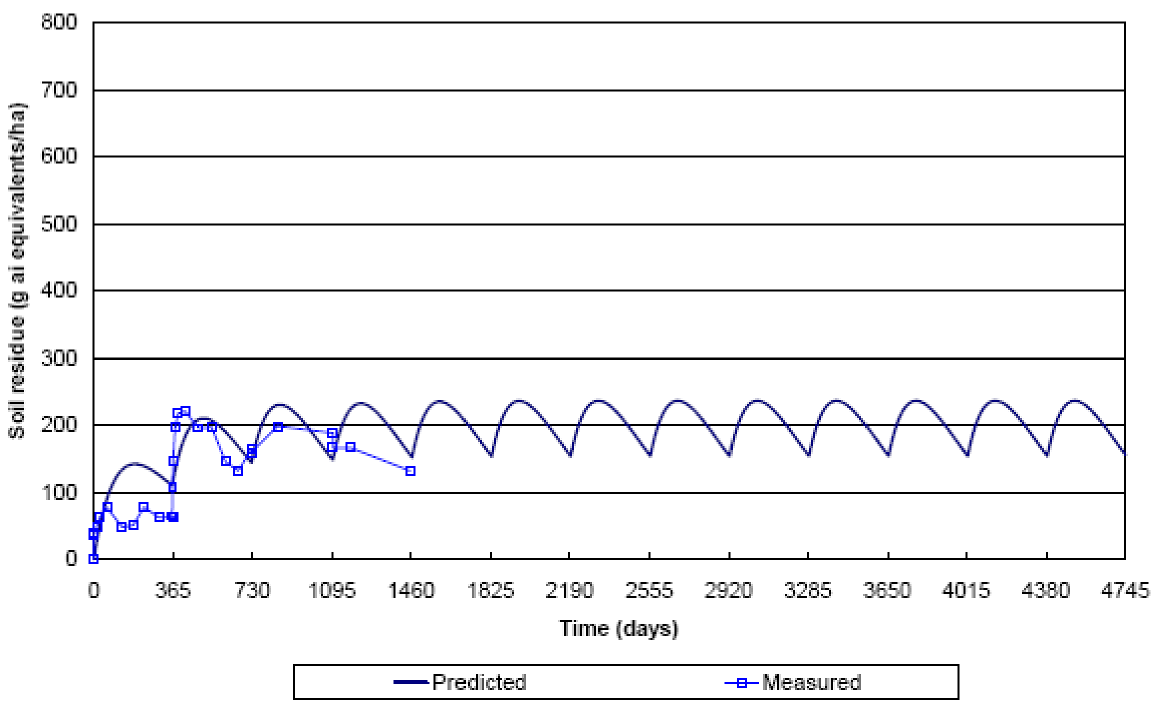
The final measured $C_{low\ max}$ value of Year 1 (26 June 2000, 368 days) was taken after the 2nd application date (20 June 2000, 362 days, Plots T1 and T3), but details of the plot layout confirmed that this sample was not affected by the Year 2 application.

Figure 8.22 Fluopicolide residues at Senas.



The applicant concluded that at the Senas trial, concentrations of fluopicolide in soil increased slightly, but reached a plateau during the study. No further increases were predicted by modelling simulations of additional applications in successive years.

Figure 8.23 Residues of M-01 at Senas.



The applicant considered that the fit to the measured concentrations of M-01 over predicted the concentration observed in the 1st year, but described the remaining years data better. There was no detailed statistical assessment of fit presented clearly in the study report. The RMS agrees that a plateau appeared to be reached for both fluopicolide and metabolite M-01 within the trial duration (4th application, with predicted peak plateau and steady state concentrations of 633 and 139 g/ha for fluopicolide, respectively).

Table 8.17 Results of the SFO evaluation at Senas.

Fluopicolide	
Initial concentration (C_0)	494ha
SFO rate constant (k)	0.00416 d^{-1}
DT50	166.7 days
$C_{high\ max}$	633 g/ha *(0.422 mg/kg over 10 cm)*
$C_{low\ max}$	139 g/ha *(0.026 mg/kg over 10 cm)*
M-01	
DT50	105.5 days
SFO rate constant (k)	0.00657 d^{-1}
$C_{high\ max}$	237 g as equivalents (117 g M-01/ha)
$C_{low\ max}$	154 g as equivalents (76 g M-01/ha)

RMS Risk Assessment and Conclusions:

Fluopicolide

To summarise the results from the three sites: At the Philippsburg site, the measured concentrations of fluopicolide from two of three trial plots (T1 and T3) indicated that a plateau concentration was likely to have been reached during the trial. For the third plot T2, the $C_{high\ max}$ values appeared to have plateaued by the study end, though $C_{low\ max}$ slightly increased at the last sampling point. However, based on mean values a plateau concentration appeared to be reached by the study end. Modelling, with SFO kinetics predicted no further increase in residues from repeated applications in successive years after the trial. This modelling underestimated measured $C_{high\ max}$ residues at the 2nd and 3rd applications, but overestimated them for the 4th and 5th applications. The plateau concentration was predicted to be reached by the 5th year with $C_{high\ max}$ and $C_{low\ max}$ values of 578 and 181 g/ha, respectively. The RMS considers that the overall data indicate that fluopicolide appeared to have reached a plateau concentration within the study duration.

At the Apilly site, the measured concentrations of fluopicolide from one of three trial plots (T3) indicated that a plateau was not reached, both $C_{high\ max}$ and $C_{low\ max}$ values were still increasing at the study end. In plot T1, $C_{high\ max}$ and $C_{low\ max}$ appeared to plateau, but only at the last sampling point and at plot T2, $C_{high\ max}$ clearly declined, though $C_{low\ max}$ values were again only level at the last point. Based on mean values the $C_{low\ max}$ values appeared to plateau but the $C_{high\ max}$ values did not. No further increase in residues beyond the trial was predicted by modelling, with SFO kinetics after repeated applications in successive years. The plateau concentration was predicted by the applicant to be reached by the 5th year with $C_{high\ max}$ and $C_{low\ max}$ values of 552and 246 g/ha, respectively. The RMS considers that this modelling underestimated measured $C_{high\ max}$ residues at last (5th) application and that the data are inconclusive as to whether a plateau was reached during the trial.

At the Senas site, the measured concentrations of fluopicolide from one of three trial plots (T3) indicated that a plateau was reached. At the T1 plot, $C_{high\ max}$ slightly increased at the last point though $C_{low\ max}$ had reached a plateau. For plot T2, $C_{high\ max}$ values had plateaued, though the $C_{low\ max}$ value was still increasing at the study end. The RMS considers that overall, based on mean $C_{high\ max}$ values, concentrations had reached a plateau and the $C_{low\ max}$ values, though close to levelling off, were very slightly increasing at the study end. Modelling, assuming SFO kinetics predicted no further increases based on repeated applications in successive years after the trial. This modelling underestimated measured $C_{high\ max}$ residues at 2nd application, but the fit to later years was reasonable. The plateau concentration was predicted by the applicant to be reached by the last (4th) year with $C_{high\ max}$ and $C_{low\ max}$ values of 633and 139 g/ha, respectively. Therefore, the RMS accepts that a plateau concentration appeared to have been reached for fluopicolide at the Senas site within the study duration.

The applicant compared the maximum residue level observed for fluopicolide at each site after 4 years ($C_{high\ max}$ 0.341-0.387 mg/kg over 10 cm) as equivalent to 1.1 -1.5 times the residue in soil after a single application. (In support of this, the RMS estimates an initial PEC_{soil} after a single application of 400-500 g a.s/ha of 0.267-

0.333 mg/kg over 10 cm soil depth, based on a simple first tier calculation with no interception assumed).

M-01
Concentrations of the metabolite M-01 (AE C653711) were not predicted by the applicant to significantly increase in soil, in successive years after the study duration at each site. However, the agreement between the concentrations predicted by SFO modelling and the measured concentrations was less robust.

The RMS considered that based on the measured data there was insufficient evidence of a plateau concentration being reached for M-01 at the Philippsburg and Apilly sites during the trials, although a plateau concentration for M-01 did appear to be reached at the Senas site. The RMS considered that for Philippsburg site the modelling clearly underestimated the concentrations of M-01 at the last time point. For the Apilly site, the RMS considered that the predicted concentrations for M-01 were closer to the measured data (except for under-estimations in the first year) and that at the Senas site, the modelling appeared to generally over predict concentrations of M-01.

The RMS proposes that further discussion is needed at the expert meeting over the general acceptability of this type of higher tier approach, versus a simple first tier calculation of PEC_{soil} accumulation. Further discussion may also be warranted over how best to interpret measured versus predicted concentrations in soil and the results of individual plots compared to mean results at each site, in reaching an overall conclusion on the potential for accumulation of an active substance.

Implications for Ecotoxicogical Assessment:

No implications for the ecotoxicolgical assessment at present. However, the PEC_{soil} may need to be reassessed on the basis of the PRAPeR expert meeting discussion.

(Kley, C; Mackenzie, E; M-267721-01-1, 2007)

B.8.6.2 Predicted environmental concentrations in groundwater.

<u>Data Requirement 4.3</u>

"Applicant to provide results with a second FOCUS model following the recommendations given in the PPR Opinion: Opinion of the Scientific Panel on Plant Health, Plant Protection Products and their Residues on a request of EFSA related to FOCUS groundwater models. The EFSA Journal (2004) 93, 1-20.

For some of the metabolites it may not be confirmed that the triggers of 0.75 µg/L and 10 µg/L are not exceeded in some scenarios. A second model is necessary to reduce the uncertainty and confirm the non relevance of the metabolites.

Applicant indicated to submit new PEC GW calculations with a second model and lower interception rate for vines by May 2007.

See reporting table 4(79)."

Data Requirement 4.4

"Applicant to repeat the FOCUS GW calculations following the GAP as reported in the Representative uses table. Applicant indicated to submit repeated PEC GW calculations with a lower interception rate for vines by May 2007.

See reporting table 4(80)."

Background:

Potential contamination of groundwater by fluopicolide was assessed with only one FOCUS model, (DAR, B.8.6.2), as the submission was made prior to the PPR Opinion[12] recommending the results of two models are needed to complete the risk assessment. The applicant was requested to provide results for FOCUS GW modelling with a second FOCUS model to reduce uncertainty and confirm the non-relevance of metabolites, following recommendations given in the PPR Opinion (EFSA Journal (2004) 93, 1-20).

For the PECgw calculation (DAR, B.8.6.2) it was assumed a one in three year crop rotation was representative of good agricultural practice in potatoes. However, as crop rotation is not mandatory and the 'representative' use concept implies the assessment is also applicable to other crops represented by the specific crop listed, the applicant was requested to repeat the FOCUS GW calculations following the GAP as reported in the Representative uses table. Similarly, the applicant was requested to repeat the FOCUS GW modelling with a lower interception rate for vines. (Reporting Table, points 4(79), 4(80) and 4(81)).

Summary of approach taken to address Data requirements 4.3 and 4.4

The applicant has performed new FOCUS groundwater modelling with PELMO. These simulations include lower interception rates for use on vines and also for modelling of use on potatoes, application of fluopicolide once every year and every 2 years, as well the previously assessed 1-in-3 year crop rotation pattern.

Furthermore, as a second FOCUS groundwater model was required, in accordance with the PPR opinion (EFSA Journal (2004) 93, 1-20), the applicant has performed PECgw calculations for use of fluopicolide on vines and potatoes, using the PEARL model.

In the original assessment, batch equilibrium studies (Rupprecht 2003 & Simmonds 2003) were previously evaluated in the DAR B.8.2.1 (a) and (b) and sorption of fluopicolide was correlated with organic carbon/matter content of the soil. However, these studies do not take into account kinetically controlled sorption behaviour and so may, in the view of the applicant, underestimate sorption and overestimate mobility.

[12] Opinion of the Scientific Panel on Plant Health, Plant Protection Products and their Residues on a request of EFSA related to FOCUS groundwater models. The EFSA Journal (2004) 93, 1-20.

Time-dependent laboratory sorption studies (Fitzmaurice, 2003, Allan, 2003b) were carried out to investigate kinetic sorption and reported in the DAR, B.8.2.1.(c) & (d), although these were not relied on for the exposure assessment presented in the original DAR. The K_{oc} was increased by a factor of *ca*. 2.1 over 23 days (Fitzmaurice, 2003) and *ca*. 2.3 over 121 days (Allan, 2003b) indicating stronger sorption of fluopicolide with time. Time-dependent sorption is proposed by the applicant, as a possible explanation for the bi-phasic behaviour of fluopicolide in some field dissipation trials. In this new assessment, the applicant has taken into account data on time-dependent sorption for fluopicolide using the PEARL model and its ability to simulate non-equilibrium sorption (PEARL NEQ).

In the PEARL NEQ model, sorption of substances in soil is described by a Freundlich type equation, with both equilibrium and non-equilibrium (kinetic) sorption being able to be considered. Sorption in the equilibrium domain of the soil system is assumed to occur instantaneously, whereas sorption in the non-equilibrium domain proceeds gradually. As pesticide is assumed to be present in both domains, 2 mass balance equations are needed.[13] The mass balance equation for sorption in the non-equilibrium domain requires additional parameters i.e. the desorption rate coefficient (k_d) and a factor describing the ratio (F_{NE}) between the Freundlich coefficients at the equilibrium ($_{EQ}$) and non-equilibrium ($_{NEQ}$) sites i.e.
($F_{NE} = Kf,_{NEQ} / Kf,_{EQ}$).

In the PEARL NEQ model, it is assumed that transformation of a pesticide only occurs in the equilibrium domain. Therefore, as the transformation half-life can only apply to the equilibrium domain, it must be obtained using an alternative approach for this purpose, (whereas DT50 values commonly reported for pesticides usually refer to the total mass content of pesticide).

An example of such an approach for transformation of compound in case of sorption/ desorption kinetics is described in section 3.2.10 of the RIVM report 711401 008.[14]

To take into account time-dependent sorption in the PEARL model, new parameters were needed for degradation rate constant for a.s. in equilibrium phases, desorption rate constant (k_d) and ratio of Freundlich coefficients for equilibrium and non-equilibrium sites (f_{NE}). Two new studies have been submitted (Kley, 2004 MEF-04/346 and MEF - 04/347) in which the applicant has used a kinetic sorption model to describe the kinetic processes influencing sorption of fluopicolide and to obtain parameter values that could be used in a higher tier assessment. (Metabolites were not considered).

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[13] From RIVM report 711401 008. Alterra report 28. Manual of FOCUS PEARL v 1.1.1. November 2000. A. Tiktak, F. van den Berg, J.j.T.I Doesten, D.van Kraalingen, M.Leistra & A.M.A. van der Linden:
2.5.3. …..two mass balances apply:
$\partial c^*_{eq} / \partial t = -R_s - \partial J_{p,L} / \partial z - \partial J_{p,g} / \partial z - R_t - R_u - R_d$ and

$\partial c^*_{neq} / \partial t = R_s$

where c^*_{eq} $(kg\ m^{-3})$ and c^*_{neq} $(kg\ m^{-3})$ are the pesticide concentrations in the equilibrium and non-equilibrium domains of the soil system, respectively. R_s (kg m^{-3} d^{-1}) is the volumic mass rate of pesticide sorption. $J_{p,L}$ and $J_{p,g}$ (kg m^{-2} d^{-1}) are the mass flux of pesticide in the liquid and gas phases, respectively. R_t and R_t (kg m^{-3} d^{-1}) are the transformation and formation rates , respectively. R_u (kg m^{-3} d^{-1}) is the rate of pesticide uptake by plant roots and R_d (kg m^{-3} d^{-1}) is the lateral discharge rate of pesticides.

[14] RIVM report 711401 008. Alterra report 28. Manual of FOCUS PEARL v 1.1.1. November 2000. A. Tiktak, F. van den Berg, J.J.T.I Boesten, D.van Kraalingen, M.Leistra & A.M.A. van der Linden.

The applicant has evaluated the time-dependent sorption of fluopicolide using batch equilibrium data (Kley, 2004, MEF-04/346), to obtain the necessary parameters and then also applied the results from this approach to an evaluation of field dissipation data to obtain a suitable field DT50, for use in the PEARL kinetic sorption model, (Kley, 2004, MEF 04/347).

These kinetic sorption parameters have then been implemented into the FOCUS PEARL modelling (Kley, C. & Ellerich C. 2007 (a) and (b)). PECgw estimates conducted with the FOCUS PEARL model, using standard degradation parameters as a first step, before implementing kinetic sorption parameters, have not been provided. It was not possible to implement sorption kinetics in the PELMO model, which was instead performed using standard degradation kinetic and sorption parameters.

Each of these studies is assessed in more detail below.

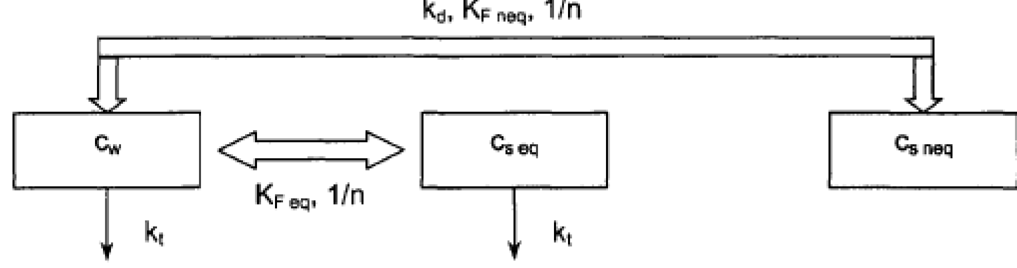
RMS Evaluation of new data – Kley, C. 2004 (MEF-04/346)
Kinetic evaluation of batch equilibrium data considering time-dependent sorption:

This report describes a kinetic sorption model used by the applicant to derive degradation rates for use in FOCUS PEARL groundwater modelling. The study author claims that "*it is equivalent to the one implemented in the PEARL model, which is used to calculate predicted environmental concentrations in groundwater*".

The following description of this kinetic evaluation is complex and as such is largely reproduced from the study report. (The complete reports are also appended for information). In order to conclude whether this is a valid approach, the RMS considers that it will be important to determine whether this is an acceptable interpretation of how the PEARL model simulates non-equilibrium sorption.

Three compartments were considered in the kinetic sorption model for a compound in a soil system: a dissolved phase (C_w); equilibrium sorbed phase ($C_{s\ eq}$) and non-equilibrium sorbed ($C_{s\ neq}$) phase. In the kinetic sorption model, only the part of the compound in the equilibrium domain (dissolved and sorbed) is considered available for degradation, so corresponding degradation rates have to be determined.

Figure 8.24 3-compartment sorption kinetic approach.



The relation between the dissolved and equilibrium sorbed phase was characterised by instantaneous equilibrium between both phases, described by the Freundlich isotherm:

$$C_{s\,eq} = K_{f\,eq} \cdot C_w^{1/n} \qquad (1)$$

where:

$C_{s\,eq}$	concentration in the equilibrium sorbed phase, mg/kg dry soil,
$C_{s\,neq}$	concentration in the non-equilibrium sorbed phase, mg/kg dry soil,
C_w	concentration in the dissolved phase, mg/L water,
$K_{f\,eq}$	Freundlich distribution coefficient for equilibrium domain, L/kg,
$K_{f\,neq}$	Freundlich distribution coefficient for non-equilibrium domain, L/kg,
1/n	Freundlich exponent

The concentration in the non-equilibrium phase ($C_{s\,neq}$) was defined as non-equilibrium sorbed mass of substance / mass of dry soil, related to $C_{s\,eq}$ by:

$$\frac{d\,C_{s\,neq}}{dt} = k_d \cdot \left(\underbrace{\frac{K_{f\,neq}}{K_{f\,eq}}}_{f_{ne}} \cdot C_{s\,eq} - C_{s\,neq} \right) \qquad (2)$$

where:

K_d	kinetic sorption rate constant,
$K_{f\,neq}$ I	Freundlich coefficient for non-equilibrium phase,
1/n	assumed valid for both the equilibrium and non-equilibrium domain

and the terms in the above equation (2):
$K_{f\,neq} / K_{f\,eq}$ } f_{ne} means $K_{f\,neq} / K_{f\,eq} = f_{ne}$ (i.e. the ratio of Freundlich coefficient for non-equilibrium phase to }Freundlich coefficient for equilibrium phase, which is larger the greater the sorption 'capacity' of the non-equilibrium domain).

$C_{s\,eq} \cdot K_{f\,neq} / K_{f\,eq}$ describes concentration in non-equilibrium phase after sufficiently long or infinite time at which $C_{s\,neq} = C_{s\,eq} \cdot K_{f\,neq} / K_{f\,eq}$ and the concentration gradient of $C_{s\,neq}$ is 0.

A number of further transformations are reproduced below (and described in further detail in the report, Kley 2004), which lead to the differential equation (12) for $C_{s\,eq.}$ Equations (2) and (12) are reported by the study author to completely define concentrations of pesticide in all three phases.

Equation (3) represents total concentration in the dissolved and equilibrium sorbed phase:

$$C_{t\,eq} = \frac{\theta_g}{\rho_w} C_w + C_{s\,eq} \qquad (3)$$

where:

θ_g	gravimetric water content (g water/g dry soil, set by experimenter)
ρ_w	density of water (assumed as 1 kg/L)

Or using the isotherm, C_w (concentration in the dissolved phase) is substituted by $(C_{s\,eq} / K_{f\,eq})^{-1/n}$ to give equation (4):

$$C_{t\,eq} = \frac{\theta_g}{\rho_w} \cdot K_{f\,eq}^{-n} \cdot C_{s\,eq}^{n} + C_{s\,eq} \qquad (4)$$

Equation (5) derived from equation (3) differentiated with respect to time:

$$\frac{d\,C_{t\,eq}}{d\,t} = \frac{\theta_g}{\rho_w} \cdot \frac{d\,C_w}{d\,t} + \frac{d\,C_{s\,eq}}{d\,t} \qquad (5)$$

To derive equation (6) the differential $dC_w/\ d_t$ is removed by use of the chain rule[15]

$dC_{s\,eq}/\ dt = dC_{s\ eq}\ /\ dC_w \cdot dC_w\ /\ dt$

(as C_w and $C_{s\ eq}$ are related via the isotherm) and substituted by $dC_{s\,eq}/dt \cdot (dC_{s\ eq}/\ dC_w)^{-1}$:

$$\frac{d\,C_{t\,eq}}{d\,t} = \frac{\theta_g}{\rho_w} \cdot \frac{d\,C_{s\,eq}}{d\,t} \cdot \left(\frac{d\,C_{s\,eq}}{d\,C_w}\right)^{-1} + \frac{d\,C_{s\,eq}}{d\,t} \qquad (6)$$

Using the sorption isotherm $dC_{s\,eq}\ /\ dC_w$ is written as:

$$\frac{d\,C_{s\,eq}}{d\,C_w} = \frac{1}{n} \cdot K_{f\,eq} \cdot C_w^{1/n-1} \qquad (7)$$

C_w is removed to give equation (8)

$$\frac{d\,C_{s\,eq}}{d\,C_w} = \frac{1}{n} \cdot K_{f\,eq} \cdot \left(\frac{C_{s\,eq}}{K_{f\,eq}}\right)^{1-n} \qquad (8)$$

which is then used to rewrite equation (6) as equation (9):

$$\frac{d\,C_{t\,eq}}{d\,t} = \frac{d\,C_{s\,eq}}{d\,t} \cdot \left(\frac{\theta_g}{\rho_w} \cdot n \cdot K_{f\,eq}^{-n} \cdot C_{s\,eq}^{n-1} + 1\right) \qquad (9)$$

The relationship between the equilibrium and non-equilibrium domain is described by equation (10), with k_t the first order, rate constant for degradation, (in the equilibrium domain only):

$$\frac{d\,C_{t\,eq}}{d\,t} = -k_t \cdot C_{t\,eq} - \frac{d\,C_{s\,neq}}{d\,t} \qquad (10)$$

Equation (10) combined with equation (2) gives equation (11):

[15] The chain rule is a formula for the derivative of the composite of two functions. If a variable, *y* depends on a second variable, *u* which in turn depends on a third variable, *x* then the rate of change of *y* with respect to *x* can be computed as the rate of change of *y* with respect to *u,* multiplied by the rate of change of *u* with respect to *x.* In Leibniz notation the chain rule is *df /dx = df /dg · dg /dx.*

$$\frac{dC_{t\,eq}}{dt} = -k_t \cdot C_{t\,eq} - k_d \cdot \left(f_{ne} \cdot C_{s\,eq} - C_{s\,neq}\right) \quad (11)$$

Equation (12) is the differential equation for $C_{s\,eq}$ (concentration in the equilibrium sorbed phase). It results from equating equations (9) and (11) and using equation (4) to substitute $C_{t\,eq}$ with $C_{s\,eq}$:

$$\frac{dC_{s\,eq}}{dt} = \frac{-k_t \cdot \left(\frac{\theta_g}{\rho_w} \cdot K_{f\,eq}^{-n} \cdot C_{s\,eq}^{n} + C_{s\,eq}\right) - k_d \cdot \left(f_{ne} \cdot C_{s\,eq} - C_{s\,neq}\right)}{1 + \frac{\theta_g}{\rho_w} \cdot n \cdot K_{f\,eq}^{-n} \cdot C_{s\,eq}^{n-1}} \quad (12)$$

where:

f_{ne} ratio between Freundlich coefficients, ($k_{f\,neq}/k_{f\,eq}$)
θg gravimetric water content (g water / g dry soil)
ρ_w density of water
k_t degradation rate constant in equilibrium phase

Equations (2) and (12) were then fitted to the kinetic-sorption model by the applicant using ACSL Optimize 1.2 software. The 3 parameters required by the model of k_d (kinetic-sorption rate constant), k_t (degradation rate constant in the equilibrium domain) and $f_{ne,}$ (ratio between the Freundlich coefficients in the non-equilibrium and in the equilibrium domain), were optimised by simultaneous fits to the experimental data ($C_{s\,eq}$ and $C_{s\,neq)}$ as described below. Initial value for non-equilibrium sorbed concentration ($C_{s\,neq\,0}$) was set to 0.

Processing of the experimental data:

Concentrations in the dissolved, equilibrium sorbed and non-equilibrium sorbed phases for use in the kinetic sorption model were calculated from the experimental data (from Fitzmaurice, 2003 and Allan, 2003b). These data were pre-processed to calculate concentrations as valid during the ageing period, i.e. at just after application without any dilution by aqueous or organic solvent and before removing supernatant in single or multiple extraction steps. The equations used are summarised below, full details and input values are described in Kley, 2004, (MEF-04/346).

The total mass of compound recovered is given by equation (13)

$$m_t = m_{OrgExtract} + \sum_{i}^{n} C_{w\,i} \cdot V_{w\,i} \quad (13)$$

where:

m_t	total mass of compound recovered
$m_{OrgExtract}$	substance mass in organic solvent (sum of substance in organic supernatant + in pore volume filled with organic solvent)
m_0	mass of dry soil
n	number of extraction steps

i	supernatant
C_{wi}	concentration in dissolved phase
V_{wi}	volume of water in supernatant
V_P	volume of water in soil (water in pore volume)

The aged sorption study in Allan (2003) involved a single aqueous extraction step, (the system after a single extraction and centrifugation with $CaCl_2$ is denoted by $_1$ in equation (14) below). The Freundlich co-efficient $K_{f\,eq}$ (valid for desorption) was calculated using day 0 values, where t = 0, $C_{s\,eq\,1}$ is calculated as difference between total mass and mass dissolved (Equation 14a), as the non-equilibrium concentration was defined as $C_{s\,neq} = 0$. The mean Freundlich exponent (1/n) from the standard batch equilibrium studies (0.9028) was used, (DAR, Table B.8.190).

$$K_{f\,eq} = \left.\frac{C_{s\,eq\,1}}{C_{w\,1}^{1/n}}\right|_{t=0} \quad (14)$$

$$C_{s\,eq\,1} = m_t / m_0 - C_{w1}\ (V_{w1} + V_{P1}) / M_0 \quad (14a)$$

The aged sorption study in Fitzmaurice (2003) involved multiple aqueous desorption steps. The mean $K_{f\,eq,\,des}$ and 1/n for the 3 desorption steps at day 0 were used, with $K_{f\,eq,\,des}$ (4.363, 4.287, 18.303 and 4.623) and corresponding 1/n (0.9237, 0.888, 0.9813 and 0.904) for each of the 4 soils.

Total substance mass in the dissolved and equilibrium sorbed phase ($m_{t\,eq}$) before and after the aqueous desorption steps has to be equal.

$$m_{teq} = \underbrace{C_w \cdot V_{p0} + C_{seq} \cdot m_0}_{\text{before desorption}} = \underbrace{\sum_i^n C_{wi} \cdot V_{wi} + C_{wn} \cdot V_{pn} + C_{seqn} \cdot m_0}_{\text{after desorption}} \quad (15)$$

The volume of water in soil pores after nth extraction step (V_{Pn}) and centrifugation is calculated by equation (16).

$$V_{P\,n} = V_{P\,0} + V_{w\,\text{extraction solution added}} - V_{w\,n} \quad (16)$$

Equation (17) is derived from equation (15), using the sorption isotherm ((equation (1) $C_{s\,eq} = K_{f\,eq} \cdot C_w^{\,1/n}$).

$$m_{teq} = C_w \cdot V_{p0} + K_{f\,eq} \cdot C_w^{1/n} \cdot m_0 = \sum_i^n C_{wi} \cdot V_{wi} + C_{wn} \cdot V_{pn} + K_{f\,eq} \cdot C_{wn}^{1/n} \cdot m_0 \quad (17)$$

The value for C_w was calculated iteratively using Microsoft Excel® Add-in Solver and $C_{s\,eq}$ calculated from C_w using equation (18), (also shown as equation 1).

$$C_{s\,eq} = K_{f\,eq}\, C_w^{1/n} \qquad (18)$$

Equation (19) describes the non-equilibrium sorbed concentration (i.e. from mass of substance in organic extracts minus remainders of other phases in soil after aqueous desorption steps):

$$C_{s\,neq} = \frac{m_{OrgExtract}}{m_0} - \frac{C_{w\,n} \cdot V_{P\,n}}{m_0} - K_{f\,eq} \cdot C_{w\,n}^{1/n} \qquad (19)$$

The total substance mass ($m_{t\,eq}$) in the dissolved and equilibrium phase is calculated with equation (20):

As in the original experiment, degradation and sorption could occur during the 24 h shaking process, as well as during the ageing process, this shaking period was treated by the study author as additional ageing time and 1 d added to the time points. Day 0 values were therefore calculated with equation (17), assuming non-equilibrium sorbed concentration at day 0 is zero ($C_{s\,neq}$ (t=0) = 0) and that applied substance is distributed in both the dissolved (C_w) and equilibrium sorbed ($C_{s\,eq}$) phase.

$$m_{t\,eq} = C_w \cdot \frac{\theta_g}{\rho_w} \cdot m_0 + K_{f\,eq} \cdot C_w^{1/n} \cdot m_0 \qquad (20)$$

where:

$m_{t\,eq}$ total substance mass in the dissolved and equilibrium phase

C_w concentration in dissolved phase (from iterative calculations in Excel Solver)

θg gravimetric water content (g water / g dry soil)

ρ_w density of water

$m0$ mass of dry soil

$K_{f\,eq}$ Freundlich distribution co-efficient for equilibrium domain

$1/n$ Freundlich exponent

$C_{s\,eq}$ = ($K_{f\,eq} \cdot C_w^{\ 1/n}$)

Results of the kinetic sorption model:

Material in the dissolved and equilibrium sorbed phases ('equilibrium domain') is in instantaneous equilibration with sorption described by the Freundlich isotherm. The kinetic sorption model assumes that fluopicolide is transferred to the non-equilibrium domain (NEQD) and vice versa by the concentration gradient between the equilibrium (EQD) and non-equilibrium (NEQD) domains. At day 0 the non-equilibrium sorbed concentration is assumed to be zero so maximum transfer to the NEQD is predicted. Once NEQD sorbed concentrations reach the same as EQD sorbed concentrations the transfer is reversed. Degradation is assumed to occur in the equilibrium domain only and is described by first-order kinetics (rate constant k_d).

Table 8.18 Parameters of the kinetic sorption model for all soils

trial	k_t d^{-1}	k_d d^{-1}	f_{ne}
Philippsburg, mean	0.0121	0.0589	0.4692
Rödelsee, mean	0.0074	0.0835	0.3701
Huntlosen, mean	ns.	0.1950	0.3657
Senas, mean	0.0118	0.1075	0.3230
Abington, both labels	0.00317	0.0362	0.4485
arithm. mean			**0.3953**
geo. mean		**0.08211**	

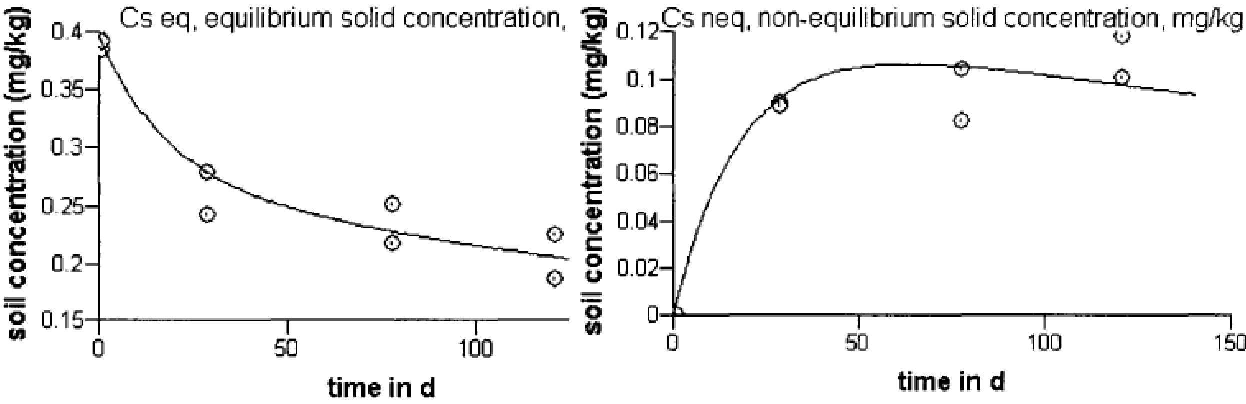
ns. Not significant

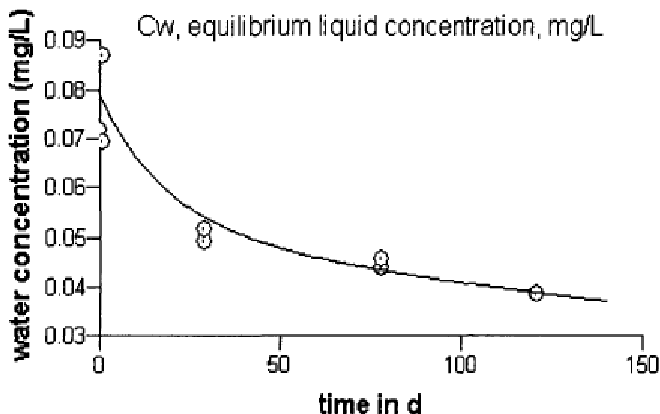
The 'capacity' of the non-equilibrium sorbed phase is stated to be characterised by the parameter f_{ne}, (defined as the ratio between the Freundlich coefficients in the non-equilibrium and the equilibrium phase). The applicant stated that mean f_{ne} ratio of 0.395 indicates moderate kinetic effects on sorption i.e. kinetically controlled 'sorption capacity' about 40% of instantaneous 'sorption capacity'.

The above rate constants (k_d) indicated 'pseudo half-lives' of 2.8-19.1 days for sorption of parent representing exchange between the equilibrium and non-equilibrium phases as shown in table 8.19. The resulting degradation rates in the equilibrium domain (k_t) based on laboratory data indicated DT50 values of 49-286 days. These degradation rates differentiate between degradation in specific phases, so these are only valid for use with the kinetic sorption model.

Curves of $C_{s\ eq}$ and $C_{s\ neq}$ concentration were provided. The applicant concluded that the kinetic-sorption model provided a good visual fit with the experimental data and sufficient goodness of fit. With regards to Table 8.19, the RMS notes that scaled errors (ε) for Chi^2 were >15% in only 3 cases, with r^2 of 0.87-0.99 and t-test values <0.05 in all but in 2 cases (k_t in Huntlosen soil), attributed by the study author to the DT50 being extrapolated beyond the short study duration of 23 d.

Figure 8.25 Curves of simulated (solid line) and measured (symbols) equilibrium and non-equilibrium sorbed, and liquid concentrations of fluopicolide, for Abington soil.





As correlation matrices showed no significant correlations between the parameters k_d, k_t and $f_{ne,}$ the applicant claimed that these parameters may be applied to other studies to calculate kinetic sorption compatible half lives. Therefore, the applicant proposed that this kinetic sorption model be applied to the field dissipation data to provide more realistic degradation rates. This approach was reported in Kley, 2004, (MEF- 04/347).

Table 8.19 Results of kinetic sorption model and statistical parameters.

mg/kg	Philippsburg 1.793	Philippsburg 0.448	Philippsburg 0.109	Rödelsee 1.793	Rödelsee 0.448	Rödelsee 0.109
DT50 sorption (d)	14.1	14.8	7.8	13.8	6.1	6.8
DT50 degrad'n (d)	49.6	59.4	63.6	84.4	80.6	122.1
Statistical parameters:						
χ^2 ε of $C_{s\,eq}$ (%)	3.2	2.4	1.8	4.5	0.5	2.4
χ^2 ε of $C_{s\,neq}$ (%)	8.3	3.9	4.3	19.8	6.9	11.0
r^2	0.952	0.984	0.992	0.870	0.993	0.961
T-probability of:						
k_t	4.3×10^{-9}	3.9×10^{-9}	6.9×10^{-10}	2.3×10^{-4}	3.4×10^{-13}	5.8×10^{-5}
k_d	2×10^{-4}	3.1×10^{-7}	4.5×10^{-9}	5.2×10^{-3}	7.7×10^{-11}	2.1×10^{-4}
f_{ne}	1.3×10^{-6}	1.4×10^{-9}	3.6×10^{-13}	5.9×10^{-4}	4.7×10^{-14}	8.5×10^{-9}

mg/kg	Huntlosen 1.793	Huntlosen 0.448	Huntlosen 0.109	Senas 1.793	Senas 0.448	Senas 0.109
DT50 sorption (d)	2.95	2.77	5.5	6.4	7.1	5.95
DT50 degrad'n (d)	286.3	349.7	$6.9 \times 10^{+07}$	48.9	52.2	79.9
Statistical parameters:						
χ^2 ε of $C_{s\,eq}$ (%)	2.8	3.1	3.3	2.2	4.2	2.8
χ^2 ε of $C_{s\,neq}$ (%)	17.3	13.5	12.5	21.0	6.6	9.2
r^2	0.872	0.907	0.939	0.955	0.932	0.963
T-probability of:						
k_t	4.2×10^{-2}	0.14	nd	9×10^{-10}	4.7×10^{-7}	2.4×10^{-6}
k_d	1.8×10^{-4}	1.7×10^{-5}	1.5×10^{-5}	2.8×10^{-3}	1.4×10^{-6}	4.9×10^{-5}
f_{ne}	9.6×10^{-9}	1.2×10^{-10}	1.1×10^{-12}	1.9×10^{-6}	1.1×10^{-10}	1.7×10^{-9}

nd "parameter could not be evaluated reliably"

mg/kg	Abington 0.41
DT50 sorption (d)	19.2
DT50 degrad'n (d)	218.5
Statistical parameters:	
χ^2 ε of $C_{s\,eq}$ (%)	4.7
χ^2 ε of $C_{s\,neq}$ (%)	14.8
r^2	0.958
T-probability of:	
k_t	2.7×10^{-7}
k_d	3.5×10^{-4}
f_{ne}	1.2×10^{-11}

RMS Evaluation of new data – Kley, C. 2004 (MEF -04/347)
Kinetic evaluation of field dissipation data considering time-dependent sorption:

For the kinetic sorption model, it is assumed that only fluopicolide in the equilibrium domain is available for degradation, so new degradation rates reflecting this are required. In this report, the kinetic sorption model described above (Kley, 2004, MEF-04/346) was applied to field dissipation data from 6 trials, (previously reported in the DAR, B.8.1.5) to determine degradation rates (k_t), that could be used with the kinetic sorption rate constant (k_d) and the ratio between the Freundlich coefficients for equilibrium and non-equilibrium domain (f_{ne}). Both the k_d and f_{ne} parameters were previously derived from the kinetic evaluation of laboratory data above (Kley, 2004, MEF-04/346).

The parameters k_t and $C_{s\,eq\,0}$ (initial value of equilibrium sorbed phase) were optimised by the applicant using ACSL Optimize 1.2 software, by fitting to measured total residue (C_t) in mg/kg. The measured total residue (Ct) included residues at depth and below the LOQ, as previously assessed and reported in the DAR (at B.8.1.5). The initial concentration for non-equilibrium sorbed phase ($C_{s\,neq\,0}$) was set to 0.

An equation was given by the applicant to describe the total soil residue (C_t) or mass balance:

$$C_t = \frac{\theta_g}{\rho_w} \cdot K_{f\,eq}^{-n} \cdot C_{s\,eq}^{n} + C_{s\,eq} + C_{s\,neq} \qquad (21)$$

where:

C_t total soil residue or concentration
θg gravimetric water content (g), *can be substituted with*
θv volumetric water content (L)
ρ_w density of water, *can be substituted with*
ρ_{bd} soil bulk density (kg/L)
$K_f{}^{-n}{}_{eq}$ Freundlich coefficient for non-equilibrium domain +
$C^n{}_{s\,eq}$ Concentration
$C_{s\,eq}$ Equilibrium sorbed concentration
$C_{s\,neq}$ Non-equilibrium sorbed concentration

Degradation rates (k_t) for use specifically with the kinetic sorption model were temperature and moisture normalised according to the time transformation approach (FOCUS 2000). Daily soil moisture and weather data used were available from the original trials reports, except in the case of Senas, for which values were simulated using FOCUS PEARL 1.1.1.

Table 8.20 Soil specific input parameters for fluopicolide.

	$\theta_{v\ ref}$ (100% field capacity)	ρ_{bd}	$K_{f\ eq,\ ads}$	1/n	considered max. depth of soil residues
	% v/v	kg/L	L/kg		m
Philippsburg	18.0	1.5	1.49	0.841	0.5
Rödelsee	35.9	1.5	2.59	0.859	0.5
Huntlosen	24.1	1.5	9.27	0.953	0.2
Appilly	34.7	1.5	*4.69*	0.9028	0.3
Valencia	24.6	1.5	*5.46*	0.9028	0.3
Senas	30.35	1.5	3.59	0.882	0.3

In italics: value recalculated, based on the mean K_{oc} of 321.1 L/kg and the corresponding organic carbon content of the top soils

Table 8.21 below shows $C_{t\,0}$ (g/ha) values, which have been calculated from the fitted $C_{s\ eq\ 0}$, alongside the nominal application rates, together with the fitted degradation rates (k_t). The kinetic sorption DT50 values range from 53-108 days (representing degradation in the equilibrium domain only, compared to the DT50 values from the standard field dissipation assessment (DAR B.8.1.5) which were 77-224 days, with a geometric mean of 138.8 days). The applicant states that total degradation is determined by both the degradation rate constant (k_t) and the transfer rate (k_d), since any substance in the non-equilibrium domain will only be degraded upon transfer to the equilibrium domain.

The applicant considered that that the geometric mean field degradation rate (k_t) of **87.8 days** above, which takes into account kinetic sorption, was more realistic than the laboratory derived values (Kley 2004, MEF -04/346). Therefore, this has been used subsequently as the appropriate degradation parameter for fluopicolide in the FOCUS PEARL model to estimate PECgw values.

The applicant considered that the SFO degradation model in, combination with sorption kinetic, was acceptable to describe the field residues of fluopicolide, with good visual fits and sufficient statistical goodness of fit between the experimental and modelled data. The results of Chi^2 statistical test, single sided t-test and coefficient of determination (r^2) were reported, see Table 8.22. Scaled errors (ε) were <15% in all but 3 cases, with r^2 of 0.81-0.97 and low t-test values, all <0.05.

Table 8.21. Results of field degradation evaluation of fluopicolide, valid for use with sorption kinetic model (20°C, 100% field capacity) and sorption kinetic input parameters.

trial	Nominal application rate	$C_{t\,0}$	k_t	DT_{50} field degradation, with kinetic sorption	used k_d	used f_{ne}
	g/ha	g/ha	d^{-1}	d	d^{-1}	
Philippsburg	400	371.4	0.006899	100.5	0.0589	0.4692
Rödelsee	400	607.3	0.007927	87.4	0.0835	0.3701
Huntlosen	400	306.8	0.00693	100.0	0.1950	0.3657
Senas, 1st year	500	298.9	*0.01051*	*66.0*	0.1075	0.3230
Senas, 2nd year	500 +	632.9	*0.01317*	*52.6*	"	"
Senas mean			0.01184	59.3		
Appilly	400	362.0	0.00846	81.9	0.0821 m	0.3953 m
Valencia	400	428.2	0.00645	107.5	0.0821 m	0.3953 m
90th percentile				104.0		
geo. mean			0.00789	**87.8**		

m = mean values from laboratory batch studies
italics = mean values of Senas used for further averaging to avoid overweighting.

Table 8.22 Results of kinetic sorption model and statistical parameters.

	Philipps-burg	**Rödelsee**	**Huntlosen**	**Senas Y 1**	**Senas Y 2**	**Apilly**	**Valencia**
DT50 degrad (d)	100.5	87.4	100	66	52.6	81.9	107.5
Statistical parameters:							
χ^2 ε of C_t (%)	16.4	14.0	15.5	9.5	13.3	14.4	15.5
r^2	0.812	0.886	0.836	0.966	0.899	0.897	0.893
T-probability of:							
k_t	4×10^{-5}	3.2×10^{-5}	5.8×10^{-5}	3.1×10^{-9}	4.9×10^{-5}	4.9×10^{-6}	1.1×10^{-5}
$Cs\ eq_{\ 0}$	8×10^{-10}	7.5×10^{-9}	1.3×10^{-9}	3.6×10^{-14}	5.8×10^{-9}	7.5×10^{-11}	1.9×10^{-10}

Figure 8.26 Degradation curve and residual plot of measured vs simulated data at **Philippsburg**

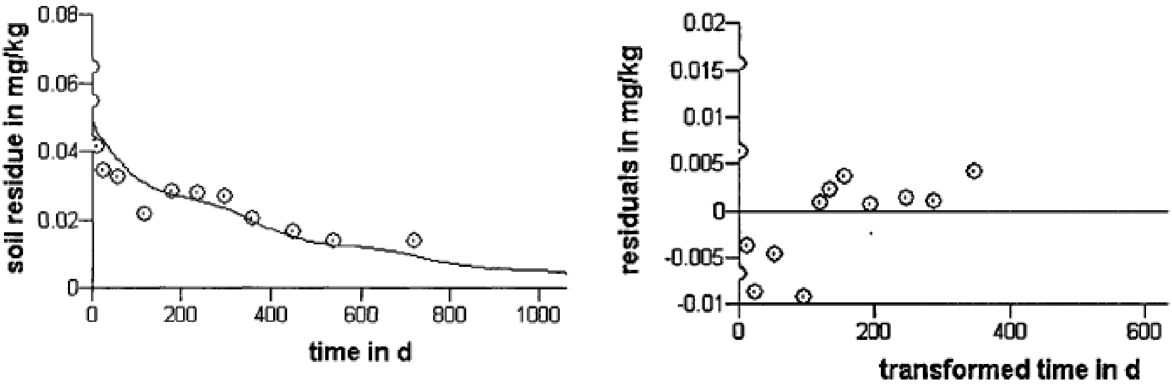


Figure 8.27 Degradation curve and residual plot of measured vs simulated data at **Rödelsee**

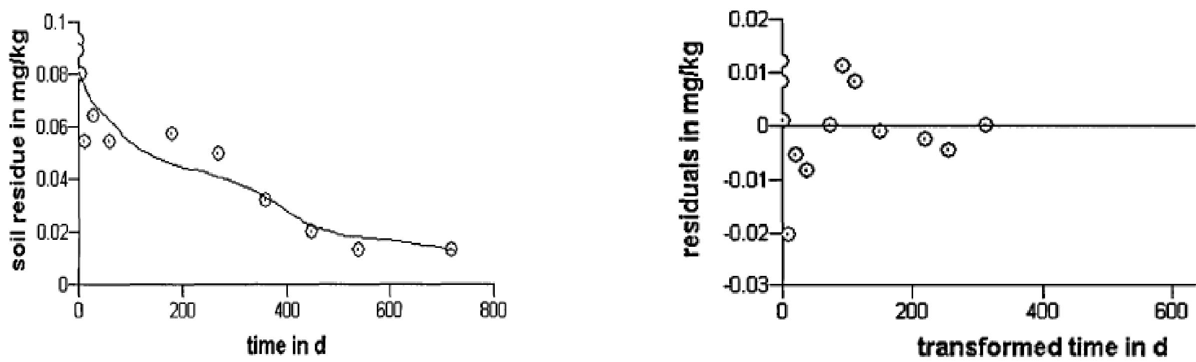


Figure 8.28 Degradation curve and residual plot of measured vs simulated data at **Huntlosen**

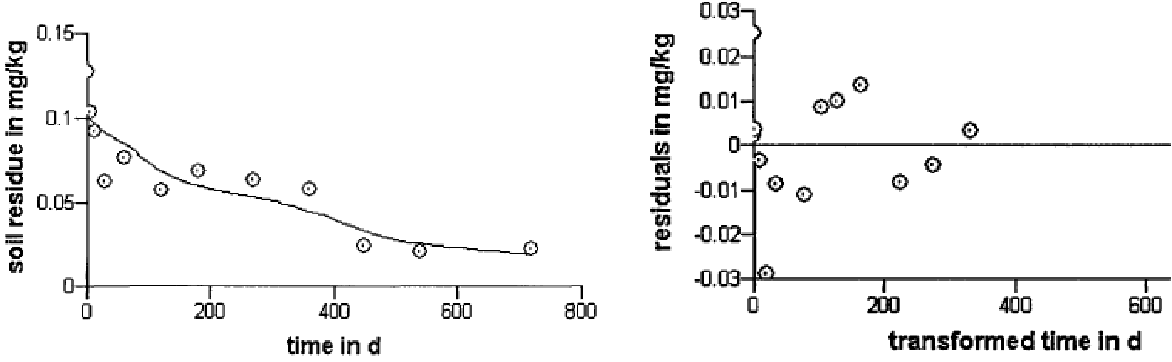


Figure 8.29 Degradation curve and residual plot of measured vs simulated data at **Senas (Yr 1)**

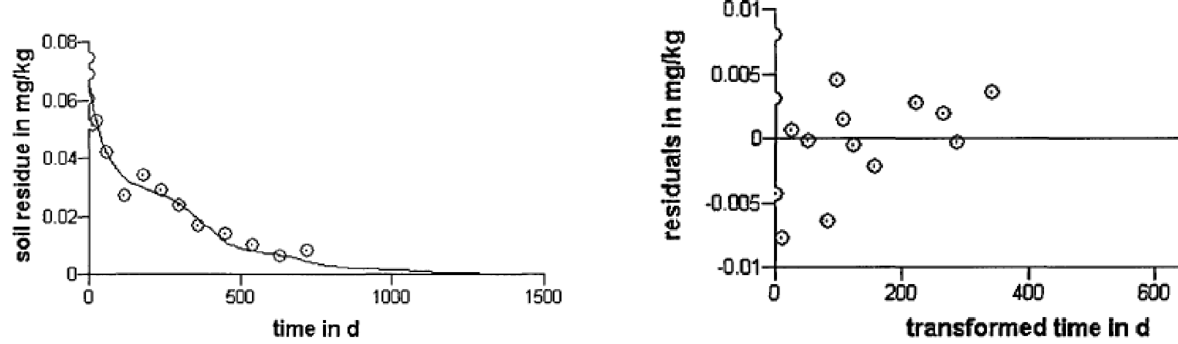


Figure 8.30 Degradation curve and residual plot of measured vs simulated data at **Senas Yr 2**

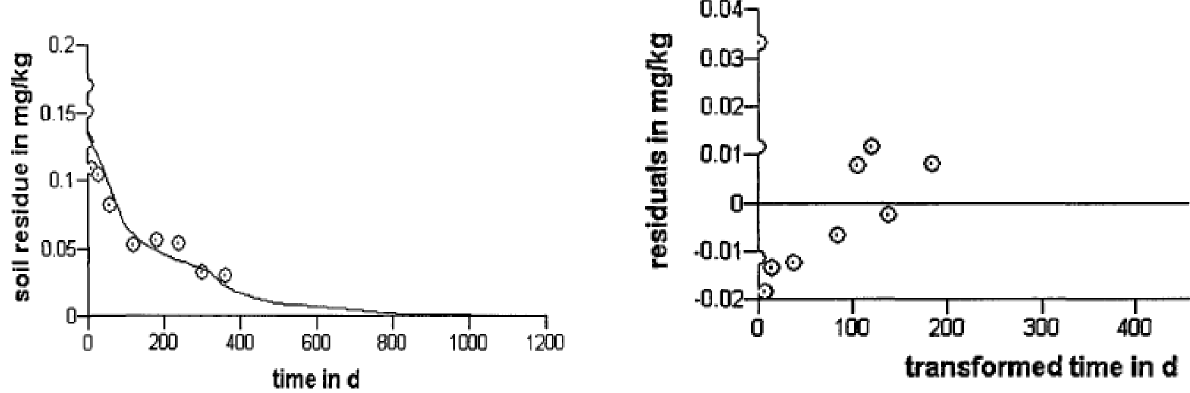


Figure 8.31 Degradation curve and residual plot of measured vs simulated data at **Apilly**

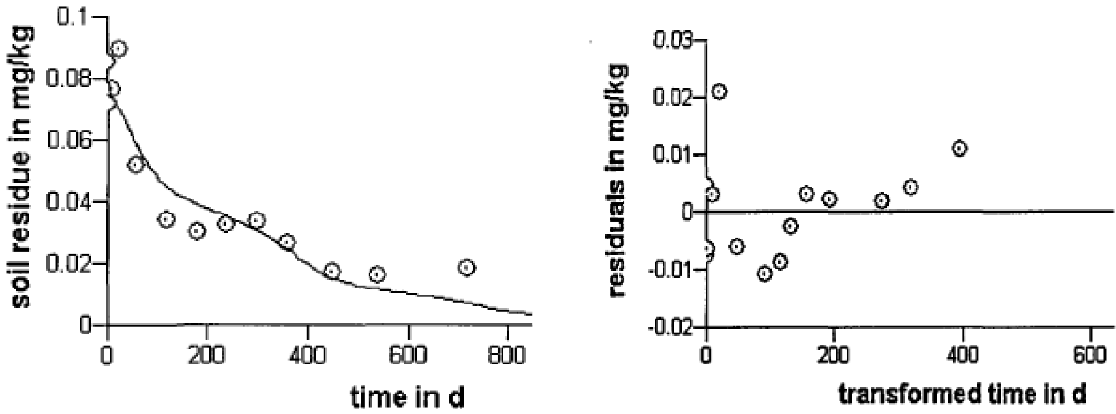
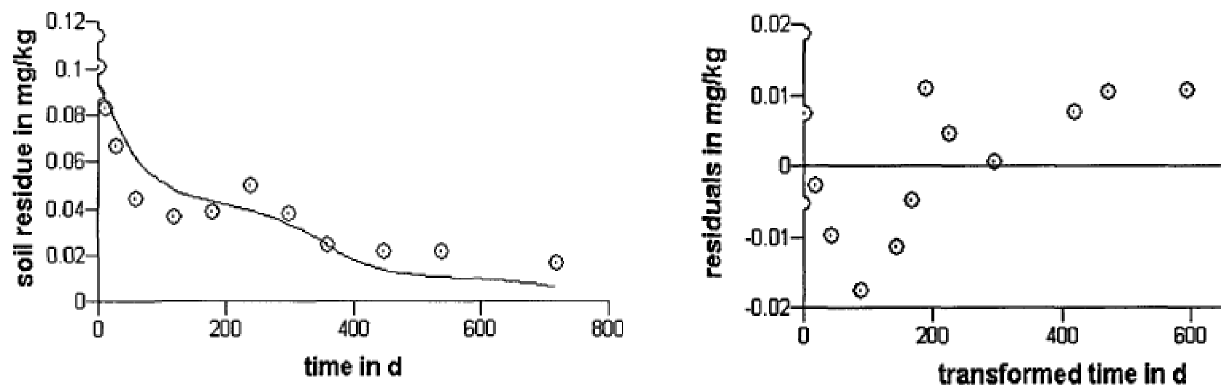


Figure 8.32 Degradation curve and residual plot of measured vs simulated data at **Valencia**



Validation of the kinetic sorption model:

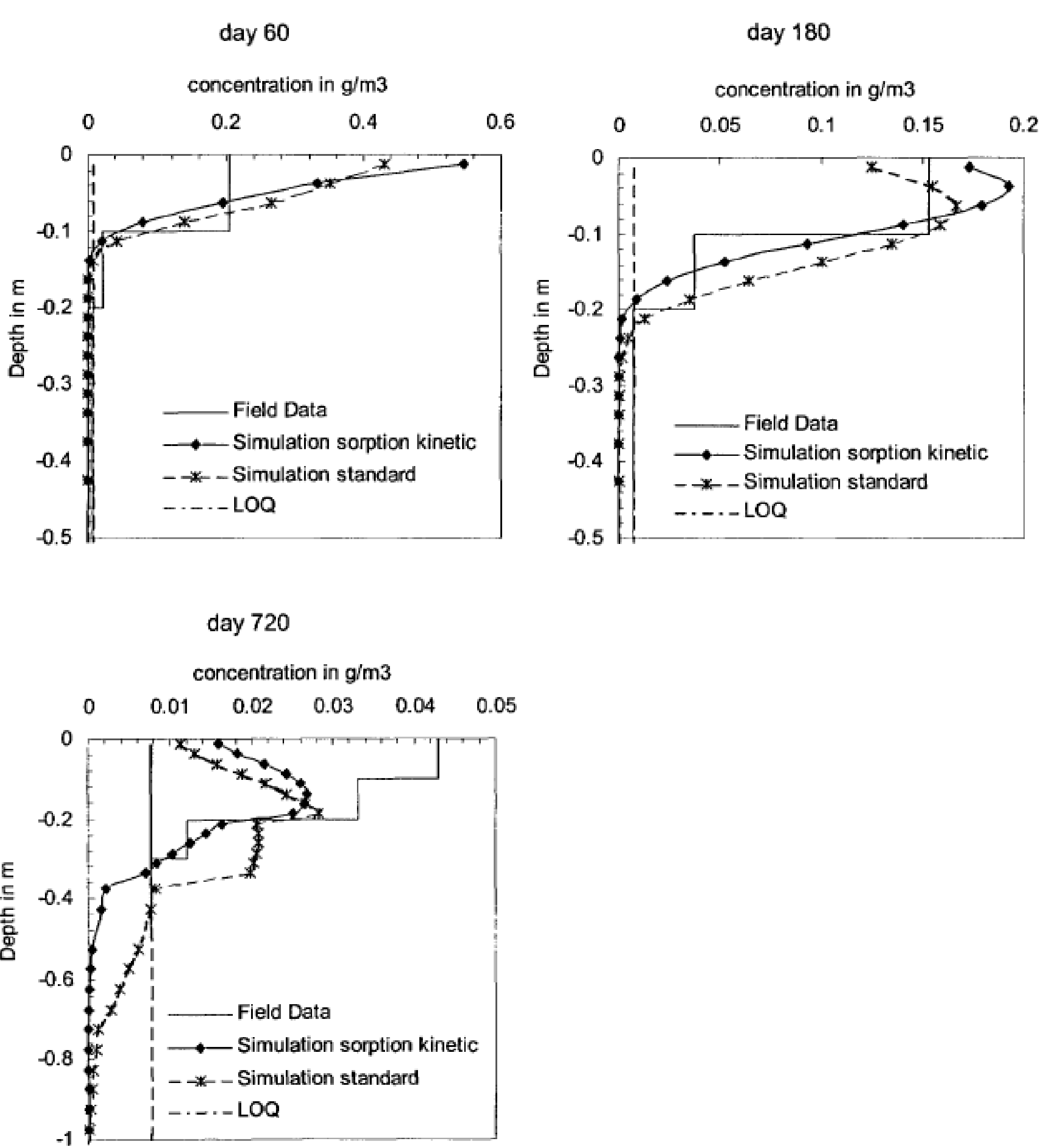
The applicant also provided validation of the kinetic sorption model, using the distribution of measured residue data with depth. The FOCUS PEARL model v 1.1.1 was used to simulate water and substance transport at four of the six field sites, (Philippsburg, Rödelsee, Huntlosen and Senas). Two PEARL simulations were run, one with the standard field degradation parameters (as assessed in the DAR) and one with the kinetic sorption parameters (k_t, k_d and f_{ne} evaluated here), to compare both approaches with the measured residue data (C_t, in mg/kg or g/m^3).

Site-specific soil, weather, irrigation, tillage, crop and application conditions, as well as hydraulic calibration were all as previously described in the DAR (B.8.1.5.1). Fitted initial soil concentrations (g/ha) were used as application rates. The input and output files for these PEARL simulations are given in Kley 2004, (MEF -04/347). Depth profiles of concentration were evaluated according to the 'method of moments' (Jury 1990[16]).

The applicant stated that both the kinetic sorption and standard degradation approaches resulted in the same overall degradation characteristics, but retardation of fluopicolide due to sorption processes lead to differences in transport velocity. The kinetic sorption simulation gave narrower depth distributions and higher peak values compared to the standard simulation. This was attributed to more substance being retained for the kinetic sorption model, with fluopicolide sorbed in the non-equilibrium domain not being available for transport. For the standard simulation, the substance is dispersed more greatly, with increasing travel depth which results in lower peak values.

[16] Jury, W.A., Roth, K (1990). Transfer functions and solute movement through soil: Theory and Applications.

Figure 8.33 Example of comparison of depth profiles for simulated and measured concentrations, from Philippsburg site. (Further concentration depth profiles provided in Kley, 2004, MEF-04/347).



From day 60-189 the standard simulation appeared to over-predict the transport velocity of fluopicolide compared to the measured data, while the kinetic sorption simulation gave a closer depth concentration profile to the measured data. The mean travel depth[17] after 2 years with the kinetic sorption simulation was also closer to the measured mean travel depth for each site, than with the standard simulation, (except, the RMS notes, for Rödelsee).

[17] mean travel depth (z_s), calculated as $z_s = \int z \cdot dm / \int dm$ where dm is substance mass at a certain depth. A description of how this was converted to soil concentration at depth is given in Kley, 2004 (MEF 04-347).

Table 8.23 Mean travel depth of fluopicolide in soil at end of trial for different soil concentration curves.

	Mean travel depth (cm) after 720 days		
	Measured	**Kinetic sorption simulation**	**Standard simulation**
Philippsburg	15.6	16.5	26.6
Rödelsee	12.5	9.4	11.6
Huntlosen	6.1	7.7	9.2
Senas	6.9	9.6	11.9

Based on these results, the applicant considered the kinetic sorption simulation was closer to the measured data than the standard simulation, and therefore more accurately described the behaviour of fluopicolide in the field.

FOCUS PEARL & PELMO modelling of PECgw for use in vines and potatoes: RMS Evaluation of New Data – Kley, C. & Ellerich C. 2007 (a) and (b)

The potential for fluopicolide and 9 of its metabolites, (M-01, M-02, M-03, M-05, M-10, M-11, M-12, M-13 and M-14) to leach to groundwater following use in Europe on vines or potatoes is assessed in the DAR (B.8.6.2), using the FOCUS PELMO 3.3.2 model.

The proposed degradation pathway (also shown in DAR, Figure B.8.6 and B.8.17) is given below. Fluopicolide is cleaved into metabolites M-01 (phenyl ring) and M-02 (pyridine ring) and the intermediate metabolite M-03, formed in acidic soils, is also cleaved into M-01 and M-02. The applicant states that where there is cleavage of a molecule, the degradation rate is equal to the formation rate for each of the resulting metabolites. Therefore, as assessed in the DAR, the same partial formation fraction k_{13} was used by the applicant for the pathways from parent to M-01 and parent to M-02 and likewise k_{23} was used for both the pathways from M-03 to M-01 and M-03 to M-02.

Figure 8.34 Applicant's proposed reaction pathways of fluopicolide in soil.

Fluopicolide (AE C638206), comp. 1

k_{13}

k_{12}

k_{13}

AE 0608000 (M-03), comp. 2

k_{23}

k_{23}

AE C657188, M-02

AE C653711, M-01

k_3

Further mineralisation

[GSH]

M-13

AE 1344123 (M-10)

AE 1344122 (M-05)

Intermediate, MW 269

M-11 and M-12

AE 1388273 (M-14)

Further mineralisation

Further mineralisation

Degradation rate used in revised PELMO modelling:

A kinetic evaluation of the field dissipation studies was performed in the DAR (B.8.1.5.1.) using a 3-tier approach. A 2nd tier approach was based on inverse modelling of dissipation curves from three field sites with PEARL and PEST models. A further kinetic assessment using ModelMaker was performed for three additional field trial sites, where detailed soil hydrology data were not available for use in PEARL. The resulting field degradation rates for fluopicolide, normalised to 20°C and field capacity, were accepted by the RMS previously for use in the groundwater assessment and are shown below. The standard geometric mean DT50 of 138.8 days has been used here in the revised PELMO groundwater modelling, since it was not possible to incorporate sorption kinetics into PELMO.

Table 8.24 Field degradation half-lives for use in PELMO exposure assessment.

	application	Estimated initial soil concentration c_0	**DT_{50} Fluopicolide**
	g a.i. / ha	g a.i. /ha	d
Philippsburg	400	400	177.7
Rödelsee	400	610	123.3
Huntlosen	400	400	117.5
Appilly	400	400	161.2
Valencia	400	400	223.6
Senas mean	500		77.0
90th percentile			200.7
arith. mean			146.7
geom. mean			**138.8**

The degradation rate constants, calculated to take into account kinetic sorption (geometric mean $DT50_{field,\ norm}$ 87.8 days for the equilibrium phase, Kley, 2004, MEF -04/347), reported in this Addendum, have been implemented into the groundwater modelling with FOCUS PEARL as described below.

Parameters assumed for the metabolites in new PEARL and PELMO modelling:

For the metabolites, almost all the parameters for degradation and sorption and formation fractions used in the modelling, were as previously accepted by the RMS in the original PELMO groundwater assessment (DAR B.8.6.2). These are summarised below.

Metabolite M-03 (AE 060800) was not detected in alkaline soils (pH>6) in field dissipation trials but was observed at up to 6.1% applied (parent equivalents) in one acidic field trial. Laboratory studies (DAR, B.8.1.2.b.) confirmed that its degradation was pH-dependent.

For alkaline scenarios (pH>6) fluopicolide was assumed to be completely degraded via M-03, with the geometric mean $DT50_{lab, norm}$ of 0.09 days. For acidic scenarios (pH<6) parallel degradation of fluopicolide to M01 and M-02 directly (k_{13}) and also via M-03 (k_{12}) was assumed, with the geometric mean $DT50_{field, norm}$ of 55.5 days for M-03.

Formation fractions assumed were 0.288 for M-03 and 0.712 for M-01/M-02. An arithmetic mean Koc of 108.8 L/kg, with mean 1/n of 0.971 was previously estimated and used in the groundwater assessment. M-03 was also rapidly hydrolysed in laboratory studies to form M-01/M-02 with DT50 from 8.1 minutes (pH 8) to 45.5 hours (pH 5), (DAR, B.8.4.1.d.).

Metabolite M-01 (AE C653711) reached up to 24.1% (parent equivalents, excluding Senas, 2nd year data) and 40.2% applied (parent equivalents) in the field and laboratory, respectively. A geometric mean $DT50_{field, norm}$ of 137.7 days, arithmetic mean Koc of 40.9 L/kg and 1/n of 0.9158 were previously accepted for use in the original PELMO groundwater assessment.

Metabolite M-02 (AE C657188) reached a maximum of 16.4% (parent equivalents, excluding Senas, 2nd year data) and 7.3% applied (parent equivalents) in field and laboratory studies, respectively. It was not possible to calculate reliable field degradation rates for M-02, as residues were only detected at low levels and early time points, so a geometric mean, $DT50_{lab, norm}$ of 2.82 days (using ModelMaker), together with an arithmetic mean Koc of 5.99 L/kg and 1/n of 0.7737, was previously used for the original PELMO groundwater assessment.

Metabolite M-05 (AE 1344122) reached a maximum of 17.99% of applied M-02 in a laboratory soil degradation study with M-02 (DAR, B.8.1.2.c). A geometric mean, $DT50_{lab, norm}$ of 42.6 days, arithmetic mean Koc of 25.9 L/kg and 1/n of 0.9182 were previously accepted for use in the original PELMO groundwater assessment. As no M-14 was formed in one of the soils tested, (attributed by the applicant as possibly due to slow degradation of M-05), the worst case formation fraction of 0.384 was selected by the applicant for M-05 into M-14. The mean formation fraction of 0.252 was used in the DAR. This also gives a slightly different partial reaction rate of 0.006248 d^{-1}, (compared to 0.0041 d^{-1} in the DAR).

Metabolite M-10 (AE 1344123) reached up 4.97% of applied M-02 in a soil degradation study with M-02 (DAR, B.8.1.2.c). A geometric mean, $DT50_{lab, norm}$ of 26.4 days, arithmetic mean Koc of 6.3 L/kg and 1/n set to 0.9 were previously accepted for use in the original PELMO groundwater assessment.

Metabolite M-11/12 (P2a/P2b) are two isomers (60:40 ratio) formed at up to 6.55% of applied M-02 in a soil degradation study with M-02 (DAR, B.8.1.2.c). A geometric mean, $DT50_{lab, norm}$ of 35.95 days, was previously accepted for use in the original PELMO groundwater assessment. No reliable Koc value could be determined and this was set at 0 (with 1/n of 0.9) as a worst case.

Metabolite M-13 (P3) reached up to 4.38% of applied M-02 in a soil degradation study with M-02 (DAR, B.8.1.2.c). A geometric mean, $DT50_{lab, norm}$ of 11.8 days was previously accepted for use in the original PELMO groundwater assessment. At pH 6

only very low sorption was observed, Koc of 0.003 L/kg so this was set at 0 L/kg, (1/n set to 0.9).

Metabolite M-14 (AE 1388273) reached up to 1.56% of applied M-02 in a soil degradation study with M-02 (DAR, B.8.1.2.c). A geometric mean, $DT50_{lab, norm}$ of 5.2 days was previously accepted for use in the original PELMO groundwater assessment. At pH 6 sorption was moderate, a Koc of 19.2 L/kg (Kom of 11.14 L/kg) were used with 1/n set to 0.9.

The parameters for fluopicolide and its metabolites input into the revised groundwater modelling with FOCUS PEARL and PELMO are summarised below.

Table 8.25 Summary of degradation and sorption parameters used in FOCUS groundwater scenarios

Compound	FOCUS scenario	DT_{50} (days)	K_{oc} (L/kg)	K_{om} (L/kg)	Freundlich exponent (1/n)
Fluopicolide	All	138.8 [a]	321.1	186.2	0.9028
		87.8 [b]			
M-03	pH < 6	55.5 [c]	108.8	63.1	0.9707
	pH > 6	0.09 [d]			
M-01	All	137.7	40.9	24	0.9158
M-02	All	2.82	5.99	3.47	0.7737
M-05 (P1x)	All	42.6	25.9	15	0.9182
M-10 (P4)	All	26.4	6.3	3.7	0.9*
M-14 (P7)	All	5.2	19.2	11.14	0.9*
M-11 and M-12	All	35.95	0	0	0.9*
M-13	All	11.8	0	0	0.9*

[a] standard overall degradation half-life used in PELMO
[b] DT50 valid only with kinetic sorption parameters K_d = 0.08211 d-1, f_{ne} = 0.3953 used in PEARL
[c] in acidic soils (Hamburg, Jokioinen, Okehampton, Porto)
[d] in alkaline soils (Châteaudun, Kremsmünster, Piacenza, Sevilla, Thiva)
[*] default 1/n

Table 8.26 Formation fractions used for FOCUS PEARL and PELMO groundwater scenarios

Compound	FOCUS scenario	Formation fraction	k_{ij} (d^{-1})
f (fluopicolide → M-02)	pH < 6	0.712	0.00356
	pH > 6	0	0
f (fluopicolide → M-03)	pH < 6	0.288	0.00144
	pH > 6	1	0.00499
f (M-03 → M-02)	pH < 6	1	0.01249
	pH > 6	1	7.7016
f (M-02 → M-05)	all	0.203	0.05
f (M-02 → M-10)	all	0.095	0.0233
f (M-02 → M-13)	all	0.062	0.0152
f (M-02 → CO_2)*	all	0.587	0.1444
f (M-02 → M-14)	all	0.053	0.013
f (M-05 → M-14)	all	0.384#	0.006248[#]
f (M-05 → CO_2)	all	0.748	0.01002[#]
f (M-14 → CO_2)	all	1	0.1333
f (M-10→ CO_2)	all	1	0.02622
f (M-13 → CO_2)	all	1	0.05864

*The formation fraction for f (M-02 → CO_2) was 0.640 with K_{ij} of 0.1574 d^{-1} in the DAR, the applicant appears to have divided this into formation fractions for f (M-02 → M-11/M-12 and → CO_2) as shown.
[#] In the DAR, K_{ij} for f (M-05 → M14) and f (M-05 → CO_2) were 0.0041 d^{-1} and 0.0122 d^{-1} respectively. The worst case formation fraction of 0.384 has been used for formation into M-14, instead of the mean of 0.252 used in the DAR.

Plant Uptake

For fluopicolide and metabolites M-01, M-02 and M-05, which were considered to be systemic, the plant uptake factor was set to 0.5 (default). For metabolites, M-03, M-10, M-11/-12, M-13 and M-14, which were not detected in plants, the uptake factor was set to 0.

GW modelling Assumptions

Simulations were performed for use of fluopicolide on vines, with a lower crop interception rate than assumed in the DAR (B.8.6). In vines, a scenario of 3 applications of 133 g fluopicolide per hectare at 10 day intervals each year was chosen as a worst-case. The applicant assumed crop interception of 60% + 70% + 70% in accordance with FOCUS (2000) and a crop growth stage of BBCH 53-77. This is, as requested, lower and more worst case than previously used in the DAR, (70%+70%+85% for Hamburg, Kremsmunster and Sevilla or 70% for other scenarios).

Groundwater modelling of use of fluopicolide on potatoes was performed assuming use on potatoes every year, once every 2 years and once every 3 years. In potatoes, a scenario of 4 applications of 100 g fluopicolide per hectare at 5 day intervals was used, with the product applied every 1, 2 or 3 years. The applicant stated three year rotation was commonly practised in many European MSs to avoid build up of potato cyst nematodes, with two year rotation possible in specific cases, but that application

every year to potatoes was unlikely. For this modelling, the applicant used the same crop interception as previously assessed in the DAR of 50, 50, 80 and 80% for the first, second, third and fourth applications, respectively. This is in accordance with FOCUS 2000 guidance of 50% interception at BBCH 20-39 and 80% at BBCH 40-89. (Although the applicant referred to application being within a slightly narrower crop growth stage band (BBCH 35-89), than reported previously in the DAR, (BBCH 20-91, for which interception was as above, but declined to 50% for BBCH 90-99)).

Simulations were performed over 26 years (including 6 year warm up period) for vines and potatoes (with application every year) and for a total period of 46 or 66 years for potatoes (applications every 2 or 3 years).

The earliest application was assumed to be 5 weeks after leaf emergence for vines and 3 weeks after emergence for potatoes, with application dates shown below.

Table 8.27 Plant development in FOCUS GW scenarios and application dates – vines.

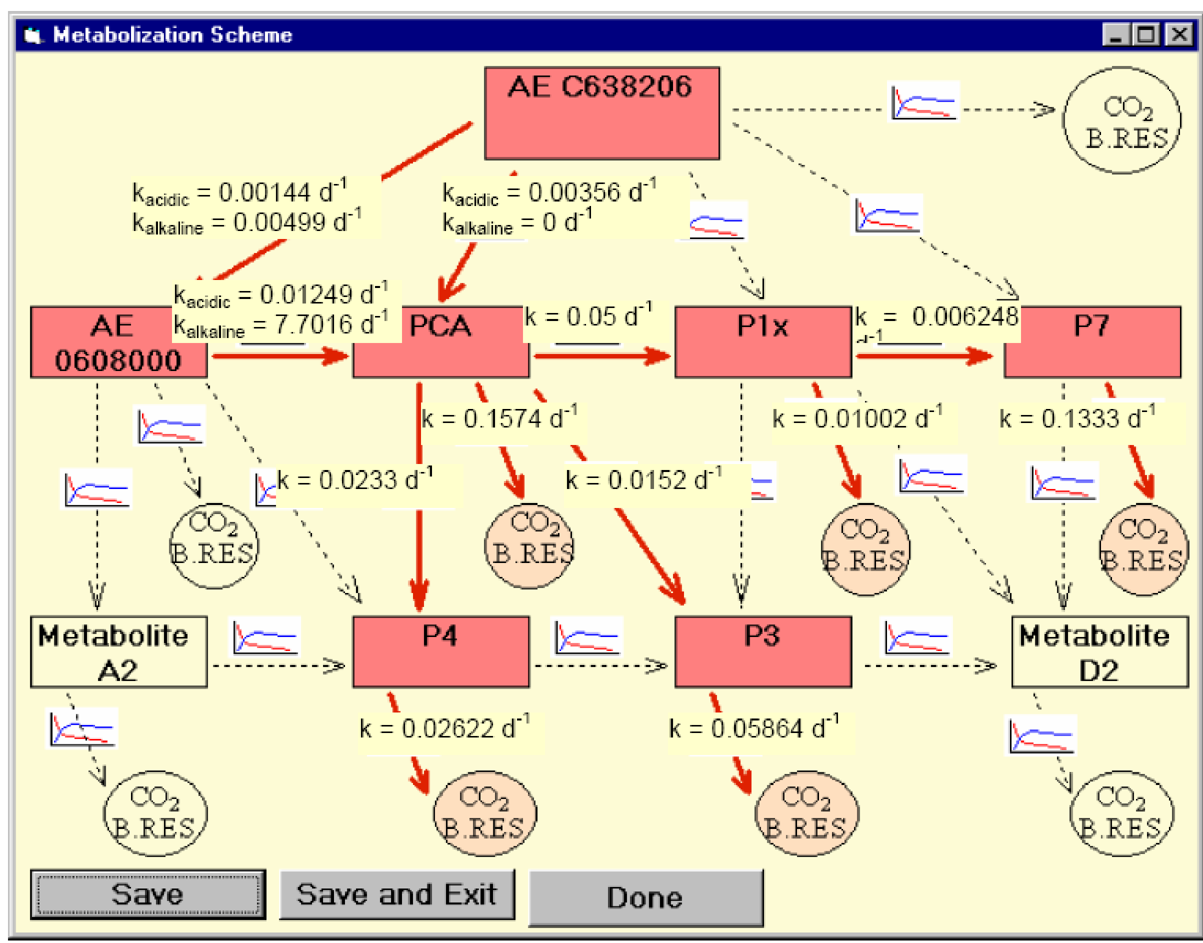
Scenario	Leaf emergence vine	LAI_{max} vine	selected application dates		
Châteaudun	1st April	31st July	6.5. (60 % Int.)	16.5. (70%)	26.5. (70%)
Hamburg	1st May	15th July	5.6. (60%)	15.6. (70%)	25.6. (70%)
Kremsmünster	1st May	15th July	5.6. (60%)	15.6. (70%)	25.6. (70%)
Piacenza	1st April	31st July	6.5. (60 %)	16.5. (70%)	26.5. (70%)
Porto	15th March	31st July	19.4. (60%.)	29.4. (70%)	9.5. (70%)
Sevilla	31st March	15th June	5.5. (60%)	15.5. (70%)	25.5. (70%)
Thiva	15th March	30th June	19.4. (60%.)	29.4. (70%)	9.5. (70%)

Table 8.28 Plant development in FOCUS GW scenarios and application dates – potatoes.

Scenario	emergence potatoes	LAI_{max} potatoes	harvest potatoes	selected application dates			
Châteaudun	30th April	15th June	1st September	21.5.	26.5.	31.5.	5.6.
Hamburg	10th May	20th July	15th September	31.5.	5.6.	10.6.	15.6.
Jokioinen	5th June	30th August	25th September	26.6.	1.7.	6.7.	11.7.
Kremsmünster	10th May	20th July	15th September	31.5.	5.6.	10.6.	15.6.
Okehampton	30th April	15th July	1st September	21.5.	26.5.	31.5.	5.6.
Piacenza	20th April	1st June	10th September	11.5.	16.5.	21.5.	26.5.
Porto	15th March	30th May	15th June	5.4.	10.4.	15.4.	20.4.
Sevilla	31st January	31st March	31st May	21.2.	26.2.	2.3.	7.3.
Thiva	1st March	30th April	30th July	22.3.	27.3.	1.4.	6.4.

As described previously in the DAR (B.8.6.2) the degradation schemes had to be implemented into FOCUS PELMO 3.3.2 in separate parts to predict the groundwater concentrations of M-01 and M-02 arising from cleavage of fluopicolide and to reflect the extensive metabolism of M-02 in soil (DAR, Figures B.8.32-34). Estimated formation fractions and partial reaction rates were used as before, except where indicated in the footnotes to the table 8.26 above.

Figure 8.35 PELMO metabolism scheme for transport of fluopicolide, M-02 and its M-02 metabolites (excluding M-11/M-12) in acidic and alkaline soils.



Where AE C638206 = fluopicolide, AE0608000 = M-03, PCA = M-02, P1x = M-05, P4 = M-10, P7 = M-14, P3 = M-13.

For both the simulation run with the FOCUS PEARL model (implementing kinetic sorption behaviour) and the simulation with the FOCUS PELMO model (no sorption kinetic included), the 80th percentile annual average concentrations of fluopicolide and its metabolites at 1 m depth are shown below.

RESULTS FOR VINES

Table 8.29 Predicted 80th percentile annual average concentrations in groundwater at 1 m depth following use to **vines (PEARL, including sorption kinetics)**

Scenario	Annual PEC_{gw} in µg/L									
	Fluo-picolide	M-03, AE 0608000	M-01, AE C653711	M-02, AE C657188	M-05, AE 1344122	M-14, AE 1388273	M-11, P2a	M-12, P2b	M-13, P3	M-10, AE 1344123
Châteaudun	0.018	< 0.001	4.887	0.002	0.510	0.025	0.241	0.161	0.083	0.313
Hamburg [a]	0.010	0.423	5.879	0.019	0.672	0.032	0.371	0.247	0.181	0.444
Krems-münster	0.008	< 0.001	4.389	0.001	0.411	0.020	0.218	0.145	0.077	0.257
Piacenza	0.147	< 0.001	4.515	0.017	0.571	0.027	0.187	0.124	0.085	0.287
Porto [a]	< 0.001	0.013	1.553	< 0.001	0.082	0.004	0.127	0.084	0.043	0.092
Sevilla	0.006	< 0.001	3.630	0.001	0.329	0.015	0.168	0.112	0.045	0.202
Thiva	0.019	< 0.001	3.875	0.002	0.339	0.016	0.132	0.088	0.032	0.163

a acidic soil, corresponding metabolism pathway used.

Table 8.30 Predicted 80th percentile annual average concentrations in groundwater at 1 m depth following use to **vines (PELMO, no sorption kinetic)**

Scenario	Annual PEC_{gw} in µg/L									
	Fluo-picolide	M-03, AE 0608000	M-01, AE C653711	M-02, AE C657188	M-05, AE 1344122	M-14, AE 1388273	M-11, P2a	M-12, P2b	M-13, P3	M-10, AE 1344123
Châteaudun	0.173	< 0.001	5.003	0.012	0.554	0.027	0.261	0.174	0.090	0.343
Hamburg [a]	0.067	0.525	6.265	0.036	0.715	0.033	0.516	0.344	0.216	0.586
Krems-münster	0.089	< 0.001	4.862	0.007	0.474	0.023	0.302	0.202	0.117	0.363
Piacenza	0.519	< 0.001	4.891	0.038	0.607	0.029	0.246	0.164	0.096	0.353
Porto [a]	< 0.001	0.018	1.981	0.001	0.126	0.006	0.208	0.138	0.069	0.140
Sevilla	0.001	< 0.001	4.118	0.001	0.236	0.011	0.296	0.197	0.037	0.203
Thiva	0.087	< 0.001	4.645	0.007	0.388	0.018	0.218	0.145	0.041	0.238

a acidic soil, corresponding metabolism pathway used

Table 8.31 Maximum 80th percentile annual average concentrations and exceedance of 0.1 µg/l limit following use to **vines – new assessment**.

	Highest 80th percentile concentrations (µg/l, *scenario*)		No. of scenarios > 0.1 µg/l (out of 7 simulated).	
	PEARL	**PELMO**	**PEARL**	**PELMO**
Parent	**0.147** (*P*)	**0.519** (*P*)	1	2
M-03	**0.423** (*H*)	**0.525** (*H*)	1	1
M-01	**5.879** (*H*)	**6.265** (*H*)	7	7
M-02	0.019 (*H*)	0.038 (*P*)	-	-
M-05	**0.672** (*H*)	**0.715** (*H*)	6	7
M-14	0.032 (*H*)	0.033 (*H*)	-	-
M-11	**0.371** (*H*)	**0.516** (*H*)	7	7
M-12	**0.247** (*H*)	**0.344** (*H*)	5	7
M-13	**0.181** (*H*)	**0.216** (*H*)	1	2
M-10	**0.444** (*H*)	**0.586** (*H*)	6	7

bold font denotes > 0.1 µg/l.
P = Piacenza, H= Hamburg, J= Jokioinen.

For comparison, Table 8.32 below provides the same results from the original groundwater assessment (reported in the DAR at B.8.6.2) using FOCUS PELMO, but assuming greater crop interception.

Table 8.32 Maximum 80th percentile annual average concentrations and exceedances of 0.1 µg/l limit following use to **vines – original assessment**

	Highest 80th percentile concentrations (µg/l, *scenario*)	No. of scenarios > 0.1 µg/l (out of 7 simulated).
	PELMO	**PELMO**
Parent	**0.452** (*P*)	2
M-03	**0.381** (*H*)	1
M-01	**4.614** (*H*)	7
M-02	0.033 (*P*)	-
M-05	**0.540** (*P*)	7
M-14	0.016 (*P*)	-
M-11	**0.386** (*H*)	7
M-12	**0.258** (*H*)	7
M-13	**0.160** (*H*)	1
M-10	**0.430** (*H*)	7

bold font denotes > 0.1 µg/l.
P = Piacenza, H= Hamburg, J= Jokioinen.

The maximum 80th percentile concentrations of parent compound and metabolites predicted in groundwater, following use on vines are shown in Table 8.31. The 80th percentile PECgw value for fluopicolide exceeded the maximum acceptable concentration of 0.1 µg/l at the Châteaudun (PEARL and PELMO) and Piacenza (PELMO) scenarios.

For metabolites M-01 and M-11, PECgw values were predicted to exceed 0.1 µg/l at every scenario, with both the FOCUS PEARL and PELMO models. Metabolites M-03, M-05, M-12 and M-13 also exceeded 0.1 µg/l at some scenarios, (shown in Table 8.31). Only metabolites M-02 and M-14 were predicted to be below 0.1 µg/l in groundwater at all 7 scenarios simulated with both models.

For both the models run, predicted concentrations of M-01 following use on vines were between >0.75 µg/l and <10 µg/l. Predicted concentrations of the other metabolites simulated were all <0.75 µg/l.

RESULTS FOR POTATOES

Table 8.33 Predicted 80th percentile annual average concentrations in groundwater at 1 m depth following use to **potatoes every year PEARL**, with sorption kinetics**)**

Scenario	Annual PEC$_{gw}$ in µg/L									
	Fluo-picolide	M-03, AE 0608000	M-01, AE C653711	M-02, AE C657188	M-05, AE 1344122	M-14, AE 1388273	M-11, P2a	M-12, P2b	M-13, P3	M-10, AE 1344123
Châteaudun	0.004	< 0.001	5.100	< 0.001	0.440	0.021	0.232	0.155	0.060	0.260
Hamburg [a]	0.007	0.386	6.628	0.019	0.697	0.033	0.490	0.327	0.202	0.508
Jokioinen [a]	< 0.001	0.146	5.658	0.007	0.477	0.021	0.669	0.446	0.312	0.525
Krems-münster	0.004	< 0.001	4.761	< 0.001	0.421	0.020	0.254	0.169	0.080	0.272
Okehamp-ton [a]	0.007	0.382	5.394	0.020	0.594	0.027	0.278	0.186	0.113	0.352
Piacenza	0.104	< 0.001	4.756	0.013	0.531	0.025	0.191	0.127	0.077	0.290
Porto [a]	< 0.001	0.011	1.576	< 0.001	0.082	0.003	0.119	0.080	0.044	0.090
Sevilla	< 0.001	< 0.001	3.448	< 0.001	0.161	0.007	0.089	0.059	0.022	0.086
Thiva	0.003	< 0.001	3.961	< 0.001	0.250	0.012	0.125	0.084	0.022	0.119

a acidic soil, corresponding metabolism pathway used.

Table 8.34 Predicted 80th percentile annual average concentrations in groundwater at 1 m depth following use to **potatoes every 2 years** (**PEARL**, with sorption kinetics)

Scenario	Annual PEC$_{gw}$ in µg/L									
	Fluo-picolide	M-03, AE 0608000	M-01, AE C653711	M-02, AE C657188	M-05, AE 1344122	M-14, AE 1388273	M-11, P2a	M-12, P2b	M-13, P3	M-10, AE 1344123
Châteaudun	0.001	< 0.001	2.428	< 0.001	0.199	0.009	0.119	0.079	0.030	0.127
Hamburg [a]	0.002	0.201	3.153	0.009	0.315	0.015	0.227	0.151	0.099	0.242
Jokioinen [a]	< 0.001	0.065	2.609	0.003	0.208	0.009	0.333	0.222	0.142	0.242
Krems-münster	0.001	< 0.001	2.281	< 0.001	0.190	0.009	0.131	0.087	0.039	0.126
Okehamp-ton [a]	0.002	0.186	2.554	0.008	0.272	0.012	0.131	0.088	0.057	0.172
Piacenza	0.036	< 0.001	2.274	0.005	0.269	0.013	0.092	0.061	0.038	0.140
Porto [a]	< 0.001	0.004	0.700	< 0.001	0.032	0.001	0.062	0.041	0.022	0.041
Sevilla	< 0.001	< 0.001	1.546	< 0.001	0.072	0.003	0.049	0.032	0.012	0.045
Thiva	0.001	< 0.001	1.950	< 0.001	0.106	0.005	0.065	0.044	0.008	0.055

a acidic soil, corresponding metabolism pathway used.

Table 8.35 Predicted 80th percentile annual average concentrations in groundwater at 1 m depth following use to **potatoes every 3 years** (**PEARL**, with sorption kinetics)

Scenario	Annual PEC$_{gw}$ in µg/L									
	Fluo-picolide	M-03, AE 0608000	M-01, AE C653711	M-02, AE C657188	M-05, AE 1344122	M-14, AE 1388273	M-11, P2a	M-12, P2b	M-13, P3	M-10, AE 1344123
Châteaudun	0.001	< 0.001	1.602	< 0.001	0.130	0.006	0.080	0.053	0.021	0.086
Hamburg [a]	0.001	0.116	2.100	0.005	0.210	0.010	0.142	0.094	0.067	0.158
Jokioinen [a]	< 0.001	0.040	1.597	0.001	0.122	0.005	0.206	0.137	0.091	0.141
Krems-münster	0.001	< 0.001	1.553	< 0.001	0.118	0.006	0.09	0.06	0.025	0.084
Okehamp-ton [a]	0.001	0.118	1.701	0.005	0.172	0.008	0.087	0.058	0.037	0.111
Piacenza	**0.024**	< 0.001	1.582	0.003	0.182	0.008	0.061	0.040	0.026	0.094
Porto [a]	< 0.001	0.003	0.436	< 0.001	0.018	0.001	0.039	0.026	0.015	0.025
Sevilla	< 0.001	< 0.001	0.910	< 0.001	0.043	0.002	0.034	0.023	0.008	0.030
Thiva	< 0.001	< 0.001	1.292	< 0.001	0.073	0.003	0.044	0.030	0.006	0.041

a acidic soil, corresponding metabolism pathway used

Table 8.36 Predicted 80th percentile annual average concentrations in groundwater at 1 m depth following use to **potatoes every year** (**PELMO**, no sorption kinetic)

Scenario				Annual	PEC$_{gw}$	in µg/L				
	Fluo-picolide	M-03, AE 0608000	M-01, AE C653711	M-02, AE C657188	M-05, AE 1344122	M-14, AE 1388273	M-11, P2a	M-12, P2b	M-13, P3	M-10, AE 1344123
Châteaudun	0.001	< 0.001	3.995	< 0.001	0.160	0.008	0.206	0.137	0.029	0.154
Hamburg [a]	0.010	0.275	6.733	0.016	0.592	0.027	0.496	0.334	0.199	0.525
Jokioinen [a]	< 0.001	0.028	4.536	0.002	0.240	0.011	0.813	0.542	0.369	0.534
Krems-münster	0.001	< 0.001	4.181	< 0.001	0.206	0.010	0.292	0.195	0.059	0.212
Okehamp-ton [a]	0.008	0.175	5.392	0.008	0.429	0.020	0.319	0.212	0.111	0.352
Piacenza	0.212	< 0.001	4.867	0.018	0.501	0.024	0.205	0.137	0.071	0.284
Porto [a]	< 0.001	0.001	1.079	< 0.001	0.021	0.001	0.165	0.11	0.055	0.093
Sevilla	< 0.001	< 0.001	0.114	< 0.001	0.001	< 0.001	0.041	0.027	0.005	0.009
Thiva	< 0.001	< 0.001	1.951	< 0.001	0.027	0.001	0.074	0.049	0.008	0.029

a acidic soil, corresponding metabolism pathway used.

Table 8.37 Predicted 80th percentile annual average concentrations in groundwater at 1 m depth following use to **potatoes every 2 years** (**PELMO**, no sorption kinetic)

Scenario				Annual	PEC$_{gw}$	in µg/L				
	Fluo-picolide	M-03, AE 0608000	M-01, AE C653711	M-02, AE C657188	M-05, AE 1344122	M-14, AE 1388273	M-11, P2a	M-12, P2b	M-13, P3	M-10, AE 1344123
Châteaudun	< 0.001	< 0.001	1.913	< 0.001	0.072	0.003	0.097	0.064	0.014	0.068
Hamburg [a]	0.003	0.119	3.152	0.006	0.271	0.012	0.242	0.162	0.099	0.243
Jokioinen [a]	< 0.001	0.013	2.073	0.001	0.097	0.004	0.371	0.247	0.177	0.214
Krems-münster	< 0.001	< 0.001	1.986	< 0.001	0.089	0.004	0.136	0.090	0.029	0.100
Okehamp-ton [a]	0.003	0.079	2.542	0.004	0.183	0.008	0.157	0.104	0.051	0.157
Piacenza	0.076	< 0.001	2.357	0.005	0.247	0.012	0.098	0.065	0.036	0.139
Porto [a]	< 0.001	< 0.001	0.471	< 0.001	0.009	0.001	0.079	0.053	0.027	0.034
Sevilla	< 0.001	< 0.001	0.056	< 0.001	< 0.001	< 0.001	0.020	0.014	0.002	0.004
Thiva	< 0.001	< 0.001	0.830	< 0.001	0.010	< 0.001	0.029	0.019	0.003	0.013

a acidic soil, corresponding metabolism pathway used

Table 8.38 Predicted 80th percentile annual average concentrations in groundwater at 1 m depth following use to **potatoes every 3 years** (**PELMO**, no sorption kinetic)

Scenario	Annual PEC$_{gw}$ in µg/L									
	Fluo-picolide	M-03, AE 0608000	M-01, AE C653711	M-02, AE C657188	M-05, AE 1344122	M-14, AE 1388273	M-11, P2a	M-12, P2b	M-13, P3	M-10, AE 1344123
Châteaudun	< 0.001	< 0.001	1.223	< 0.001	0.043	0.002	0.065	0.044	0.009	0.043
Hamburg [a]	0.002	0.079	2.003	0.004	0.170	0.008	0.151	0.101	0.068	0.160
Jokioinen [a]	< 0.001	0.008	1.331	< 0.001	0.060	0.003	0.249	0.166	0.117	0.131
Krems-münster	< 0.001	< 0.001	1.224	< 0.001	0.051	0.003	0.091	0.060	0.019	0.061
Okehamp-ton [a]	0.001	0.049	1.627	0.002	0.113	0.005	0.104	0.069	0.034	0.099
Piacenza	**0.041**	< 0.001	1.526	0.003	0.166	0.008	0.065	0.043	0.024	0.093
Porto [a]	< 0.001	< 0.001	0.303	< 0.001	0.005	< 0.001	0.053	0.035	0.017	0.018
Sevilla	< 0.001	< 0.001	0.034	< 0.001	< 0.001	< 0.001	0.010	0.007	0.001	0.002
Thiva	< 0.001	< 0.001	0.559	< 0.001	0.006	< 0.001	0.019	0.013	0.002	0.008

[a] acidic soil, corresponding metabolism pathway used

Table 8.38 Maximum 80th percentile annual average concentrations and exceedance of 0.1 µg/l limit following use to **potatoes with PEARL**.

	Highest 80th percentile concentrations (µg/l, *scenario*)			**No. of scenarios > 0.1µg/l (out of 9 simulated).**		
Application:	**Every yr**	**1 in 2 yrs**	**1 in 3 yrs**	**Every yr**	**1 in 2 yrs**	**1 in 3 yrs**
Parent	**0.104** (*P*)	0.036 (*P*)	0.024 (*P*)	1	-	-
M-03	**0.386** (*H*)	**0.201** (*H*)	**0.118** (*N*)	3	2	2
M-01	**6.628** (*H*)	**3.153** (*H*)	**2.10** (*H*)	9	9	9
M-02	0.02 (*N*)	0.009 (*H*)	0.005 (*H/N*)	-	-	-
M-05	**0.697** (*H*)	**0.315** (*H*)	**0.210** (*H*)	8	7	6
M-14	0.033 (*H*)	0.015 (*H*)	0.01 (*H*)	-	-	-
M-11	**0.669** (*J*)	**0.333** (*J*)	**0.206** (*J*)	8	5	2
M-12	**0.446** (*J*)	**0.222** (*J*)	**0.137** (*J*)	6	2	1
M-13	**0.312** (*J*)	**0.142** (*J*)	0.091 (*J*)	3	1	-
M-10	**0.525** (*J*)	**0.242** (*H/J*)	**0.158** (*H*)	7	6	3

bold font denotes > 0.1 µg/l

Table 8.39 Maximum 80th percentile annual average concentrations and exceedance of 0.1 µg/l limit following use to **potatoes with PELMO**.

	Highest 80th percentile concentrations (µg/l, *scenario*)			**No. of scenarios > 0.1µg/l (out of 9 simulated).**		
Application:	**Every yr**	**1 in 2 yrs**	**1 in 3 yrs**	**Every yr**	**1 in 2 yrs**	**1 in 3 yrs**
Parent	**0.212** (*P*)	0.076 (*P*)	0.041 (*P*)	1	-	-
M-03	**0.275** (*H*)	**0.119** (*H*)	0.079 (*H*)	2	1	-
M-01	**6.733** (*H*)	**3.152** (*H*)	**2.003** (*H*)	8	8	7
M-02	0.018 (*P*)	0.006 (*H*)	0.004 (*H*)	-	-	-
M-05	**0.592** (*H*)	**0.271** (*H*)	**0.170** (*H*)	6	3	3
M-14	0.027 (*H*)	0.012 *H/P*)	0.008 (*H/P*)	-	-	-
M-11	**0.813** (*J*)	**0.371** (*J*)	**0.249** (*J*)	7	4	3
M-12	**0.542** (*J*)	**0.247** (*J*)	**0.166** (*J*)	7	3	2
M-13	**0.369** (*J*)	**0.177** (*J*)	**0.117** (*J*)	3	1	1
M-10	**0.534** (*J*)	**0.243** (*H*)	**0.160** (*H*)	6	5	2

bold font denotes > 0.1 µg/l.
P = Piacenza, H= Hamburg, J= Jokioinen.
The results of Table 8.39 for application 1 in 3 years are equivalent to those reported for the original groundwater assessment in the DAR, Table B.8.260, with very slight differences for metabolite M-14 (highest 80th percentile concentration was 0.005 µg/l).

The maximum predicted 80th percentile concentrations of fluopicolide and metabolites in groundwater following use on potatoes, and the number of scenarios where 0.1 µg/l is exceeded, are shown in Tables 8.38 and 8.39.

Following use to potatoes, PECgw values for fluopicolide exceeded the maximum acceptable concentration of 0.1 µg/l at Piacenza (PEARL and PELMO), when application was assumed every year. If crop rotation was taken into account (application assumed every 2 or 3 years), then PECgw values for fluopicolide were less than 0.1 µg/l.

PECgw values of metabolite M-01 exceeded 0.1 µg/l at every scenario (PEARL and PELMO) when application was assumed every year. When application to potatoes was assumed every 2 or 3 years instead, M-01 still exceeded 0.1 µg/l at every scenario (PEARL) and all but Sevilla (PELMO).

Only metabolites M-02 and M-14 were predicted to be below 0.1 µg/l in groundwater at every scenario (and application regime simulated), for both models.

For both models, following use in potatoes, PECgw for all the metabolites simulated were <0.75 µg/l, with these exceptions, which were between >0.75 µg/l and <10 µg/l:

M-11 at Jokioinen (PELMO, application every year)
M-01 at every scenario/ application regime simulated, except Sevilla (PELMO, application every 1, 2 and 3 years which were <0.75 µg/l).

Comparison of results with original groundwater assessment in DAR, B.8.6.2.

Metabolites exceeding 0.1 µg/l

The original groundwater assessment for fluopicolide and its metabolites (reported in the DAR, B.8.6.2) was carried out using FOCUS PELMO with standard degradation and sorption parameters and for use on vines, assumed greater crop interception than considered here. The results indicated that parent and the metabolites, M-01, M-03, M-05, M-10, M-11, M-12 and M-13 had potential to exceed 0.1 µg/l at various scenarios (see Table 8.32).

The new groundwater modelling with PELMO (assuming less crop interception for vines) and PEARL (incorporating sorption kinetics), results in the same metabolites being predicted to have potential to contaminate groundwater above 0.1 µg/l. No additional metabolites are predicted to exceed 0.1 µg/l, following proposed use of fluopicolide to vines.

The original groundwater assessment (DAR, B.8.6.2) with FOCUS PELMO assumed application to potatoes, once every 3 years. It resulted in predicted concentrations of fluopicolide being < 0.1 µg/l, but metabolites M-01, M-5, M-10, M-11, M-12 and M-13 were predicted to have potential to contaminate groundwater > 0.1 µg/l.

The new groundwater modelling with PELMO (assuming application to potatoes also every 2 and every 3 years) and with PEARL (incorporating sorption kinetics), results in the same metabolites being predicted to have potential to contaminate groundwater above 0.1 µg/l. However, for application every year, parent compound and M-03 are also predicted to exceed 0.1 µg/l for certain scenarios.

Predicted concentrations of M-03 exceed 0.1 µg/l in both the PEARL and PELMO models, following application to potatoes every 2 years, and also in PEARL after application every 3 years, (though not in PELMO). Following application to potatoes every 3 years, M-13 did not exceed 0.1 µg/l in PEARL, though it did at one scenario in PELMO.

Number of scenarios where 0.1 µg/l is exceeded

For use of fluopicolide on vines, the number of scenarios where 0.1 µg/l was exceeded by parent or metabolites is almost the same, when comparing the results of new and previous PELMO modelling. Incorporation of sorption kinetics in PEARL modelling, gave slightly fewer scenarios exceeding 0.1 µg/l for parent, M-05, M-10 and M-12, but otherwise was similar.

For use of fluopicolide on potatoes, the results of PELMO modelling for application once every 3 years are essentially the same as previously reported in the DAR. Assuming more frequent application, i.e. every year or every 2 years, modelling with PELMO gave a greater number of scenarios where 0.1 µg/l was exceeded, as shown in Table 8.39.

Incorporation of sorption kinetics in PEARL modelling for use on potatoes generally gave an increased number of scenarios at which concentrations of metabolites exceeded 0.1 µg/l, (increasing with frequency of application). There were some exceptions: for M-13, the number of scenarios with concentrations > 0.1 µg/l were similar to those with PELMO and for application every 3 years were all <0.1 µg/l in PEARL. For M-12, the number of scenarios with concentrations >0.1µg/l were slightly fewer in PEARL, than those with PELMO. For M-11, the number of scenarios with concentrations >0.1µg/l were one less than in PEARL, for application once every 3 years).

Differences in 80th percentile concentrations of parent and metabolites

For use of fluopicolide on vines, the assumption of less crop interception in PELMO modelling resulted in higher 80th percentile annual average concentrations for parent and metabolites, as would be expected. The incorporation of sorption kinetics in PEARL modelling gave lower PECgw values for parent fluopicolide, than in the original PELMO assessment, but in some cases concentrations of metabolites were higher (e.g. M-01, M-03, M-05, M-10, M-11, M-13 and M-14. Compare Tables 8.31 and 8.32).

For use of fluopicolide on potatoes, revised PELMO modelling assuming more frequent application (every year or every 2 years) gave higher PECgw values for parent and metabolites, as would be expected. Incorporating sorption kinetics into PEARL modelling generally gave similar or slightly lower PECgw, compared to the results of PELMO modelling, with application every 3 years. (See Table 8.38 compared with the column for “application 1 in 3 years” of Table 8.39, the results of which are equivalent to those originally reported in the DAR).

For application to potatoes every 2 years, PEARL modelling gave a slightly higher 80th percentile concentration for M-05, but similar or lower concentrations for parent and other metabolites, compared to corresponding results with PELMO. For application every 3 years, PEARL gave higher 80th percentile concentrations for M-03, M-05 and M-14, but similar or lower concentrations for parent and the other metabolites, compared to corresponding results with PELMO.

RMS Risk Assessment and Conclusions:

For use on vines, fluopicolide is predicted to contaminate groundwater above the maximum acceptable concentration (0.1 µg/l) at one or two of the 7 scenarios modelled, (Châteaudun and or Piacenza). Concentrations of the metabolites M-01, M-05, M-10, M-11, M-12 and M-13 were predicted to exceed 0.1 µg/l in groundwater. Of these, M-01, M-05, M-10, M-11 and M-12 exceeded 0.1 µg/l in all, or almost all of the scenarios simulated in both PELMO and PEARL. In particular, predicted concentrations of M-01 were many orders of magnitude higher than this limit (range 1.6-6.3 µg/l). Metabolites M-03 and M-13 only exceeded 0.1 µg/l at a couple of scenarios, (and for M-03 the scenarios were those with acidic soils). Therefore, the relevance of these metabolites needs to be assessed further, in accordance with the EU Guidance Document on the assessment of the relevance of metabolites in groundwater.[18]

In the view of the RMS, application every year to potatoes is considered to be extreme and not representative in the vast majority of cases. For use of fluopicolide as proposed on potatoes, assuming application every 2 or 3 years, fluopicolide was not predicted to contaminate groundwater above 0.1 µg/l. However, M-01 exceeded 0.1 µg/l in all or almost all of the modelled scenarios (up to 2 µg/l and 3.2 µg/l, for application every 2 and 3 years, respectively). Metabolites M-03, M-05, M-10, M-11, M-12 and M-13 also exceeded the 0.1 µg/l limit for various scenarios. Therefore, as above for vines, the relevance of these metabolites need to be assessed further, in accordance with the EU Guidance Document.

Overall, it can be seen that the revised modelling has not resulted in any additional metabolites being predicted to occur at >0.1 µg/l on an annual average basis. The highest concentrations of fluopicolide metabolites from either modelling or lysimeter study seen in the original DAR compared to the highest results from modelling in this addendum are presented below. These have been tabulated simply on the basis of concentration and ignore the GAP used to produce the PEC values and the model used. However, it should be noted that some of the highest concentrations from modelling in this addendum are from use every year on potatoes which the RMS considers to be extreme worst-case and inappropriate as a regulatory scenario.

[18] EU Guidance Document on the assessment of the relevance of metabolites in groundwater of substances regulated under Council Directive 91/414/EEC – Sanco/221/2000-rev 10, 25 February 2003.

Table B.8.40 Comparison of highest metabolite groundwater PEC values from original DAR and this addendum for regulatory decision-making (µg/l)

	Highest concentrations in original DAR	Highest concentrations in addendum
M-03	0.381 (H)	**0.525** (H)
M-01	4.614 (H)	**6.733** (H)
M-02	0.033 (P)	0.038 (P)
M-05	0.90 (L)	0.715 (H)
M-14	0.19 (L)	0.033 (H)
M-11	0.55 (L)	**0.813** (J)
M-12	0.36 (L)	**0.542** (J)
M-13	0.160 (H)	**0.369** (J)
M-10	0.83 (L)	0.586 (H)

Values in **bold** are increases from the original DAR values
P = Piacenza; H = Hamburg; L = lysimeter; J = Jokioinen

Thus it can be seen that the highest concentrations of regulatory significance for most metabolites have increased as a result of this new assessment. It should be noted that for M-11, the revised concentration is >0.75 µg/l, whereas in the original DAR the concentration was <0.75 µg/l. This has implications for the relevance assessment. However, it must be realised that the highest concentration occurred on potatoes assuming that the crop was grown every year. In the opinion of the RMS, this is an extreme and unrepresentative GAP for potato, and in GAP assuming a rotation of 1 in 2 years or longer, 0.75 µg/l was not exceeded.

Implications for Ecotoxicogical Assessment:

Based on assessment of new FOCUS groundwater modelling, the following metabolites are predicted to have potential to exceed 0.1 µg/l in groundwater: M-01, M-03 (acidic soils), M-05, M-10, M-11, M-12 and M-13. (M-02 and M-14 were predicted at concentrations less than 0.1 µg/l).

The only metabolite predicted to contaminate groundwater at > 0.1 µg/l, not previously identified in the original assessment (DAR, B.8.6.2) is M-03 following use on potatoes. However, M-03 was considered in the previous assessment to exceed the 0.1 µg/l limit, following use of fluopicolide on vines.

Table 8.41 Summary of predicted potential of fluopicolide and metabolites to contaminate groundwater above 0.1 µg/l.

	Vines	Potatoes (every yr)	Potatoes (every 2 yr)	Potatoes (every 3 yr)
Parent	X	X	-	-
M-01	X	X	X	X
M-02	-	-	-	-
M-03	X	X	X	X
M-05	X	X	X	X
M-10	X	X	X	X
M-11	X	X	X	X
M-12	X	X	X	X
M-13	X	X	X	X
M-14	-	-	-	-

X 80th percentile annual average concentration > 0.1 µg/l for at least one FOCUS scenario in PEARL and/or PELMO model(s).

- 0.1 µg/l not exceeded at any FOCUS scenario modelled.

Concentrations of parent and metabolites following use on vines have been modelled assuming less crop interception, so the results from the PELMO model are higher (Table 8.30) than previously assessed. The concentrations predicted from the PEARL model (for vines), taking into account sorption kinetics, were higher for metabolites M-01, M-03, M-05, M-10, M-11, M-13 and M-14.

Concentrations of parent and metabolites have been modelled using the FOCUS PELMO and PEARL models assuming application to potatoes every year, every 2 and every 3 years. The previous assessment (DAR, B.8.6.2) used only the PELMO model and assumed application only once every 3 years. Therefore, concentrations from the PELMO model (application every 3 years) are equivalent to those previously assessed (Table 8. 39). However, taking sorption kinetics into account in the PEARL model (Table 8.38) gave higher 80th percentile concentrations for the metabolites M-01, M-03, and M-05, (and only very fractionally higher for M-02 and M-14).

Assuming more frequent application to potatoes, every year or every 2 years, the predicted 80th percentile concentrations of all the metabolites are increased over those previously considered in the DAR. (The RMS considers that application every year to potatoes is extreme and not representative in the vast majority of cases).

(Kley, C. & Ellerich, C. 2007 (a) & (b), Kley 2004, MEF-04/346 and MEF-04/347)

B.9 ECOTOXICOLOGY

This addendum addresses ecotoxicological issues raised during the EU peer review of the Draft Assessment Report (DAR) prepared by the RMS, UK, for EU consideration of inclusion of the fungicide new active substance (NAS), fluopicolide, in Annex I of EU Directive 91/414/EEC on plant protection products.

Ecotoxicological issues to be addressed were identified in Section 5 of the Evaluation Table which were derived from EU peer review comments and responses compiled in Section 5 of the Reporting Table [Reporting Table, fluopicolide, rev.1 (26.01.2007)].

For ease of reference the proposed EU uses of fluopicolide are re-presented in Table B.9.0.1.

Table B.9.0.1 Summary of intended EU uses of fluopicolide

Crop (formulation)	Maximum individual fluopicolide application rate (kg a.s./ha)	Maximum no. of applications	Maximum fluopicolide total dose (kg a.s./ha)	Spray water volume (L/ha)	Application timings (d - min. spray interval)	PHI (d)
Vine ('EXP 11074B')	0.133	3	0.4 kg/ha	100-1500	BBCH 53-81[1] (10)	21-35
Potato ('EXP 11120A')	0.1	4	0.4 kg/ha	200-400 (NMS) 400-1000 (SMS)	BBCH 20-91[2] (7)	7

PHI pre harvest interval
[1] inflorescences clearly visible - to beginning of ripening
[2] first basal side shoot visible- to beginning of leaf yellowing

B.9.1 Non-target vertebrates - birds and mammals (DAR B.9.1 & B.9.3)

Two issues **Evaluation Open pts. 5.1 & 5.2** were raised pertinent to the dietary risk posed to birds and mammals from proposed uses of fluopicolide in potato and vine.

Evaluation Table Open pt. 5.1

'RMS to clarify in an addendum how the MAF for different vegetation was calculated and used in the assessment of risk to birds'

5.1 RMS response:

For clarity, the Tier 1 bird and mammal risk assessment for use on potato, undertaken in accordance with SANCO 4145/2000-24/Sep/2002, is re-presented in Table B.9.1.1.

Table B.9.1.1 Tier I avian and mammalian dietary risk from proposed fluopicolide use on potato

RISK Crop / food	Indicator spp. (bw kg)	FIR /bw	a.s. app. rate kg/ha	RUD	MAF	ftwa	PT	PD	AV	ETE mg a.s./kg bw/d	Tox. end pt. mg a.s./kg bw/d	TER	Ann. VI
AVIAN - ACUTE											**LD50**		
Leafy (E/L) / s. insects	insectivore (0.01)	1.04	0.100	52	n.a.	n.a.	1.00	1.00	1.00	5.408	>2250.00	**>416.05**	10
Leafy (E/L) / leaves	herbivore (0.3)	0.76	0.100	87	**1.8**[1]	n.a.	1.00	1.00	1.00	11.879	>2250.00	**>189.41**	10
AVIAN - SHORT TERM											**LC50**		
Leafy (E/L) / s. insects	insectivore (0.01)	1.04	0.100	29	n.a	n.a.	1.00	1.00	1.00	3.016	>1744.00	**>578.25**	10
Leafy (E/L) / leaves	herbivore (0.3	0.76	0.100	40	2.2	n.a.	1.00	1.00	1.00	6.772	>1744.00	**>257.52**	10
AVIAN - LONG TERM											**NOEC**		
Leafy (E/L) / s. insects	insectivore (0.01)	1.04	0.100	29	n.a.	n.a.	1.00	1.00	1.00	3.016	88.90	**29.48**	5
Leafy (E/L) / leaves	herbivore (0.3)	0.76	0.100	40	2.2	0.53	1.00	1.00	1.00	3.567	88.90	**24.92**	5
MAMMALIAN - ACUTE											**LD50**		
Leafy (E/L) / leaves	herbivore (3.0)	0.28	0.100	87	**1.8**[1]	n.a.	1.00	1.00	1.00	4.377	>5000.00	**>1142.46**	10
Leafy (E/L) / l. insects	Insectivore[2] (0.01)	0.63	0.100	14	n.a.	n.a.	1.00	1.00	1.00	0.882	>5000.00	**>5668.93**	10
MAMMALIAN - LONG TERM											**NOEC**		
Leafy (E/L) / leaves	herbivore (3.0)	0.28	0.100	40	2.2	0.53	1.00	1.00	1.00	1.314	20.00	**15.22**	5
Leafy (E/L) / l. insects	Insectivore[2] (0.01)	0.63	0.100	5	n.a.	n.a.	1.00	1.00	1.00	0.321	20.00	**62.25**	5

n.a. not applicable; [1]amended to SANCO 4145/2000 value

[2] indicator insectivorous species included as potato leaves are not grazed by herbivorous mammals

The TERs in Table B.9.1.1 are all above Annex VI thresholds indicating low risk to herbivorous and insectivorous birds and mammals following fluopicolide ('EXP 11120A') application to potato. It should also be noted that potato foliage is unattractive food for birds and mammals.

The issue (Open pt. 5.1) concerned selection of the acute MAF value in the risk assessment. The acute MAF value (1.8) used in Table B.9.1.1 was derived from Table 3 of SANCO 4145/2000 reflecting the proposed application regime (Table B.9.0.1) for potato (4 applications with a 7d spray interval). A discrepancy was noted in the acute MAF value (2.0) for a leafy crop application regime when derived from mathematical formula by the RMS. However, this had no impact on the conclusion of the risk assessment which now includes the SANCO 4145/2000 value.

Evaluation Table Open pt. 5.2

'RMS to include the corrected calculations and the refined RA in an addendum. List of endpoints has been amended. No discussion in expert meeting required unless required by MS.'

5.2 RMS response:

For clarity, the Tier 1 bird and mammal risk assessment for use on vine, undertaken in accordance with SANCO 4145/2000-24/Sep/2002, is re-presented in Table B.9.1.2.

Table B.9.1.2 Tier I avian and mammalian dietary risk from proposed fluopicolide use on vine

RISK Crop / food	Indicator spp. (bw kg)	FIR /bw	a.s. app. rate kg/ha	RUD	MAF	ftwa	PT	PD	AV	ETE mg a.s./kg bw/d	Tox. end pt. mg a.s./kg bw/d	TER	Ann. VI
AVIAN - ACUTE											**LD50**		
Vine (E/L) / s. insects	insectivore (0.01)	1.04	0.133	52	n.a.	n.a.	1.00	1.00	1.00	7.193	>2250.00	**>312.82**	10
AVIAN - SHORT TERM											**LC50**		
Vine (E/L) / s. insects	insectivore (0.01)	1.04	0.133	29	n.a.	n.a.	1.00	1.00	1.00	4.011	>1744.00	**>434.77**	10
AVIAN - LONG TERM											**NOEC**		
Vine (E/L) / s. insects	insectivore (0.01)	1.04	0.133	29	n.a.	n.a.	1.00	1.00	1.00	4.011	88.90	**22.16**	5
MAMMALIAN - ACUTE											**LD50**		
Vine (E/L) / s. insects	herbivore (0.025)	1.39	0.133	85	1.5	n.a.	1.00	1.00	1.00	23.445	>5000.00	**>213.26**	10
MAMMALIAN - LONG TERM											**NOEC**		
Vine (E/L) / s. insects	herbivore (0.025)	1.39	0.133	46	1.5	0.53	1.00	1.00	1.00	6.761	20.00	***2.96***	5

n.a. not applicable;

The TERs in Table B.9.1.2 are all above Annex VI thresholds except for the long term TER for small herbivorous mammals which indicates potential risk. Hence this risk requires further Tier II refinement (Table B.9.1.3).

Table B.9.1.3 Tier II mammalian refined dietary risk from proposed fluopicolide use on vine

RISK Crop / food	Indicator spp. (bw kg)	FIR /bw	a.s. app. rate kg/ha	RUD	MAF	ftwa	PT	PD	AV	ETE mg a.s./kg bw/d	Tox. end pt. mg a.s./kg bw/d	TER	Ann. VI
MAMMALIAN - LONG TERM											**NOEC**		
Vine (E/L) / s. insects	herbivore (0.025)	1.39	0.133	**23**[1]	1.5	0.53	1.00	1.00	1.00	3.38	20.00	**5.92**	5

[1] amended to reflect 70% vine canopy spray interception

In vines small herbivorous mammals consume sub-canopy (ground) vegetation, assessed as short grass, and the issue (Open pt. 5.2) addresses risk refinement via canopy spray interception. For vine fungicides, Tier 1 bird and mammal risk assessment (SANCO 4145/2000) assumes a canopy 40% spray interception. The proposed fluopicolide ('EXP 11074B') use on vine is between growth stages BBCH 53-91 (Table B.9.0.1), i.e. from inflorescence clearly visible through to start of grape ripening. These growth stages represent canopy interception from 60% (end of foliage development) through to 85% (start of ripening) and a 70% spray interception was considered an appropriate precautionary refinement. The long term risk to herbivorous mammals has been refined taking account of the higher canopy interception (Table B.9.1.3).

The TER>5 in Table B.9.1.3 indicates a low risk to small herbivorous mammals consuming sub-canopy vegetation following fluopicolide ('EXP 11074B') application to vine.

B.9.2 Aquatic organisms

Four issues **(Evaluation Table Open points 5.3, 5.4, 5.5 & 5.12)** were raised pertinent to the risk to aquatic organisms from proposed uses of fluopicolide in potato and vine crops:

Evaluation Table Open pt. 5.3

'RMS to include the information on Log Pow values for the metabolites in an addendum (only data for M02 and M03 are available in Vol.B.2.1 of the DAR). No discussion in an experts meeting is required.'

5.3 RMS response:

Log Pow values of parent and metabolites are considered in ecotoxicological risk assessment of potential bioconcentration potential.

A fish bioconcentration study was conducted (see DAR B.9.2.3.4) as fluopicolide has a logPow of 2.9, i.e. close to the logPow Annex VI threshold of >3.0 for bioconcentration assessment. The results showed that fluopicolide had bioconcentration factor (BCF) in fish of 121. Clearance of fluopicolide from fish tissues, CT50 and CT90, were 0.51 and 1.7d, respectively, and 5% of total residue remained after 18d depuration.

The overall conclusion for fluopicolide was that the low BCF and rapid clearance times of fluopicolide from fish tissues indicated a low propensity to bioaccumulate in fish. Potential for fluopicolide bioconcentration in worm- and fish-eating bird and mammals was also considered to be low (DAR B.9.15 & B.9.3.3).

Key aquatic metabolites M03, M02 and M01 (see Appendix 1) have log Pow values of 2.34, -2.0 and 0.51 (DAR Volume B.2.1), respectively, and a lower propensity for bioaccumulation than parent fluopicolide would hence be expected. M03 is also not stable in aquatic systems at environmental pH and degraded to form M02 and M01 and hence also likely to have a very low bioavailability. All other aquatic metabolites M05, M10, M11, M12, M13 and M14 are similarly structured hydroxylated and/or sulphonated derivatives of M02 (see Appendix 2). Thus such derivatives will likely have logPow values approximating to that of M02 and hence it can also be concluded that all the key aquatic fluopicolide metabolites will have little potential to bioaccumulate.

Evaluation Table Open pt. 5.4

'RMS to include the correction in a corrigendum and to update the list of endpoint. Since threshold values are different for algae and fish/invertebrates we would prefer to have TER values also for fish and invertebrates in the list of endpoints even if algae was the most sensitive organism group.'

5.4 RMS response:

List of endpoints have been updated and for purposes of clarification the corrected aquatic spray drift risk assessment for EXP 11074B and EXP11120A following respective uses on vine and potato is presented in Table B.9.2.1 below (DAR B.9.2.4.1i and B.9.2.4.2i).

Table B.9.2.1 Spray drift aquatic risk assessment for EXP 11074B and EXP11120A

Organism	Time scale	Tox. end pt.	PEC initial [1]@ 3m vine use [2]@ 1m potato use		PEC initial @ 5m vine use		Annex VI
		EXP 11074B (mg/L)	EXP 11074B (mg /L[1])	TER	EXP 11074B (mg /L)	TER	
O. mykiss	96h LC50	8.54	0.0802	106.5	-	-	100
D. magna	**21d NOEC**	**>25.0**	**0.0802**	**>311.7**	**-**	**-**	**10**
N. pelliculosa	72h EbC50 72h ErC50	0.58 0.91	0.0802 0.0802	***7.2*** 11.3	0.0362 -	16.0 -	10 10
		EXP 11120A (mg/L)	EXP 11120A (mg /L[2])	TER			
O. mykiss	96h LC50	6.57	0.0167	393.4	-	-	100
D. magna	21d NOEC	>100.0	0.0167	>5988.0	-	-	10
N. pelliculosa	72h EbC50 72h ErC50	0.40 0.63	0.0167 0.0167	24.0 37.7	- -	- -	10 10

EXP 11074B and EXP 11120A exposure was considered only likely to constitute an acute aquatic risk from spray drift following application when formulation integrity is most conserved. In Table B.9.2.1 acute TERs >Annex VI indicate that low acute aquatic risk can be expected from spraydrift following EXP 111120A on potato, however, a 5m buffer zone is required to mitigate the acute (worse case) aquatic risk to *N. pelliculosa* from EXP 11074B following use on vine.

Evaluation Table Open pts. 5.5

'RMS to include the information and argumentation regarding the ecotoxicological relevance of GW metabolites presented in column 3 in an addendum for the sake of completeness. We agree that since the TER for M05 is >18519 (vine) and >58824 (potato) for algae and this metabolite is the one of highest concentration in the FOCUS$_{gw}$ modelling, apart from M01, the risk from M10, M11, M12 and M13 to aquatic organisms can be considered to be low. The information presented is however of value for the assessment of "pesticidal activity".'

5.5 RMS response:

For aquatic ground water risk assessment of the parent, fluopicolide, the green algal diatom, *N. pelliculosa*, was by far the most sensitive species (see DAR Tables B.9.2.78 and B.9.2.85). Available aquatic data on fluopicolide metabolites also indicated *N. pelliculosa* to be most sensitive species tested, but >300x less than parent (see DAR Table B.9.2.75). The *N. pelliculosa* aquatic endpoint was used to assess the potential risk to aquatic organisms from principal leachate and groundwater average annual contaminants >0.1µg/L (Tables B.9.2.2 and B.9.2.3) (DAR B.9.2.4.1iii and B.9.2.4.2iii). It should also be noted that Env Fate (Section B.8.6.2, Addendum 1 (Nov 2007)) have recalculated PECgw based on revised modelling and the aquatic risk has been assessed in Table B.9.2.4 below.

Table B.9.2.2 Maximum fluopicolide and metabolites detected in lysimeter leachates

Leachate component[1]	µg/L	TER	Annex VI
Parent	1.69	17.15[2]	10
M01	6.69	>1495[3]	10
M02	0.10	29.0[4]	10
M03	n.d.	-	-
M05	0.90	1111[5]	10
M10	0.83	***3.5***[4]	10
M11	0.55	***5.3***[4]	10
M12	0.36	***8.1***[4]	10
M13	0.14	20.7[4]	10
M14	0.19	15.3[4]	10

n.d. not detected;
[1] see DAR B.8.2.3.3
[2] based on parent *N. pelliculosa* 72h EbC50 = 29 µg/L
[3] based on M01 *N. pelliculosa* 72h EbC50 = >10000 µg/L
[4] based on parent *N. pelliculosa* 72h EbC50 (x 0.1) = 2.9 µg/L
[5] based on M05 *N. pelliculosa* 72h EbC50 = >10000 µg/L

Table B.9.2.3 FOCUSgw PECs - *N. pelliculosa* Aquatic risk assessment

EXP 11074B use on vine (see DAR Table B.8.259)										
PEC_{gw} (80%ile)	**parent**		**M01**		**M02**		**M03**		**M05**	
Scenarios	**µg/L**	**TER[2]**	**µg/L**	**TER[3]**	**µg/L**	**TER**	**µg/L**	**TER[4]**	**µg/L**	**TER[5]**
Châteaudun	0.147	197	4.466	>2239	< 0.1	n.r.	< 0.1	n.r.	0.492	>20325
Hamburg[1]	< 0.1	n.r.	4.614	>2167	< 0.1	n.r.	**0.381**	***7.6***	0.515	>19417
Kremsmünster	< 0.1	n.r.	3.570	>2801	< 0.1	n.r.	< 0.1	n.r.	0.340	>29412
Piacenza	0.452	64	4.374	>2286	< 0.1	n.r.	< 0.1	n.r.	0.540	>18519
Porto[1]	< 0.1	n.r.	1.755	>5698	< 0.1	n.r.	< 0.1	n.r.	0.111	>90090
Sevilla	< 0.1	n.r.	3.016	>3316	< 0.1	n.r.	< 0.1	n.r.	0.168	>59524
Thiva	< 0.1	n.r.	4.131	>2421	< 0.1	n.r.	< 0.1	n.r.	0.343	>29155
	M10		**M11**		**M12**		**M13**		**M14**	
	µg/L	**TER[4]**	**µg/L**	**TER[4]**	**µg/L**	**TER[4]**	**µg/L**	**TER[4]**	**µg/L**	**TER**
Châteaudun	**0.306**	*9.5*	0.235	12.3	0.156	18.6	< 0.1	n.r.	< 0.1	n.r.
Hamburg[1]	**0.430**	***6.7***	**0.386**	***7.5***	0.258	11.2	0.160	18.1	< 0.1	n.r.
Kremsmünster	0.267	10.9	0.226	12.8	0.151	19.2	< 0.1	n.r.	< 0.1	n.r.
Piacenza	**0.316**	***9.2***	0.221	13.1	0.147	19.7	< 0.1	n.r.	< 0.1	n.r.
Porto[1]	0.125	23.2	0.187	15.5	0.124	23.4	< 0.1	n.r.	< 0.1	n.r.
Sevilla	0.148	19.6	0.221	13.1	0.147	19.7	< 0.1	n.r.	< 0.1	n.r.
Thiva	0.212	13.7	0.196	14.8	0.130	22.3	< 0.1	n.r.	< 0.1	n.r.
EXP 11120A use on potato (see DAR Table B.8.260)										
PEC_{gw} (80%ile)	**parent**		**M01**		**M02**		**M03**		**M05**	
Scenarios	**µg/L**	**TER[2]**	**µg/L**	**TER[3]**	**µg/L**	**TER**	**µg/L**	**TER[4]**	**µg/L**	**TER[5]**
Châteaudun	< 0.1	n.r.	1.223	>8177	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Hamburg[1]	< 0.1	n.r.	2.003	>4993	< 0.1	n.r.	< 0.1	n.r.	0.170	>58824
Jokioinen[1]	< 0.1	n.r.	1.331	>7513	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Kremsmünster	< 0.1	n.r.	1.224	>8170	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Okehampton[1]	< 0.1	n.r.	1.627	>6146	< 0.1	n.r.	< 0.1	n.r.	0.113	>88496
Piacenza	< 0.1	n.r.	1.526	>6553	< 0.1	n.r.	< 0.1	n.r.	0.166	>60241
Porto[1]	< 0.1	n.r.	0.303	>33003	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Sevilla	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Thiva	< 0.1	n.r.	0.559	>17889	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
	M10		**M11**		**M12**		**M13**		**M14**	
	µg/L	**TER[4]**	**µg/L**	**TER[4]**	**µg/L**	**TER[4]**	**µg/L**	**TER[4]**	**µg/L**	**TER**
Châteaudun	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Hamburg[1]	0.160	18.1	0.151	19.2	0.101	28.7	< 0.1	n.r.	< 0.1	n.r.
Jokioinen[1]	0.131	22.1	0.249	11.6	0.166	17.5	0.117	24.7	< 0.1	n.r.
Kremsmünster	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Okehampton[1]`	< 0.1	n.r.	0.104	27.9	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Piacenza	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Porto[1]	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Sevilla	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.
Thiva	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.	< 0.1	n.r.

n.r. not relevant; **bold / grey highlight** = scenarios TER<Annex VI threshold

[1] acidic soil modelling

[2] based on parent *N. pelliculosa* 72h EbC50 = 29 µg/L

[3] based on M01 *N. pelliculosa* 72h EbC50 = >10000 µg/L

[4] based on parent *N. pelliculosa* 72h EbC50 (x 0.1) = 2.9 µg/L

[5] based on M05 *N. pelliculosa* 72h EbC50 = >10000 µg/L

Table B.9.2.4 FOCUSgw PECs (see Addendum Section B.8.6.2)- *N. pelliculosa* Aquatic risk assessment

VINE	Highest 80th percentile concentrations (µg/l, *scenario*)		TER Based on *N. pelliculosa*	
	PEARL	PELMO	PEARL	PELMO
Parent[1]	0.147 (*P*)	0.519 (*P*)	197.2	55.9
M-01[2]	5.879 (*H*)	6.265 (*H*)	1701.0	1596.2
M-02	0.019 (*H*)	0.038 (*P*)	n.r.	n.r.
M-03[3]	**0.423 (*H*)**	**0.525** (*H*)	***6.9***	***5.5***
M-05[4]	0.672 (*H*)	0.715 (*H*)	14881.0	13986.0
M-10[3]	**0.444 (*H*)**	**0.586 (*H*)**	***6.5***	*4.9*
M-11[3]	**0.371 (*H*)**	**0.516 (*H*)**	***7.8***	***5.6***
M-12[3]	0.247 (*H*)	**0.344 (*H*)**	11.7	***8.4***
M-13[3]	0.181 (*H*)	0.216 (*H*)	16.0	13.4
M-14	0.032 (*H*)	0.033 (*H*)	n.r.	n.r.

POTATO	Highest 80th percentile concentrations (PEARL; µg/l, *scenario*)			TER Based on *N. pelliculosa*		
Application	**1 p.a.**	**1 in 2y**	**1 in 3y**	**1 p.a.**	**1 in 2y**	**1 in 3y**
Parent[1]	0.104 (*P*)	0.036 (*P*)	0.024 (*P*)	278.8	n.r.	n.r.
M-01[2]	6.628 (*H*)	3.153 (*H*)	2.10 (*H*)	1508.8	3171.6	4761.9
M-02	0.02 (*N*)	0.009 (*H*)	0.005 (*H/N*)	n.r.	n.r.	n.r.
M-03[3]	**0.386 (*H*)**	0.201 (*H*)	0.118 (*N*)	***7.5***	14.4	24.6
M-05[4]	0.697 (*H*)	0.315 (*H*)	0.210 (*H*)	14347.2	31746.0	47619.0
M-10[3]	**0.525 (*J*)**	0.242 (*H/J*)	0.158 (*H*)	***5.5***	12.0	18.4
M-11[3]	**0.669 (*J*)**	**0.333 (*J*)**	0.206 (*J*)	***4.3***	***8.7***	14.1
M-12[3]	**0.446 (*J*)**	0.222 (*J*)	0.137 (*J*)	***6.5***	13.1	21.2
M-13[3]	**0.312 (*J*)**	0.142 (*J*)	0.091 (*J*)	***9.3***	20.4	n.r.
M-14	0.033 (*H*)	0.015 (*H*)	0.01 (*H*)	n.r.	n.r.	n.r.

POTATO	Highest 80th percentile concentrations (PELMO; µg/l, *scenario*)			TER Based on *N. pelliculosa*		
Application	**1 p.a.**	**1 in 2y**	**1 in 3y**	**1 p.a.**	**1 in 2y**	**1 in 3y**
Parent[1]	0.212 (*P*)	0.076 (*P*)	0.041 (*P*)	136.8	n.r.	n.r.
M-01[2]	6.733 (*H*)	3.152 (*H*)	2.003 (*H*)	1485.2	3172.6	4992.5
M-02	0.018 (*P*)	0.006 (*H*)	0.004 (*H*)	n.r.	n.r.	n.r.
M-03[3]	0.275 (*H*)	0.119 (*H*)	0.079 (*H*)	10.5	24.4	36.7
M-05[4]	0.592 (*H*)	0.271 (*H*)	0.170 (*H*)	16891.9	36900.4	58823.5
M-10[3]	**0.534 (*J*)**	0.243 (*H*)	0.160 (*H*)	***5.4***	11.9	18.1
M-11[3]	**0.813** (*J*)	**0.371 (*J*)**	0.249 (*J*)	***3.6***	***7.8***	11.6
M-12[3]	**0.542** (*J*)	0.247 (*J*)	0.166 (*J*)	***5.4***	11.7	17.5
M-13[3]	**0.369** (*J*)	0.177 (*J*)	0.117 (*J*)	***7.9***	16.4	24.8
M-14	0.027 (*H*)	0.012 (*H/P*)	0.008 (*H/P*)	n.r.	n.r.	n.r.

P = Piacenza, H= Hamburg, J= Jokioinen, N= Okehampton

n.r. not relevant; **bold / grey highlight** = scenarios TER<Annex VI threshold

[1] based on parent *N. pelliculosa* 72h EbC50 = 29 µg/L

[2] based on M01 *N. pelliculosa* 72h EbC50 = >10000 µg/L

[3] based on parent *N. pelliculosa* 72h EbC50 (x 0.1) = 2.9 µg/L

[4] based on M05 *N. pelliculosa* 72h EbC50 = >10000 µg/L

From TERs (Table B.9.2.2) based on available parent and metabolite *N. pelliculosa* toxicological end points (see DAR Table B.9.2.75) and, in the absence of data, the parent *N. pelliculosa* endpoint with a 10x safety factor (SANCO 3268/2001 rev. 4), a low aquatic risk was indicated for parent and metabolites, M01, M02, M05, M13 and M014, detected in lysimeter leachate. However, metabolites, M10, M11 and M12, gave TERs < Annex VI threshold using the surrogate parent endpoint, requiring further consideration.

It is considered that M10, M11 and M12 are not structurally related to fluopicolide and do not contain the biological toxophore (see pt. 5.12 below). They are derived from M02, and are structurally related to, M05 and for these metabolites *N. pelliculosa* is >100x less sensitive than fluopicolide, therefore the toxicity profile of M10, M11 and M12 will likely be closer to M02/M05 than parent and a risk assessment based these endpoints (with a 10x safety factor) would indicate low risk. Furthermore, theoretical FOCUS estimations predict lower GW contamination than that detected in lysimeter leachate by these metabolites in all scenarios (see below).

The GW risk assessment (Table B.9.2.3) was conducted using PECs derived from FOCUS groundwater modelling (DAR Tables B.8.259 & B.8.260) following proposed respective uses of EXP11074B and EXP 11120A on vine and potato at the proposed EU GAPs. A further aquatic risk assessment was undertaken (Table B.9.2.4) using refined PECgws derived from further environmental modelling (see Addendum 1, Section B.8.6.2).

Following EXP11074B use on vine (Tables B.9.2.3-4), PECgws for M02 and M14 from all modelled scenarios were <0.1µg/L and hence were not considered further. For parent, metabolites, M01, M05, M12 (PEARL) and M13, in scenarios where the PECgw >0.1µg/L, all TERs were > Annex VI threshold indicating low risk to aquatic organisms. In two scenarios M10 was the only metabolite with TERs (9.5 & 9.2) < Annex VI threshold (10) and in one scenario (Hamburg) metabolites M03, M10, M11 and M12 (PELMO) had respective TERs (7.6, 6.7, 7.5 & 8.4) < Annex VI threshold.

Following EXP11120A use on potato (Table B.9.2.3), PECgws for parent (fluopicolide), M02, M03 and M14 from all scenarios were <0.1µg/L and hence were not considered further. For metabolites, M01, M05, M10-M13, in scenarios where the PECgw >0.1µg/L, all TERs were > Annex VI threshold indicating low risk to aquatic organisms. However, further PECgw modelling refinement (Table B.9.2.4) gave TERs <10 for M03 (PEARL - Hamburg), M10, M11, M12 & M13 in PEARL and PELMO GW modelling in Hamburg and Jokioinen scenarios based on one treatment regime per annum. From biennial treatment only TERs for M11 (PEARL and PELMO - Jokioinen) were < Annex VI threshold and following triennial treatment no TERs were < Annex VI threshold.

However, it should be noted that the risk is assessed presuming aquatic organisms will be exposed at the groundwater PECs, whereas it is reasonable to assume that at least a 10x dilution would likely occur (SANCO 3268/2001). For vine application a correction for 60% canopy interception would also have further reduced potential exposure (see DAR B.8.2.3.3). It should also be noted that M10 and M11 are chemically structurally related to M05 and M11 is purported to be an isomer of M12

(Appendix 2), hence these metabolites would likely exhibit similar aquatic toxicity, i.e. approximately 300x less toxicity than parent. M03 is structurally closely related to parent (Appendix 2) and was not seen in lysimeter leachate and only detected in two PECgw scenarios; it is unstable in water at most environmental pH and therefore negligible exposure via groundwater is expected.

Therefore, overall the weight of evidence indicates a low risk to aquatic organisms from predicted exposure to fluopicolide and principal metabolites occurring in groundwater following proposed uses of EXP 11074B and EXP 11120A on vine and potato.

Evaluation Table Open pt. 5.12

'RMS to present the complete assessment for the relevance of ground water metabolites in and addendum. Special attention should be paid to the fact that at this stage for metabolites M01, M05 and M10 the threshold of 0.75 µg/L is also exceeded either in the lysimeter or the FOCUS modelling.'

(See also Section B.6.1.4.1, Addendum 1 (Nov 2007) for full Relevance Assessment of Groundwater Metabolites).

5.12 RMS response:
Environmental relevance of GW metabolites

Formation of metabolites

Fluopicolide is a pyridinyl-benzamide fungicide (see Appendix 2). In soil the proposed fluopicolide degradation is initiated via cleavage at the amide bridge to a pyridinyl (M02) and a benzyl (M01) derivative after formation of transient hydroxylated fluopicolide intermediate (M03). M01 is relatively stable before undergoing mineralization but M02 undergoes further transformation. M02 can be sulphated by substitution of the chlorine group at the 3' position on the pyridine ring forming M05 and M10. Further ring hydroxylation of M02, M05 and M10 can also occur forming M13, M11/M12 (isomers) and M14 derivatives (Appendix 2). Parent and metabolites, M01, M02, M05, M10, M11, M12, M13 and M14, were identified in lysimeter leachate at an annual average >0.1µg/L (Table B.9.2.2) whereas parent and metabolites, M01, M03 (2 scenarios only), M05, M10, M11, M12 and M13, were predicted to occur at an annual average >0.1µg/L in some groundwater scenarios by FOCUS modelling (Table B.9.2.3); hence consideration of overall environmental relevance is required (SANCO 221/2000 rev.10).

Biological activity

Initial efficacy active substance screening and numerous tests on vegetative vigour and seedling emergence indicated that fluopicolide has no significant herbicidal activity (see DAR B.9.9.1.1). In laboratory screening (see DAR B.9.9.4) fluopicolide also did not exhibit insecticidal activity. Furthermore, in screens on 5 soil fungal species of different classes fluopicolide fungicidal sensitivity was specific to only one species, Phytophora (oomycetes) [Lechelt-Kunze, 2003e-m]. In tests on fluopicolide-sensitive fungi, grape downy mildew (*Plasmopara viticola*) and potato late blight (*Phytophthora*

infestans), fluopicolide metabolites M01, M02, M05, M10, M14 and M15 were all shown to be <<50% active compared with parent [Lechelt-Kunze, 2003e-m, Latorse & Flahout, 2004]. The fact that M01and M02, benzyl and pyridinyl derivatives formed from fluopicolide cleavage at the amide bridge (and their derivatives M05, M10 and M14) all retain no fungicidal activity is strongly indicative that the fluopicolide biological activity toxophore comprises of the intact pyridinyl-benzamide molecule.

Untested GW pyridinyl metabolites M11 and M12 (isomers), tentatively identified as hydroxylated derivatives of M10, and M13, a hydroxylated derivative of M02, are structurally similar and hence do not contain the toxophore and will not retain biological activity. M03 is a structurally-related transient hydroxylated-derivative of fluopicolide and is an unstable intermediate prior to cleavage of fluopicolide to M01 and M02. It is very unstable in water and at environmental pH will rapidly degrade to M01 and M02 and the RMS considers it inconceivable that significant exposure to M03 will occur via GW. Thus the RMS concludes that all metabolites theoretically occurring in GW >0.1µg/L will not retain or express biological activity of the parent, fluopicolide.

Other GW metabolite ecotoxicological testing

All GW metabolites were considered to be irrelevant in terms of mammalian risk (see DAR B.6.1.4 and B.6.80, Addendum 1, B.6.1.4.1) and M01 was considered of low ecotoxicological risk to mammals (B.9.1, DAR B.9.3). M01 was formed in the hen metabolism study (DAR B.7.2.2) indicating that fluopicolide avian toxicity test encompass M01 effects, and, on a molar basis, M01 was not more acutely toxic to birds and low avian risk from M01 was also indicated (DAR B.9.1). In aquatic tests M01, M02 and M05 were at least 10x < toxic than fluopicolide which included the most sensitive species, *N. Pelliculosa* (DAR Table B.9.2.75). M10, M11, M12 and M13 are GW metabolites not tested on aquatic species, are structurally similar to M02 and M05, which were significantly less toxic than fluopicolide when tested on most sensitive fish and algae species (DAR Table B.9.2.75). Low aquatic risk was concluded for all fluopicolide GW metabolites (B.9.2.1.2, DAR B.9.2). Furthermore, M01, M02 and M03 were not more toxic to worms than fluopicolide and constituted less overall risk (DAR B.9.6). Folsomia, soil microbes, soil fungi and litter decomposition, non-target plants were not more sensitive to M01 than fluopicolide and hence low ecotoxicological terrestrial risk was indicated (DAR B.9.7-9). None of the GW metabolites is predicted to have bioconcentration/bioaccumulation potential. Thus overall fluopicolide metabolites were considered unlikely to express significant ecotoxicological activity and the RMS considers that the weight of evidence suggests that GW metabolites can be regarded as not ecotoxicologically relevant.

Conclusion

From an ecotoxicological viewpoint, sufficient evidence is considered available to support the contention that all metabolites identified in groundwater at an average annual concentration >0.1µg/L can be considered environmentally 'non-relevant'.

B.9.3 Non-target vertebrates - mammals (see B.9.1 above)

B.9.4 Bees - no Open pts. to address.

B.9.5 Non Target Arthropods

One issue **(Evaluation Table Open point 5.6)** was raised pertinent to the risk to NTAs from proposed uses of fluopicolide in potato and vine crops:

Evaluation table Open pt. 5.6

RMS to correct the list of endpoint with exact %-age effect on fecundity instead of <50%. Note that highest conc. with effects <50% for A. rhopalosiphi was 2 L/ha.

5.6 RMS response

For clarity the results from NTA fluopicolide data are re-presented in Table B.9.5.1 below.

Table B.9.5.1 Summary of results from fluopicolide testing on NTAs.

SPECIES	TEST	MORTALITY LR50	FECUNDITY	DAR SECTION
Fluopicolide - applied as 'AE C638206 SC 480 A2' (487 g fluopicolide/L)				
		mL product/ha [CL 95%] (fluopicolide g/ha)	**mL product/ha (fluopicolide g/ha) [% control]**	
Aphidius rhopalosiphi	Laboratory (glass plate)	>861 [n.c.] (>419)	861 (419) [-15.7]	B.9.5.1.1 i)
Typhlodromus pyri	Laboratory (glass plate)	642 [591 - 698] (312)	574 (279) [-3.5]	B9.5.1.1 ii)
'EXP 11074B'containing fluopicolide (45.1g/kg) + fosetyl Al (671g/kg)				
		Kg product/ha [CL 95%] (fluopicolide g/ha)	**Kg product/ha (fluopicolide g/ha) [% control]**	
Aphidius rhopalosiphi	Laboratory (glass plate)	8.23 [7.81 - 8.67] (371) in/off-field HQ = 0.84/0.06	4.6 (207) [-44.1] 6.9 (311) [-66.4]	B.9.5.1.2a i)
Typhlodromus pyri	Laboratory (glass plate)	7.13 [6.62 - 7.67] (322) in/off-field HQ = 0.97/0.07	4.6 (207) [-23.9] 6.9 (311) [-19.9]	B.9.5.1.2a ii)
'EXP 11120A' containing fluopicolide (64.7 g/L) + propamocarb HCl (634 g/L)				
		L product/ha [CL 95%] (fluopicolide g/ha)	**L product/ha (fluopicolide g/ha) [% control]**	
Aphidius rhopalosiphi	Laboratory (glass plate)	2.48 [1.76 - 3.76] (161) in/off-field HQ = 1.74/0.03	0.43 (27.8) [-46.8] 0.81 (52.4) [-72.7] 2.92 (188.9)[-89.6]	B.9.5.1.2b i)
Typhlodromus pyri	Laboratory (glass plate)	3.24 [2.69 - 4.14] (210) in/off-field HQ = 1.33/0.03	0.4 (25.9) [-7.7] 0.72 (46.6) [-19.7] 1.29 (83.5) [-41.0] 2.32 (150.1) [-49.6] 4.17 (269.8) [-86.3]	B.9.5.1.2b iii)
Aphidius rhopalosiphi	Ext. lab. (leaf)	>8.0 [n.c.] (>518)	1.0 (64.7) [-7.6] 2.0 (129.4) [-20.3] 4.0 (258.8) [-50.0] 8.0 (517.6) [-98.7]	B.9.5.1.2b ii)
Typhlodromus pyri	Ext. lab. (leaf)	>4.17 [n.c.] (>270)	0.4 (25.9) [-12.9] 0.72 (46.6) [-17.9] 1.29 (83.5) [-27.6] 2.32 (150.1) [-29.8] 4.17 (269.8) [-34.3]	B.9.5.1.2b iv)
Chrysoperla carnea	Laboratory (glass plate)	>6.4 [n.c.] (>414)	6.4 (414.1) [-2.7]	B.9.5.1.2b v)

n.c. not calculable

Under extended laboratory (leaf) conditions *Aphidius rhopalosiphi* fecundity was most sensitive with 50 and 20% inhibition seen at 4.0 and 2.0L EXP 11120A/ha dosing, respectively, thus a <50% fecundity inhibition would be expected at >2x the proposed maximum individual application rate. However, all in-field and off-field HQ values, which take account of multiple applications, for both potato and vine uses (DAR 9.5.2) were below Annex VI thresholds indicating low NTA risk.

B.9.6-8 Effects on soil organisms

Four issues **(Evaluation Table Open points 5.7, 5.8, 5.9, 5.10)** were raised pertinent to the risk to soil organisms from proposed uses of fluopicolide in potato and vine crops.

B.9.6 Earthworm

Evaluation Table Open pt. 5.7

'RMS to update the list of endpoints for earthworms. It is still not clear if the values for the formulation are based on a.s. or formulation concentrations. Furthermore, values should be given as mg/kg DS.'

5.7 RMS response:

For clarity, the revised earthworm endpoints and risk assessment are presented in Table B.9.6.1 (DAR B.9.6.3.1 and B.9.6.3.2).

Table B.9.6.1 Summary of acute and chronic toxicity end points, PEC_{soil} values and TERs for earthworms from EXP 11074B use on vine

APPLICATION Test Substance	Toxic end point mg/kg DS (corrected)	Max. PEC_{soil} (mg/kg DS)	Toxicity exposure ratio	Annex VI threshold
EXP 11074B on vine				
Acute	**14dLC50**		**TER_a**	
Fluopicolide (log Pow = 2.9)	>1000 (>500[2])	0.268[1]	>1866	10
M-01 (log Pow = 0.51)	750	0.043[1]	17442	10
M-02 (logPow = -2.0)	>1000	0.026[1]	>19230	10
M-03 (logPow = 2.34)	>1000 (>500[2])	0.017[1]	>29412	10
EXP 11074B (fluopicolide)	>43.5 (>21.75[2])	0.268[1]	>81.1	
Chronic	**28/56dNOEC**		**TER_{lt}**	
Fluopicolide	62.5[3,5]	0.268[1]	233	5
M-01	250[4]	0.046[1]	5435	5
EXP 11074B (fluopicolide)	2.435[5]	0.268[1]	9.1	5
EXP 11120A on potato				
Acute	**14dLC50**		**TER_a**	
Fluopicolide (log Pow = 2.9)	>1000 (>500[2])	0.2016[1]	>2480	10
M-01 (log Pow = 0.51)	750	0.0174[1]	43103	10
M-02 (logPow = -2.0)	>1000	0.019[1]	>26316	10
M-03 (logPow = 2.34)	>1000 (>500[2])	0.013[1]	>38462	10
EXP 11120A (fluopicolide)	>57.3 (>28.65[2])	0.2016[1]	>142	10
Chronic	**28/56dNOEC**		**TER_{lt}**	
Fluopicolide	62.5[3,5]	0.2016[1]	233	5
M-01	250[4]	0.0174[1]	5435	5
EXP 11120A (fluopicolide)	2.587[5]	0.2016[1]	12.8	5

DS = dry soil
[1] peak accumulated 5cm depth (see DAR Table B.8.198)
[2] value reduced by a factor of 2 (logKow>2/10% soil OM)
[3] based on growth (28d)
[4] based on reproduction
[5] conducted in 5% soil OM (correction not required)

For Table B.9.6.1 correction (x0.5) was required for fluopicolide, M03 and product acute endpoints as log Pow > 2 and 10% soil OM was used, in chronic studies 5% soil OM was used and no correction was required. Revised TERs indicate low acute and chronic risk to earthworms from fluopicolide and principal soil metabolites, M01, M02 and M03, following application of EXP 11074B and EXP 11120A to vine and potato.

B.9.7 Other soil non-target macro-organisms

Evaluation Table Open pts. 5.8 & 5.9

'Pending on the discussion on the PECsoil in the section on Fate and behaviour, a revision of the risk assessment for soil organisms might be necessary.'

(Reporting Table comment at 5(45) and 5(47)

5.8 & 5.9 RMS response:

RMS will address, as appropriate, if evaluation/discussion of Applicant's response by Environmental Fate results in PECsoil amendment.

B.9.8 Non-target soil microorganisms

Evaluation Table Open pt. 5.10

'RMS to include the argumentation for why no studies with soil micro-organisms are required with M 03 in an addendum for the sake of completeness. No discussion in an expert meeting is required.'

5.10 RMS response:

OECD 216/217 guidance for soil microbial activity recommends tests to be undertaken at soil pH 5.5 - 7.5. At these pHs M03 has a DT50 <1.0d and in acidic soils pH5.0 - 5.5 M03 has a DT50 of <5d (DAR B.8.1.8). Therefore rapid decay would be expected in these soils and any resulting toxicity mostly expressed via M01 and M02 derivatives of M03. Furthermore, it is likely that soil microorganisms could be exposed transiently to M03 in fluopicolide and product soil microorganism studies which were all conducted at soil pH5.4 - 5.9 over 28d where no effects were reported at up to 10x proposed application rate (DAR B.9.8.1). M03 has a very similar chemical structure to fluopicolide (Appendix 2) and significantly increased toxicity would not be anticipated. Moreover, no effects of M03 on earthworm at 1000 mg/kg DS (pH 5.7-6.0) over 14d were reported and TERs for acute (14d) and long term fluopicolide effects > Annex VI (soil pH 6 -7) over 56d, where some transient M03 formation may be expected. Fluopicolide also did not affect straw litter bag decomposition in soil (pH 6.72) over 184d again where some transient exposure to M03 might be assumed. Where tested M01 and M02, both M03 soil degradation products, also had no significant impact on soil organisms and processes. Therefore overall the RMS considered that there was sufficient weight of evidence to suggest that M03 would not have a significant effect on soil organisms and processes in the absence of a specific soil microbial M03 study.

B.9.9 Non-target flora

One issue **(Evaluation Table Open point 5.11)** was raised pertinent to the risk to off-field non-target plants from proposed uses of fluopicolide in potato and vine crops:

Evaluation Table Open pt. 5.11

'RMS to include the argumentation regarding risk to non-target plants from exposure to M 01 in an addendum for the sake of completeness. No discussion in an expert meeting is required.'

5.11 RMS response:

For non-target plants off-field risk is only considered and contamination will result primarily from spray drift. M01 is a soil metabolite and not present in spray applications. Hence, pre-emergent effects on non-target plants following M01 formation in off-field soil contaminated with fluopicolide by spray drift are most relevant.

The pre-emergent M01 non-target plant study revealed no effects >50% on seedling germination and growth at rates ≤ 0.0121 mg/kg soil and an ER50 of >0.0121 mg M01/kg DS (5 cm) was established. From theoretical in-field PECsoils (Table B.8.198) for fluopicolide and M01 and spray drift values (6.9% for vine; 1.9% for potato), max. M01 off-field PECsoils of 0.00196 and 0.00039 mg/kg (5cm) can be derived for vine and potato use, respectively. Thus TERs of >6 and >31 can be established for M01 off-field pre-emergent effects on non-target plants indicating low risk. This is considered to be a worse case scenario as the ER50 is > highest dose tested and no off-field interception of spray drift deposition is assumed.

B.9 ECOTOXICOLOGY - CORRIGENDUM

Please note the following correction to the original DAR:

i) Tables B.9.2.60 and B.9.2.63 need amendment (2nd a.s. is propamocarbHCl not fosetyl-Al as reported)

ii) Tables B.9.5.1-4; B.9.9.3-4. 'SC 40' should be 'SC480'

iii) B.9.2.2.1 S phrases (and Vol 1) should be amended to 'S60 This material and its container must be disposed of as hazardous waste' and 'S61 Avoid release to the environment. Refer to special instructions/safety data sheets'
Justification 'Recommended for substances that may cause effects in the environment'.

iv) Tables B.9.5.10, 9.5.12 'kg/ha' should be 'L/ha'

v) B.9.5.1.2b iii) 2nd para '2.04-10.35 kg/ha' should be '0.4-4.7 L/ha)

vi) B.9.7.3.2/9.8.3.2 '10cm' should be '5 and 10cm'

vii) B.9.7.3.1/9.8.3.1 '10cm' should be '5cm'

viii) Table B.9.9.15 M01 '0.046' should be '0.043'.

ix) Table B.9.2.77 Heading 'AE F05361606 WG71 A1' should be 'EXP 11074B'

APPENDIX 1

Summary of the significant metabolites of fluopicolide identified in studies in animals, plants and the environment

M-Code number (Company code number)	Other identifiers	Structure	Formula	Presence in metabolism studies
AE C638206	Fluopicolide (parent)	F3C, Cl, Cl, NH, N, O, Cl	2,6-dichloro-N-{[3-chloro-5-(trifluoromethyl)-2-pyridinyl]methyl}benzamide $C_{14}H_8Cl_3F_3N_2O$ MW = 383.59	
M-01 (AEC653711)	BAM	Cl, H_2N, O, Cl	2,6-dichlorobenzamide $C_7H_5Cl_2NO$ MW = 190.0	rat liver, laying hen, crop, soil, lysimeter leachate, rotational crop
M-02 (AEC657188)	PCA UMET/2	F3C, Cl, N, OH, O	3-chloro-5-trifluoromethyl-pyridine-2-carboxylic acid $C_7H_3ClF_3NO_2$ MW = 225.6	rat, crop, rotational crop, soil, water
M-05 (AE 1344122)	P1x RPA433497	F3C, $SOCH_3$, N, COOH	3-methylsulfinyl-5-trifluoro-methylpyridine-2-carboxylic acid $C_8H_6F_3NO_3S$ MW = 253	rotational crop, lysimeter leachate,
M-04 (AEC657378)	3-hydroxy BAM	Cl, H_2N, O, Cl, OH	2,6-dichloro-3-hydroxy-benzamide $C_7H_5Cl_2NO_2$ MW = 206	rotational crop rat (BAM ADME study)

List of metabolites continued

Company code number	Other identifiers	Structure	Formula	Presence in metabolism studies
M-06 (AEC643890)	3-OH 206 MET IV MET.F/16 FMET/38 UMET/51 FMET/8 UMET/44 UMET/53 FMET/33	F3C, Cl, Cl, NH, N, OH, O, Cl	2,6-dichloro-N-[(3-chloro-5-trifluoromethylpyridin-2-yl) methyl]-3-hydroxybenzamide $C_{14}H_8Cl_3F_3N_2O_2$ MW = 399	laying hen, lactating cow crop, confined rotational crop, rat
M-07 (AE 0712556)	4-OH 206 UMET/54 UMET/26	F3C, Cl, Cl, OH, NH, N, O, Cl	2,6-dichloro-N-[(3-chloro-5-trifluoromethylpyridin-2-yl) methyl]-4-hydroxybenzamide $C_{14}H_8Cl_3F_3N_2O_2$ MW = 399	laying hen, lactating cow rat
M-08 (AEC653598)		F3C, Cl, NH2, N, O	3-chloro-5-trifluoromethyl pyridine-2-carboxamide $C_7H_4ClF_3N_2O$ MW = 224.57	confined rotational crop
M-09 (AE B102859)		F3C, Cl, N, OH	3-chloro-2-hydroxy-5-trifluoromethylpyridine $C_6H_3ClF_3NO$ MW = 197.54	confined rotational crop
M-03 (AE060800)	RPA427967	F3C, Cl, Cl, H, N, N, OH, O, Cl	4-N-[3-chloro-5-trifluoro-methylpyridin-2-yl) (hydroxyl)methyl]-2,6-dichlorobenzamide $C_{14}H_8Cl_3F_3N_2O_2$ MW = 399.58	soil
M-10 (AE 1344123)	P4 RPA433965	F3C, SO3H, N, COOH	3-sulfo-5-trifluoromethyl pyridine-2-carboxylic acid $C_7H_4F_3NO_5S$ MW = 271.17	lysimeter leachate, soil (PCA soil degradation study)

List of metabolites continued

Company code number	Other identifiers	Structure	Formula	Presence in metabolism studies
M-11 M-12	P2 Mixture of 2 isomers (P2a and P2b)	F_3C, SO_3H, HO, N, COOH	isomers x-hydroxy -y-sulfo-5-trifluoromethylpyridine-2-carboxylic acid $C_7H_4F_3NO_6S$ MW = 287.17	lysimeter leachate, soil (PCA soil degradation study)
M-13	P3	F_3C, Cl, HO, N, COOH proposal not confirmed	$C_7H_3ClF_3NO_3$ MW = 241.3	lysimeter leachate,
M-14 (AE 1388273)	P7 RPA433986	F_3C, SO_2CH_3, N, OH	3-mesyl-5-(trifluoromethyl) pyridin-2-ol $C_7H_6F_3NO_3S$ MW = 241.19	lysimeter leachate, soil (PCA soil degradation study)
M-15 (AE 1413903)	P8	F_3C, Cl, Cl, SO_3H, H, N, N, O, Cl	3,5-dichloro-4-[3-chloro-5-trifluoromethylpyridine-2-yl-methyl) carbamoyl]benzene sulfonic acid $C_{14}H_8Cl_3F_3N_2O_4S$ MW = 463.65	lysimeter leachate,
M-16	P9 UMET/40 FMET/23	F_3C, Cl, Cl, H, N, N, OH, O, SO_3H	3-chloro-2-[({3-chloro-5-trifluoromethylpyridine-2-ylmethyl}amino) carbonyl]-6-hydroxybenzene sulfonic acid $C_{14}H_9Cl_2F_3N_2O_5S$ MW = 444	lysimeter leachate, rat
M-17	Metabolite 1	F_3, Cl, Cl, H, N, N, S, O, O, CH_3, O, Cl	2,6-dichloro-N-{[3-chloro-5-(trifluoromethyl)pyridin-2-yl]methyl}-3-(methylsulfonyl)benzamide $C_{15}H_{10}Cl_3F_3N_2O_3S$ MW = 462	laying hen

List of metabolites continued

Company code number	Other identifiers	Structure	Formula	Presence in metabolism studies
M-18	HS (hydroxy sulphate of fluopicolide) UMET/45 UMET/47	F3C, Cl, Cl, H, N, N, OSO3H, O, Cl or F3C, Cl, Cl, OSO3H, H, N, N, O, Cl	2,4-dichloro-3-[({[3-chloro-5-(trifluoromethyl)pyridin-2-yl]methyl}amino)carbonyl] phenyl hydrogen sulfate or 3,5-dichloro-4-[({[3-chloro-5-(trifluoromethyl)pyridin-2-yl]methyl}amino)carbonyl] phenyl hydrogen sulfate $C_{14}H_7Cl_3F_3N_2O_5S$ MW = 477	laying hen lactating cow rat
M-19	DHS (dihydroxy sulphate of fluopicolide) UMET/23 UMET/39 UMET/46 UMET/49	F3C, Cl, Cl, OH, H, N, N, OSO3H, O, Cl	3,5-dichloro-4-[({[3-chloro-5-(trifluoromethyl)pyridin-2-yl]methyl}amino)carbonyl] hydroxyphenyl hydrogen sulfate $C_{14}H_7Cl_3F_3N_2O_6S$ MW = 493	laying hen lactating cow rat

APPENDIX 2

Fluopicolide soil degradation pathway proposed by Applicant

fluopicolide MOO

MO3

MO2

MO1

M13

M1O

MO5

M11/M12

M14

APPENDIX 3

[NB. Section B.10 is the UK Efficacy assessment which is not included in the EU DAR. The following section relates to the biologically activity assessment and is presented for completeness in response to the Reporting Table point 2(25). It has not been updated, therefore for a full assessment of the relevance of groundwater metabolites - please see Section B.6.1.4.1, Addendum 1 (November 2007)].

B.10.7.5 Effects of Metabolites in Ground Water

The applicant identified the potential metabolites in groundwater as M-01, M-02, M05, M10, M14 and M-15. Evidence had been provided from the initial screening data to indicate that fluopicolide has no significant insecticidal or herbicidal activity (B10.7.1). The applicant therefore tested these metabolites for fungicidal activity only. Reference was made to fate and behaviour metabolite studies submitted under Annex II.

(Latorse & Flahaut 2004)

In vitro tests for powdery mildew and late blight showed no activity for any of these metabolites tested at 100 mg/l (equivalent to 100 g/ha). This included a range of doses for the major metabolite, AE C653711. These results were summarised further in a position paper on the non-relevance of metabolites found in lysimeter leachate and field leaching studies. The paper argued that the data from the biological screens indicated that both the pyridine and phenyl rings of the molecule are required for fungicidal activity. Any metabolites without both these rings would be predicted to have no fungicidal activity. It was also noted that functional groups, especially polar ones to the phenyl ring causes loss of fungicidal activity.

(Leake & Payraudeau 2004a)

The position paper also summarised further studies with M-01 against five species of fungi. These showed M-01 gave no inhibition of growth at rates between 0.3 and 30 mg/kg dry soil.

(Lechelt –Kunze 2003k, 2003f, 2003m)

Further supporting evidence of the lack of activity of AE C653711 was referenced from a Tier II seedling emergence and vigour study showing no effects on ten different non-target plants.

(Pallett & Gosch, 2004)

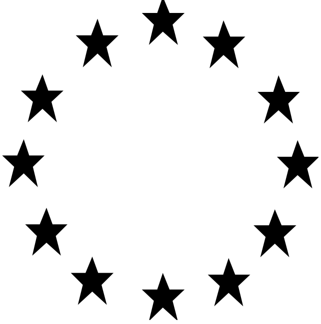
Further evidence of lack of insecticidal activity was provided in the Ecotoxicology studies with no effect against Collembola.

(Klein & Luhrs 2003a)

Assessment

Various biological screening data confirmed that neither fluopicolide nor its metabolites have herbicidal or insecticidal activity. Metabolites were therefore investigated for any fungicidal effects, and the studies indicate that none of those tested had any significant biological activity. The rapporteur differed in their assessment of which metabolites have the potential to occur in groundwater above the 0.1 µg/L level, specifying M-01, M-05 and M-10 to M-14 (see B8.6.2). Of these only M11 and M12 (mixture of 2 isomers) and M13 were not tested for fungicidal activity. These three are all single pyridine ring structures and are unlikely to have any significant fungicidal activity.

Council Directive 91/414/EEC



Fluopicolide (AE C638206)

Volume 4
ADDENDUM 1

Annex C

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

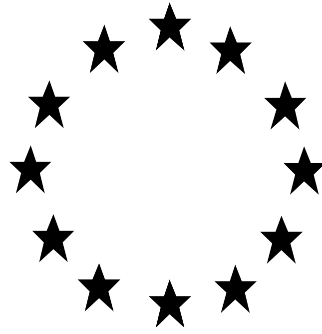
Confidential Information

November 2007

CONFIDENTIAL BUSINESS INFORMATION:

available at RMS

Council Directive 91/414/EEC



Fluopicolide

ADDENDUM 2
TO THE DRAFT ASSESSMENT REPORT PREPARED BY THE UNITED KINGDOM

Draft: November 2007

PESTICIDES SAFETY DIRECTORATE
Mallard House, Kings Pool,
3 Peasholme Green,
York YO1 7PX, UK
Website: www.pesticides.gov.uk

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B.8 ENVIRONMENTAL FATE AND BEHAVIOUR

B.8.1 Route and rate of degradation in soil

Open point 4.8

"MS experts to discuss in an experts meeting the kinetic evaluation of field dissipation studies.

See reporting table 4(36)."

Background

This open point was the result of the following comment from EFSA in the Reporting Table in relation to the kinetics of the field dissipation studies, "*for some of the sites "measured initial concentration" is relatively far of the "nominal application rate" and the "calibrated application rate". Reasons for these differences are not clear. Also the selection of the fixed "initial concentration" may need to be examined case by case in order to confirm the reliability of the results obtained in this fitting exercise.*" The RMS agreed that discussion of the kinetic approaches at an expert meting would be useful.

Open point 4.1

"Half lives for metabolites derived in the studies where they are dosed as starting material are seen by the RMS as more reliable, specially with respect to M14 (see DAR p 661). Therefore, only these DT50 should be reported in the list of end points. RMS to amend the list of end points accordingly.

MS experts to discuss if the half lives derived from the study dosed with M02 may however still be used for modelling.

See reporting table 4(6)"

Background

This open point arose from discussion following the Applicants request to include degradation endpoints for metabolites which were derived from a study where M-02 had been the starting material, rather than simply metabolite endpoints where specific metabolites had been used as starting material.

In response, the Applicant has submitted two position papers. The first is in relation to evaluation of the field dissipation studies to derive end points for comparison against regulatory 'trigger' values. The second is in relation to derivation of soil degradation parameters from field studies for use in modelling and relevance of the M-02 study for derivation of degradation endpoints for other metabolites.

i) Kley & Mackenzie 2007a, Evaluation of soil Degradation Parameters for Fluopicolide (AE C638206) for use as Trigger Values (Report No MEF-07/265)

This position paper is an evaluation of field dissipation data originally presented in the DAR under Volume 3, Section B.8.1.5. It appears to build on arguments submitted by the Applicant which were presented by the RMS in the Summary and Assessment section (B.8.1.8) in relation initial concentration fixing issues. It has been noted that some, but not all DT50 and DT90 values presented in this position paper are the same as those in Table B.8.143a of the DAR for Hockey Stick kinetics.

In summary, the Applicant has evaluated the field dissipation data using Hockey Stick (HS) kinetics and excluding the step of fixing the initial concentration to assess the impact on statistical fit. The step of fixing initial concentration was the subject of discussion between RMS and Applicant in the original evaluation, and subsequently attracted comments from EFSA and MSs. In addition to removal of the step whereby initial concentration was fixed, the principles of curve fitting evaluation (as recommended by the FOCUS Degradation Kinetics guidance document) have been applied, in the form of visual fit, plotting of residual fit and chi^2 evaluation. The t-test has not been assessed, however, from the magnitude of fitted parameters presented, it is assumed by the RMS than these will be significantly different from 0.

It should be noted that the FOCUS Degradation Kinetics guidance document states that the HS model is not recommend in the core set of models to be used for the evaluation of kinetic parameters for assessment against regulatory trigger values (see section 7.1, pages 108 – 112 of the FOCUS Kinetics guidance document). However, the document demonstrates what may be achieved without initial concentration fixing.

Evaluation of the field dissipation data started with addition of residues (in mg/kg) at each individual sample time from all soil horizons where quantifiable residues were found. The summed residue was then converted to a g/ha basis assuming a soil bulk density of 1.5 g/cm^3. The summed residues for each field site were then subjected to kinetic modelling with the HS model allowing free optimisation of initial concentration (C_0).

Following initial HS modelling, the statistical and graphical outputs were compared with those from a subsequent kinetic evaluation using SFO kinetics on the same dataset. As with the HS evaluation, free optimisation of C_0 was allowed with SFO. As a follow-up step, SFO fitting was also attempted with C_0 fixed to the optimised initial concentration from the HS modelling. It should be noted that the re-evaluation does not follow FOCUS Kinetics advice, which is to start with an initial assessment using both SFO and FOMC, and then to follow with DFOP if necessary.

Summaries of the optimised parameters from HS with free-fitting (Table B.8.1), SFO with free optimisation (“free fitting”) (Table B.8.2) and SFO with C_0 fixed (Table B.8.3) are presented below. Example graphical outputs associated the kinetic assessments from the Philippsburg site are also presented in Figures B.8.1 - 3. Graphical outputs for the other sites are presented in Appendix 1.

Table B.8.1 Optimised HS kinetic parameters for fluopicolide from field dissipation studies, free fitting of C_0

Hockey Stick fit								
	C_0 free fitted	**DT_{50}**	DT_{90}	k_1	k_2	tb	r^2	Error of χ^2 test
	g/ha	d	d	d^{-1}	d^{-1}	d		%
Philippsburg	408	99.1	1184.4	0.0206	0.00148	28.6	0.879	15.9
Rödelsee	695	132.5	863.1	0.0309	0.0022	14.0	0.940	12.2
Appilly	368	103.5	1133.6	0.0067	0.0014	136.0	0.916	14.9
Hüntlosen	353	171.9	999.6	0.0440	0.0019	8.5	0.893	14.0
Valencia	456	50.2	972.8	0.0138	0.00163	60.4	0.953	11.8
Senas Year 1	319	115.1	619.1	0.0608	0.0032	5.6	0.977	8.7
Senas Year 2	656	58.4	679.3	0.0119	0.00242	69.8	0.928	12.7
Geometric mean [a]		99.3	949.1	-	-	-	-	-
Median [a]		101.3	986.2	-	-	-	-	-

[a] Mean values for Senas were used for further averaging

Table B.8.2 Optimised SFO kinetic parameters for fluopicolide from field dissipation studies, free fitting of C_0

Simple First Order fit					
	C_0 free fitted	**DT_{50}**	DT_{90}	r^2	Error of χ^2 test
	g/ha	d	d		%
Philippsburg	339	247.9	823.3	0.721	22.6
Rödelsee	574	239.6	796.0	0.837	18.6
Appilly	346	187.7	623.5	0.883	16.3
Hüntlosen	290	276.2	917.4	0.798	17.9
Valencia	396	172.2	572.0	0.816	21.7
Senas Year 1	272	173.6	576.8	0.926	14.7
Senas Year 2	609	121.4	403.4	0.862	16.0
Geometric mean [a]		206.3	685.1	-	-
Median [a]		213.7	709.8	-	-

[a] Mean values for Senas were used for further averaging

Table B.8.3 Optimised SFO kinetic parameters for fluopicolide from field dissipation studies, C_0 fixed to optimised value from HS

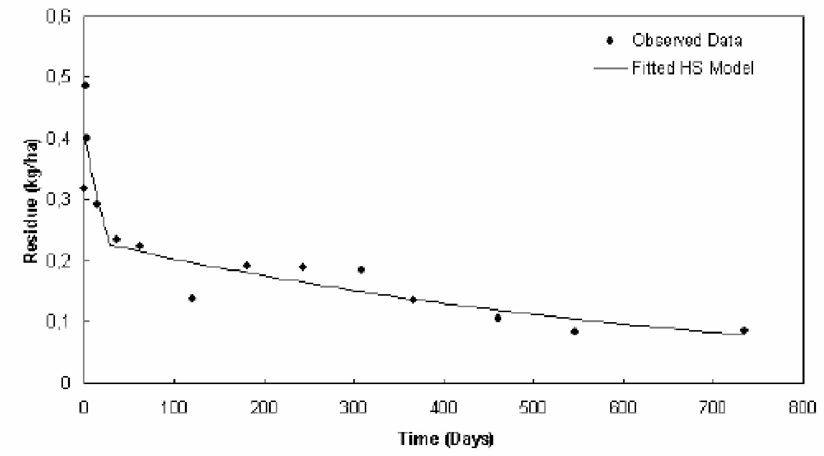
	Simple First Order fit				
	C_0 fixed to C_0 hockey stick	**DT_{50}**	DT_{90}	r^2	Error of χ^2 test
	g/ha	d	d		%
Philippsburg	408	179.2	595.3	0.603	26.1
Rödelsee	695	184.5	613.0	0.701	24.3
Appilly	368	170.5	566.4	0.872	16.6
Hüntlosen	353	200.3	665.5	0.628	23.4
Valencia	456	133.5	443.4	0.763	23.9
Senas Year 1	327 [a]	130.6	433.7	0.783	21.6
Senas Year 2	656	106.8	354.8	0.842	16.4
Geometric mean [b]		161.6	536.8	-	-
Median [b]		174.9	580.9	-	-

[a] C_0 fixed to first measurement

[b] Mean values for Senas were used for further averaging

Figure B.8.1 HS graphical output for fluopicolide at Philippsburg, free fitting

Figure 5.1: Philippsburg, Hockey Stick fit, C_0 free fitted



Hockey Stick fit							
	DT_{50}	C_0 free fitted	k_1	k_2	tb	r^2	Error of χ^2 test
	d	g/ha	d^{-1}	d^{-1}	d		%
Philippsburg	99.1	408	0.0206	0.00148	28.6	0.879	15.9

Figure 5.2: Residual plot for Philippsburg, Hockey Stick fit, C_0 free fitted

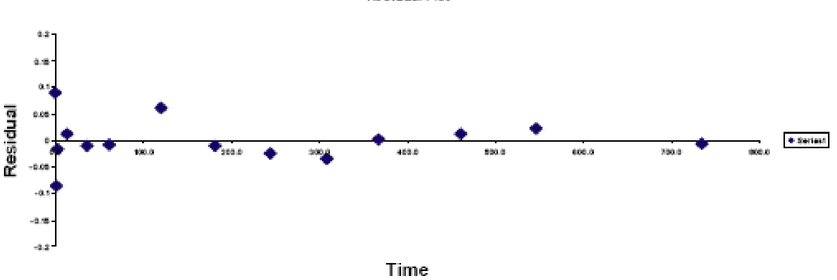
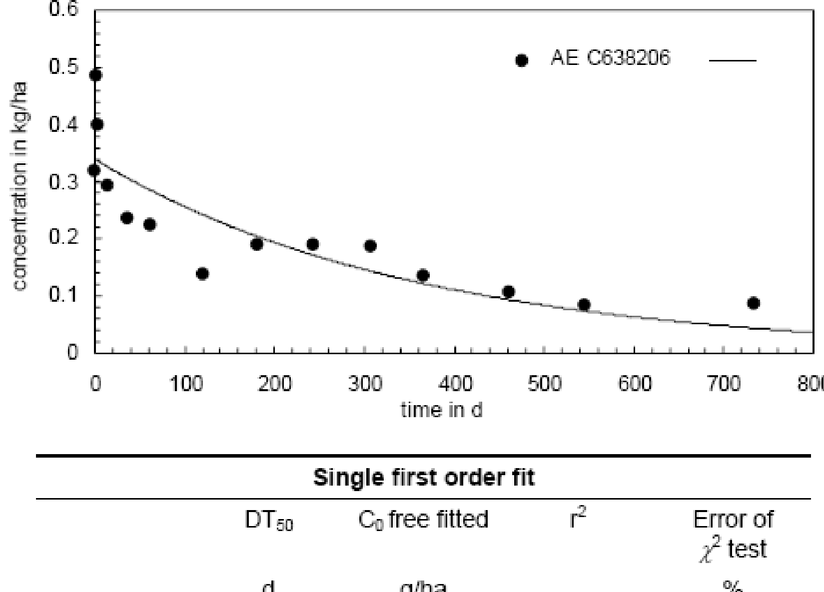


Figure B.8.2 SFO graphical output for fluopicolide at Philippsburg, free fitting

Figure 5.3: Philippsburg, Single First Order fit, C_0 free fitted



Single first order fit				
	DT_{50}	C_0 free fitted	r^2	Error of χ^2 test
	d	g/ha		%
Philippsburg	247.9	339	0.721	22.6

Figure 5.4: Residual plot for Philippsburg, SFO fit, C_0 free fitted

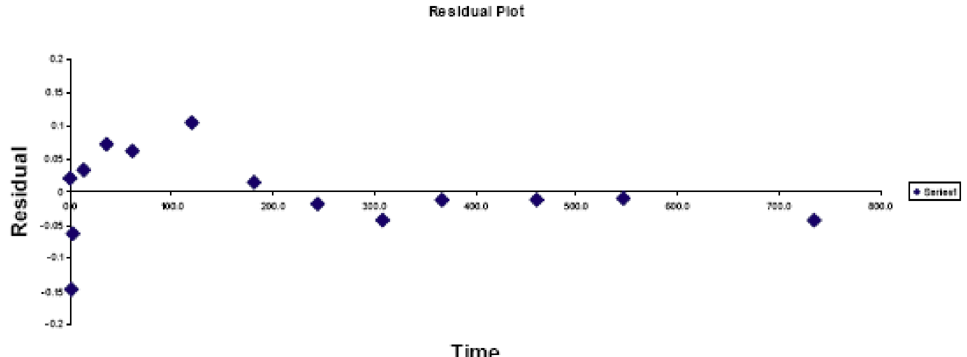
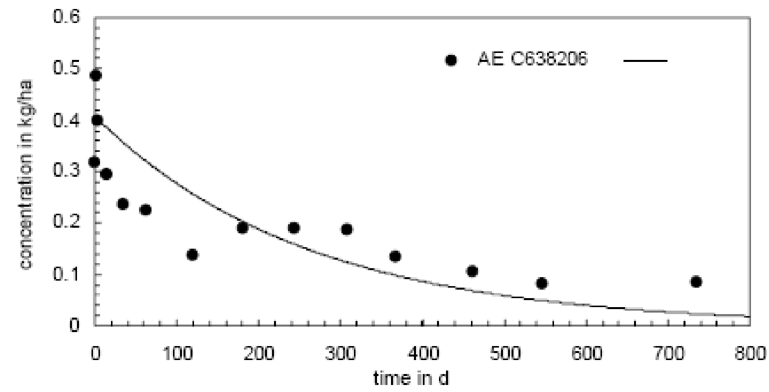


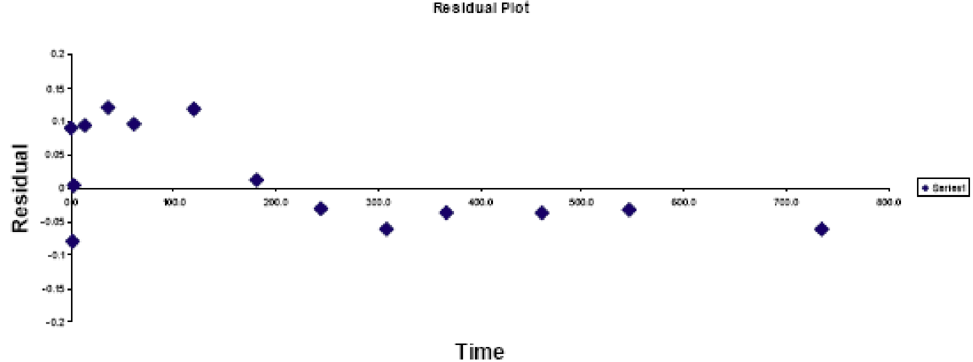
Figure B.8.3 SFO graphical output for fluopicolide at Philippsburg, C_0 fixed

Figure 5.5: Philippsburg, Single First Order fit, C_0 fixed



Single first order fit				
	DT_{50}	C_0 fixed to C_0 of hockey stick	r^2	Error of χ^2 test
	d	g/ha		%
Philippsburg	179.2	408	0.603	26.1

Figure 5.6: Residual plot for Philippsburg, SFO fit, C_0 fixed



The RMS consideration of the kinetic evaluation is that the HS model delivers a better representation of the residue decline than SFO. In particular, optimised initial concentration from free fits appear to be closer to measured concentrations with HS compared to SFO, chi^2 values from HS are superior, and there is a greater incidence of random distribution of data points in the residual plots for HS. Calculated HS DT50/DT90 in this position paper are the same as those presented in the DAR for four of the seven sites (for Philippsburg, Rodelsee, Valencia and Senas year 2, see Table B.8.144 of original DAR). It should be noted that the longest HS DT90 remains unchanged from that originally presented in the DAR (1184 days at Philippsburg), although the longest DT50 has increased from 132 days to 172 days. Note that the DT50 of 172 days is not associated with the longest DT90.

Unfortunately, whilst this position paper was written in 2007, its origins date back to HS assessments conducted before agreement of the FOCUS kinetics guidance document. Thus, whilst the graphical and statistical evaluation are more in line with the current guidance, the step-wise procedure is not as recommended by FOCUS. However, it demonstrates that bi-phasic kinetics seem to be more appropriate than SFO for assessment of the un-normalised field data. In addition, for the Huntlosen site, which was originally assessed in the DAR with HS with fixed C_0, the new assessment demonstrated that free fitting of C_0 can have a significant influence on the outcome of the kinetic assessment (new DT50/DT90 172/1000 days, DAR DT50/DT90 122/893 days).

(Kley & Mackenzie 2007a)

i) Kley & Mackenzie 2007b, Evaluation of Soil Degradation Parameters for Fluopicolide and its Metabolites from Laboratory and Field Trials for Modelling Purposes (Report No MEF-07/266)

The first area that this position paper addresses is the reliability of the normalisation procedure for the field dissipation studies presented in the original DAR in Volume 3, Section B.8.1.5.1. In general, the position paper revisits the original assessment, rather than applying the fully agreed FOCUS degradation kinetics approaches to fitting parent and metabolite data to support the outcome of the original assessment. Reasoning for fixing initial a.s. concentration was re-iterated, i.e. to achieve the best overall fit for both parent and metabolites. The Applicant gave additional statistical values for goodness of fit for the Huntlosen site which was a more complex situation due to the need to simulate the metabolite M-03 in addition to M-01 and M-02. It appears that these values are meant to serve as an illustration of the complexity of the decision making process with respect to the kinetics for the field studies. These statistics demonstrated the overall impact of fixing initial a.s. concentration at various values as compared to a free-fitting approach. Note that the calibrated dose in this study was calculated to be 399 g/ha, and the measured initial soil concentration was 375 g/ha. It appears that M-02 data are not given here because the there was only one detection >LOQ at this particular site. Plots of the a.s. and metabolite fits for this site ate included in Appendix 2 for information. Note that no plots of residuals have been presented.

Table B.8.4 Statistics from kinetic analysis for fluopicolide and metabolites at Huntlosen field dissipation site

M_0 fit	ModelMaker	Fluopicolide		M-03, AE 0608000		M-01, AE C653711	
	r^2	χ^2	r^2	χ^2	r^2	χ^2	r^2
Fixed 400g/ha	0.82	29.0	0.90	31.3	0.86	19.7	0.95
Free 321g/ha	0.92	16.5	0.97	57.9	0.51	19.5	0.95
Fixed 350g/ha	0.91	19.1	0.95	41.4	0.73	17.6	0.96
Fixed 375g/ha	0.89	22.8	0.94	39.1	0.80	15.5	0.97
Fixed 425g/ha	0.79	32.1	0.87	33.0	0.85	15.4	0.97
Fixed 450g/ha	0.72	37.1	0.83	35.2	0.78	17.3	0.96

At the end of this fitting procedure it can be seen that there is no one solution that provides an improved fit of all of the simulated substances and therefore, with the procedure used, a compromise was required.

In reading back through the original study report, this position paper and correspondence exchanged at the time of DAR presentation, it appears that the fitting procedure used was a simultaneous fit for parent and metabolites, whereas FOCUS degradation kinetics advocates a step-wise approach of fitting parent first, followed by adding metabolites in subsequent steps. It is possible that had the step-wise approach been taken that a better solution may have been found. However, only draft versions of the FOCUS kinetics guidance document were available to the Applicant and RMS at time of Dossier preparation and writing of the DAR.

The Applicant also attempts to address the lack of a flow from parent to sink compartment in compartment modelling (a concern raised in Open point 4.7 of the evaluation table). It is presumed that the origin of this comment is that the FOCUS degradation kinetics guidance indicates that in the first instance, the conceptual metabolic pathway modelled should include a direct flow from parent to sink. The Applicant states that given unextracted residues of 5-13% with phenyl labelled a.s. and less than 3% mineralisation with both labels, they considered that direct transfer of a.s. to sink to be a minor process. The Applicant recognises that there were higher levels of unextracted residues with the pyridyl-labelled material (up to 23% at 1 year). In addition, they considered that inclusion of a direct flow from a.s. to sink in the evaluation of field studies and in subsequent predictive modelling would have over-parameterised the conceptual model. RMS notes that FOCUS degradation kinetics guidance advocates a general principle that the preferred model should have the minimum number of parameters necessary to obtain a satisfactory analysis. This does not in itself justify the omission of the direct flow from parent to sink, but should be borne in mind.

With respect to open point 4.1 and the issue relating to reliability of metabolite half-lives derived from the M-02 study, the Applicant collated information to compare metabolite DT50 values from the M-02 study against DT50 values from studies where they had been applied as starting substances. The tables are shown below.

Table 8.5 Laboratory DT50 values for M-05 normalised to FOCUS temperature and moisture conditions of 20˚C and pF2

AE 1344122, M-05						
Study	**Soil**	**Original DT_{50}**	**Actual Temp**	**Moisture Content Study**	**Moisture Content pF2**	**Normalised DT_{50}**
		d	°C	g / 100g	g / 100g	d
[14C]-M-05 Study (Arthur et al., 2003a)	Abington	60.4	20	10.8	19	40.6
	Sarotti	33.6	20	14.4	26	22.2
	Münster	130.4	20	9.6	14	100.1
[14C]-M-02 Study (Hardy, 2004)	Abington	30.8	20	10.8	19	20.7
	Sarotti	53.3	20	14.4	26	35.2
	Münster	118.1	20	9.6	14	90.7
Geometric mean (n = 6)						**42.6**

Table 8.6 Laboratory DT50 values for M-10 normalised to FOCUS temperature and moisture conditions of 20˚C and pF2

AE 1344123, M-10						
Study	**Soil**	**Original DT_{50}**	**Actual Temp**	**Moisture Content Study**	**Moisture Content pF2**	**Normalised DT_{50}**
		d	°C	g / 100g	g / 100g	d
[14C]-M-10 Study (Arthur et al., 2003b)	Abington	35.8 [a]	20	10.8	19	24.1
	Sarotti	24.1 [a]	20	14.4	26	16.0
	Münster	252.5	20	9.6	14	193.9
[14C]-M-02 Study (Hardy, 2004)	Abington	4.5	20	10.8	19	3.0
	Sarotti	9.7	20	14.4	26	6.4
	Münster	307	20	9.6	14	235.7
Geometric mean (n = 6)						**26.4**

[a] The degradation of M-10 in Abington and Sarotti soil treated with [14C]-M-10 showed a lag-phase, with a more rapid degradation rate observed after two to three weeks.

The Applicant stated that M-10 applied as starting material showed a lag phase for the first 2-3 weeks in Abington and Sarotti soils. Checking back to Tables B.8.83 and B.8.84 of the DAR and plotting the concentrations in Excel, the RMS concurs that rate of degradation appeared to significantly increase (i.e. get faster) after the initial two or three data points.

Table 8.7 Laboratory DT50 values for M-14 normalised to FOCUS temperature and moisture conditions of 20˚C and pF2

AE 1388273, M-14						
Study	**Soil**	**Original DT_{50}**	**Actual Temp**	**Moisture Content Study**	**Moisture Content pF2**	**Normalised DT_{50}**
		d	˚C	g / 100g	g / 100g	d
M-14 Study (Nicolaus Brumhard, 2003)	Abington	4.9	20	10.8	19	3.3
	Sarotti	5.8	20	14.4	26	3.8
	Münster	8.2	20	9.6	14	6.3
[^{14}C]-M-02 Study (Hardy, 2004)	Abington	7.9	20	10.8	19	5.3
	Sarotti	13.7	20	14.4	26	9.1
	Münster	-	20	9.6	14	-
Geometric mean (n = 5)						**5.2**

Overall, the RMS concurs with the Applicant that there is generally a good agreement between the calculated DT50 values for the M-05, M-10 and M-14 metabolites from the M-02 study compared to the DT50 values where the metabolites were applied as starting materials. Thus, the RMS considers that the use of metabolite kinetic parameters combined from the M-02 study and the studies where individual metabolites were used as starting materials is reasonable.

(Kley & Mackenzie 2007b)

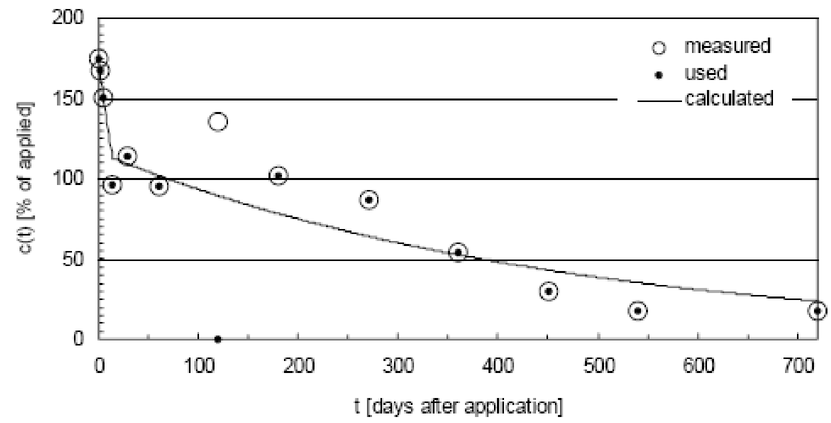
Appendix 1

Graphical output of kinetic assessment of fluopicolide residue decline in field dissipation studies, non-normalised data (from Kley & Mackenzie 2007a)

Note graphical output for Philippsburg presented in main body of Addendum.

Figure B.8.4 HS graphical output for fluopicolide at Rodelsee, free fitting

Figure 5.7: Rödelsee, Hockey Stick fit, C_0 free fitted



Hockey Stick fit							
	DT_{50}	C_0 free fitted	k_1	k_2	tb	r^2	Error of χ^2 test
	d	g/ha	d^{-1}	d^{-1}	d		%
Rödelsee	132.5	695	0.0309	0.0022	14.0	0.940	12.2

Figure 5.8: Residual plot for Rödelsee, Hockey Stick fit, C_0 free fitted

Residual Plot

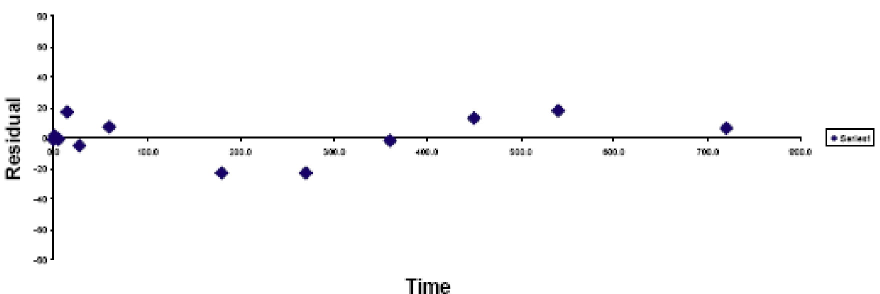
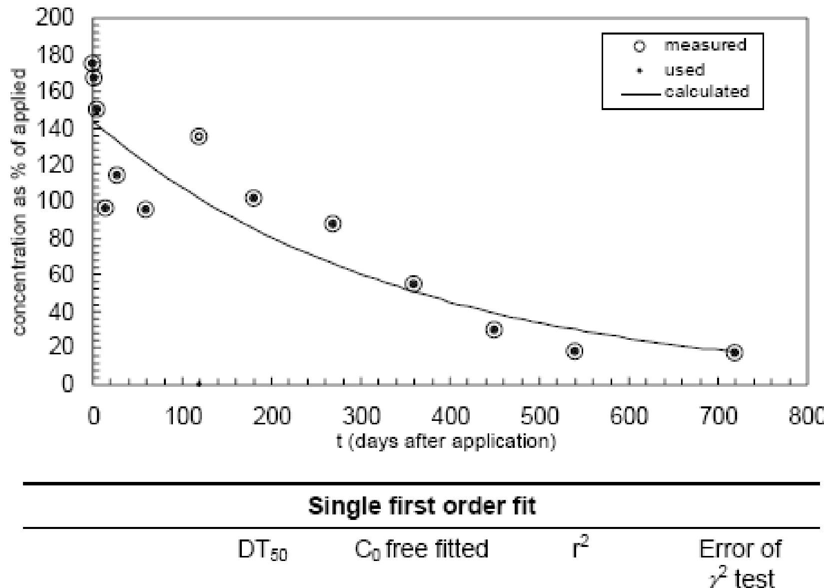


Figure B.8.5 SFO graphical output for fluopicolide at Rodelsee, free fitting

Figure 5.9: Rödelsee, Single First Order fit, C_0 free fitted



Single first order fit				
	DT_{50}	C_0 free fitted	r^2	Error of χ^2 test
	d	g/ha		%
Rödelsee	239.6	574	0.837	18.6

Figure 5.10: Residual plot for Rödelsee, SFO fit, C_0 free fitted

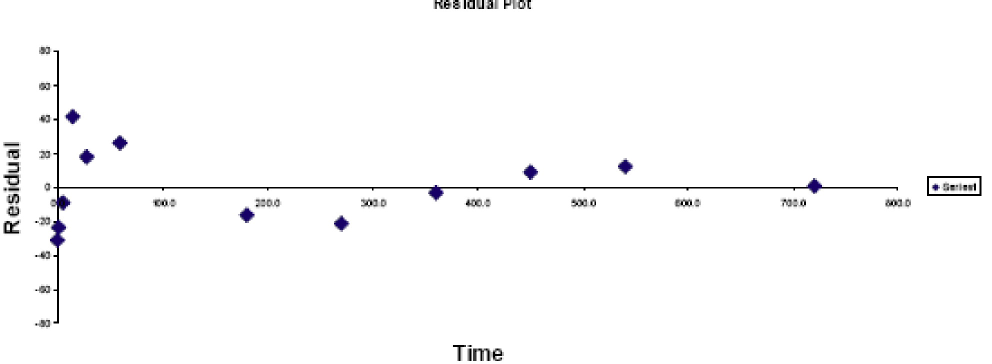
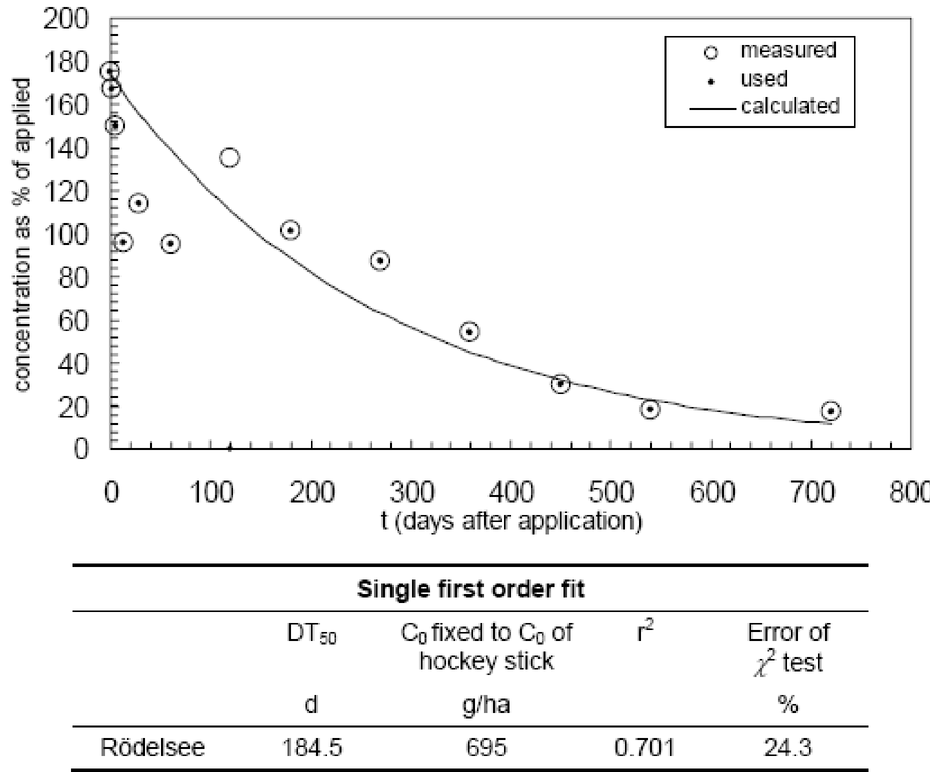


Figure B.8.6 SFO graphical output for fluopicolide at Rodelsee, C_0 fixed

Figure 5.11: Rödelsee, Single First Order fit, C_0 fixed



Single first order fit				
	DT_{50}	C_0 fixed to C_0 of hockey stick	r^2	Error of χ^2 test
	d	g/ha		%
Rödelsee	184.5	695	0.701	24.3

Figure 5.12: Residual plot for Rödelsee, SFO fit, C_0 fixed

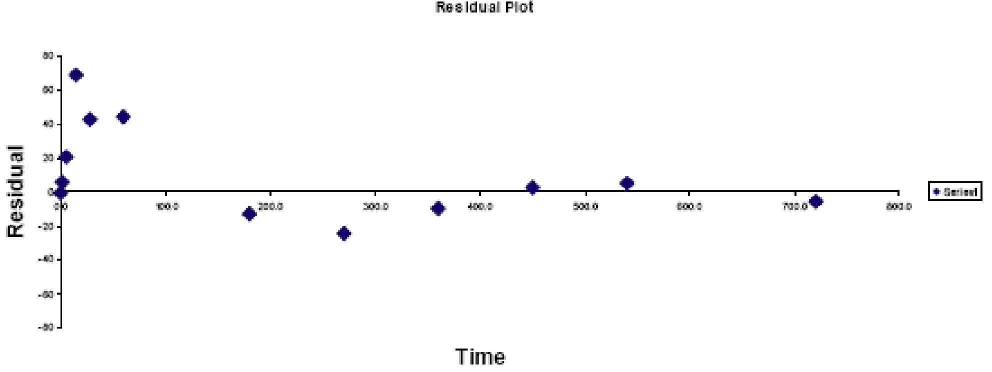
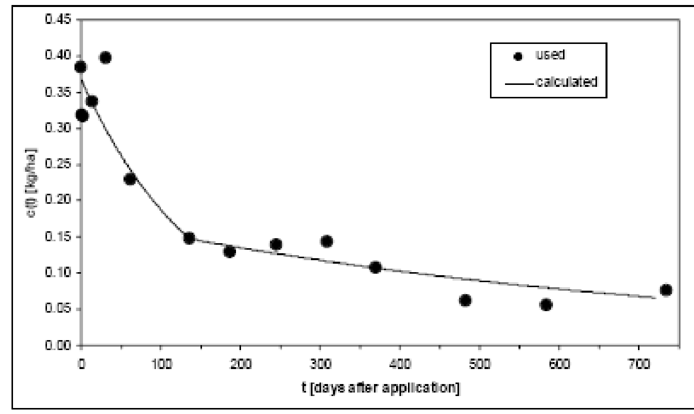


Figure B.8.7 HS graphical output for fluopicolide at Appilly, free fitting

Figure 5.13: Appilly, Hockey Stick fit, C_0 free fitted



	Hockey Stick fit						
	DT_{50}	C_0 free fitted	k_1	k_2	tb	r^2	Error of χ^2 test
	d	g/ha	d^{-1}	d^{-1}	d		%
Appilly	103.5	368	0.0067	0.0014	136.0	0.916	14.9

Figure 5.14: Residual plot for Appilly, Hockey Stick fit, C_0 free fitted

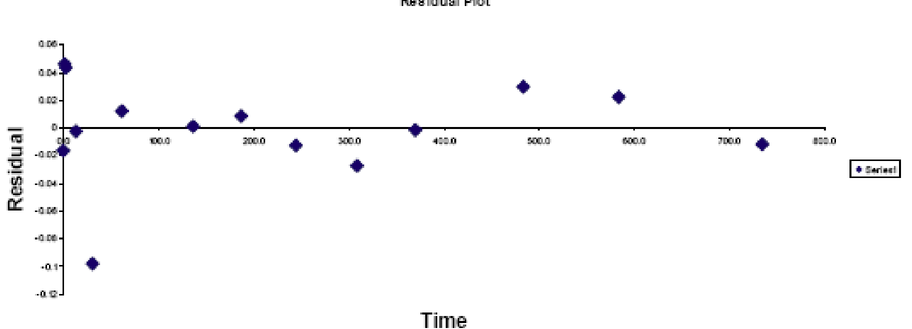
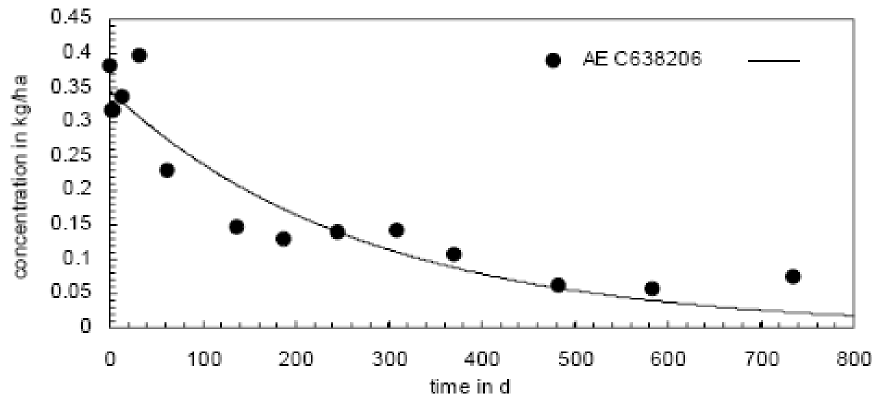


Figure B.8.8 SFO graphical output for fluopicolide at Appilly, free fitting

Figure 5.15: Appilly, Single First Order fit, C_0 free fitted



Single first order fit				
	DT_{50}	C_0 free fitted	r^2	Error of χ^2 test
	d	g/ha		%
Appilly	187.7	346	0.883	16.3

Figure 5.16: Residual plot for Appilly, SFO fit, C_0 free fitted

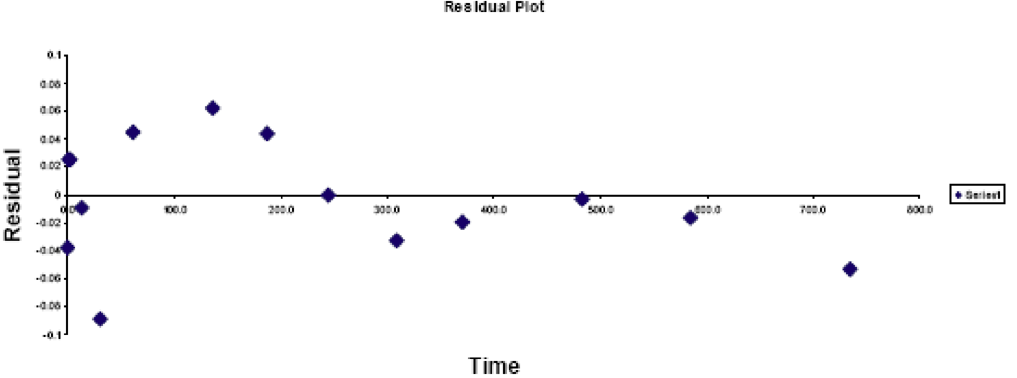
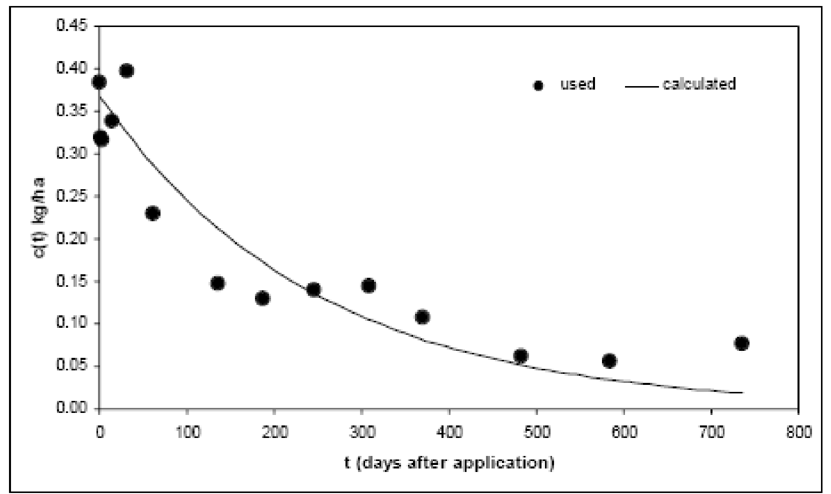


Figure B.8.9 SFO graphical output for fluopicolide at Appilly, C_0 fixed

Figure 5.17: Appilly, Single First Order fit, C_0 fixed



Single first order fit				
	DT_{50}	C_0 fixed to C_0 of hockey stick	r^2	Error of χ^2 test
	d	g/ha		%
Appilly	170.5	368	0.872	16.6

Figure 5.18: Residual plot for Appilly, SFO fit, C_0 fixed

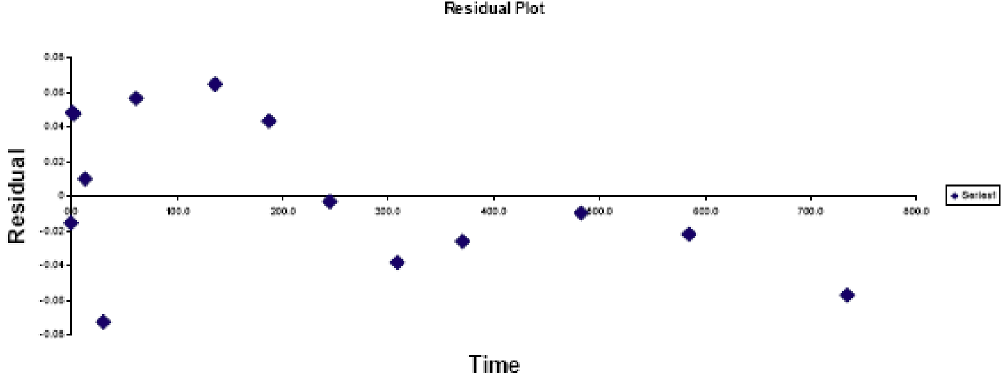
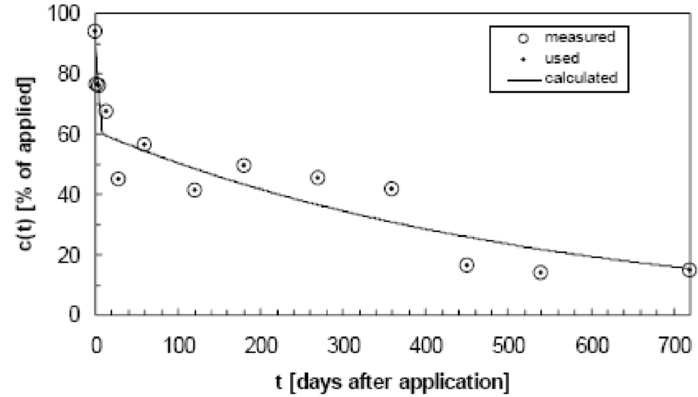


Figure B.8.10 HS graphical output for fluopicolide at Huntlosen, free fitting

Figure 5.19: Huntlosen, Hockey Stick fit, C_0 free fitted



	Hockey Stick fit						
	DT_{50}	C_0 free fitted	k_1	k_2	tb	r^2	Error of χ^2 test
	d	g/ha	d^{-1}	d^{-1}	d		%
Hüntlosen	171.9	353	0.0440	0.0019	8.5	0.893	14.0

Figure 5.20: Residual plot for Huntlosen, Hockey Stick fit, C_0 free fitted

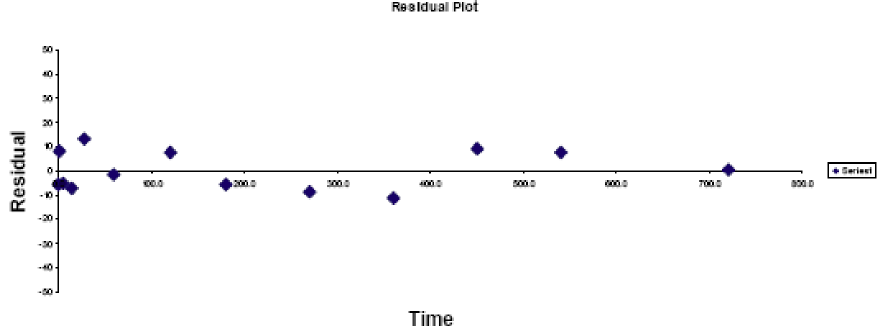
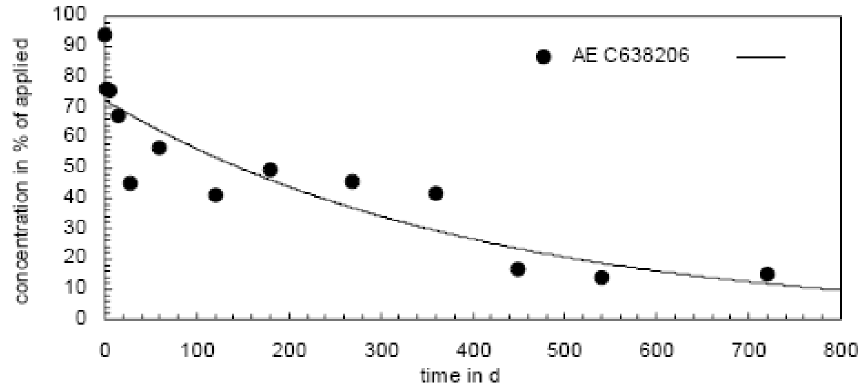


Figure B.8.11 SFO graphical output for fluopicolide at Huntlosen, free fitting

Figure 5.21: Huntlosen, Single First Order fit, C_0 free fitted



Single first order fit				
	DT_{50}	C_0 free fitted	r^2	Error of χ^2 test
	d	g/ha		%
Huntlosen	276.2	290	0.798	17.9

Figure 5.22: Residual plot for Huntlosen, SFO fit, C_0 free fitted

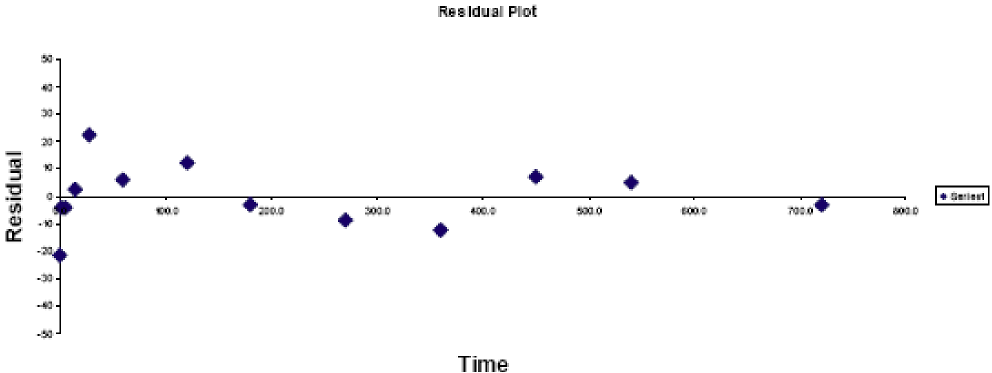
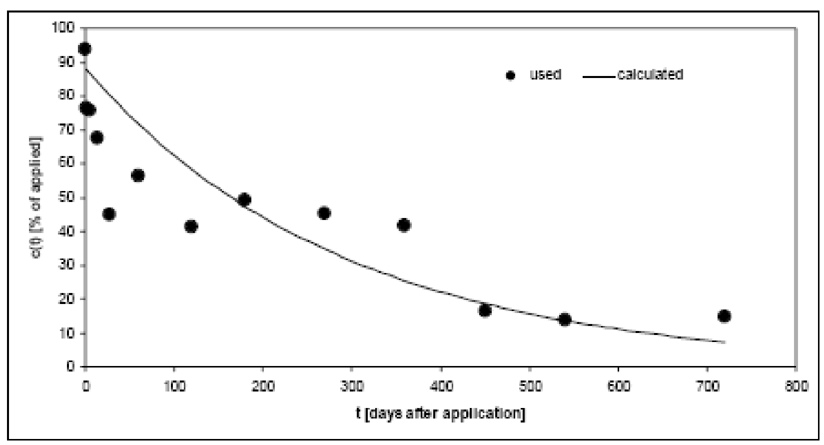


Figure B.8.12 SFO graphical output for fluopicolide at Huntlosen, C_0 fixed

Figure 5.23: Huntlosen, Single First Order fit, C_0 fixed



Single first order fit				
	DT_{50}	C_0 fixed to C_0 of hockey stick	r^2	Error of χ^2 test
	d	g/ha		%
Huntlosen	200.3	353	0.628	23.4

Figure 5.24: Residual plot for Huntlosen, SFO fit, C_0 fixed

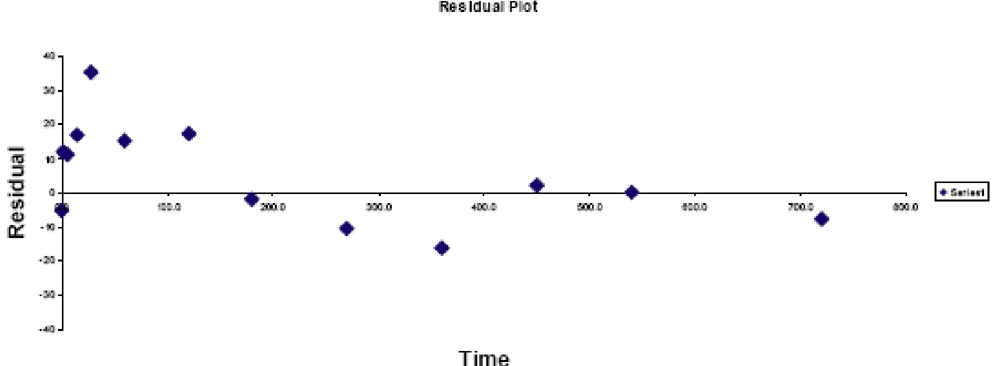
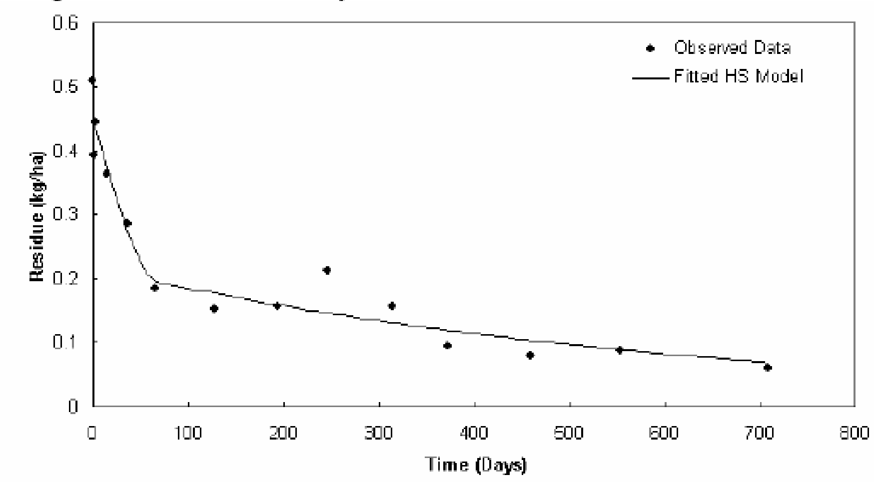


Figure B.8.13 HS graphical output for fluopicolide at Valencia, free fitting

Figure 5.25: Valencia, Hockey Stick fit, C_0 free fitted



Hockey Stick fit							
	DT_{50}	C_0 free fitted	k_1	k_2	tb	r^2	Error of χ^2 test
	d	g/ha	d^{-1}	d^{-1}	d		%
Valencia	50.2	456	0.0138	0.00163	60.4	0.953	11.8

Figure 5.26: Residual plot for Valencia, Hockey Stick fit, C_0 free fitted

Residual Plot

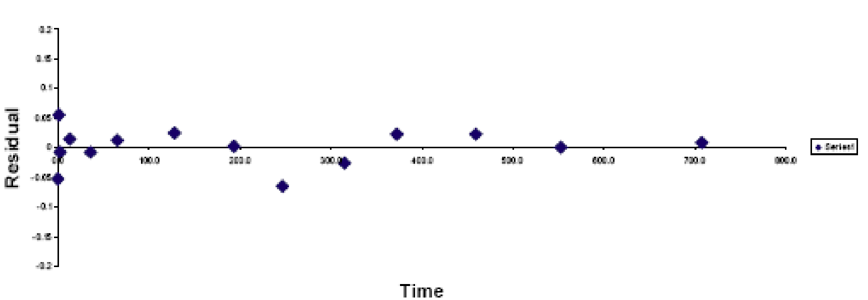
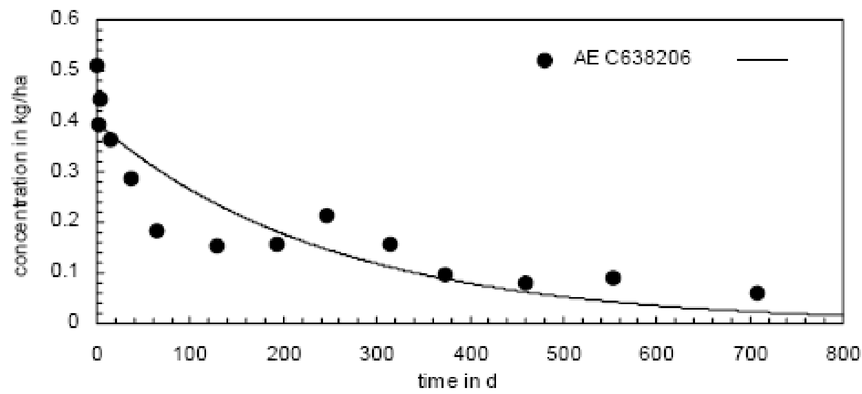


Figure B.8.14 SFO graphical output for fluopicolide at Valencia, free fitting

Figure 5.27: Valencia, Single First Order fit, C_0 free fitted



Single first order fit				
	DT_{50}	C_0 free fitted	r^2	Error of χ^2 test
	d	g/ha		%
Valencia	172.2	396	0.816	21.7

Figure 5.28: Residual plot for Valencia, SFO fit, C_0 free fitted

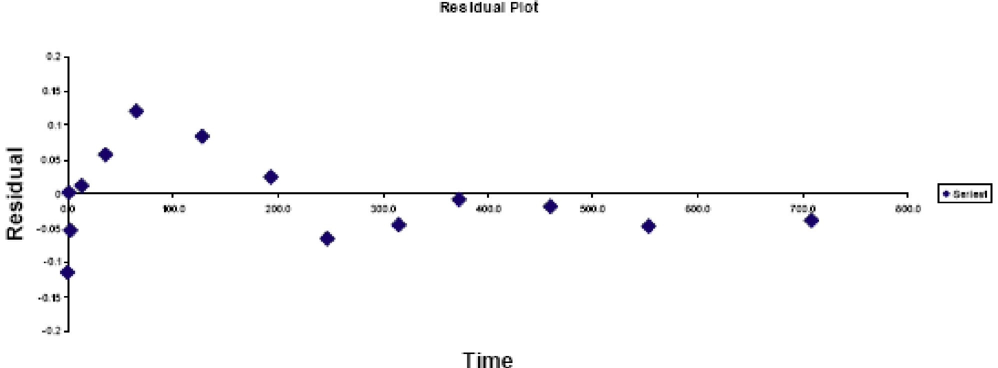
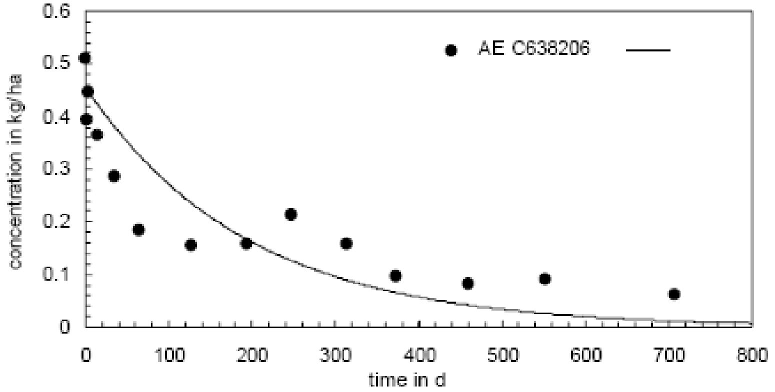


Figure B.8.15 SFO graphical output for fluopicolide at Valencia, C_0 fixed

Figure 5.29: Valencia, Single First Order fit, C_0 fixed



Single first order fit				
	DT_{50}	C_0 fixed to C_0 of hockey stick	r^2	Error of χ^2 test
	d	g/ha		%
Valencia	133.5	456	0.763	23.9

Figure 5.30: Residual plot for Valencia, SFO fit, C_0 fixed

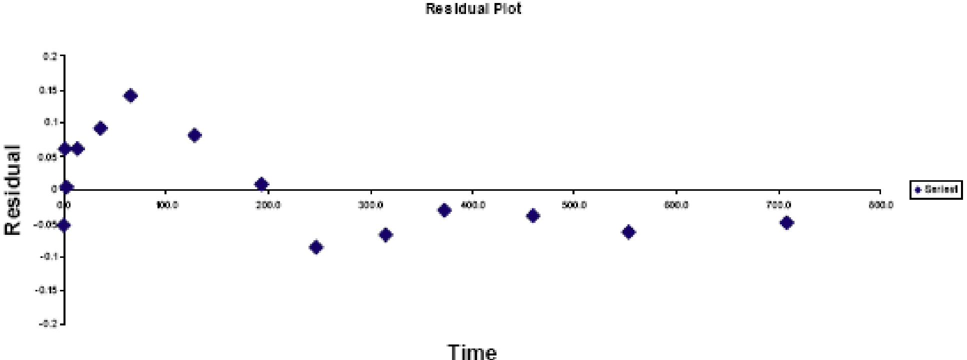
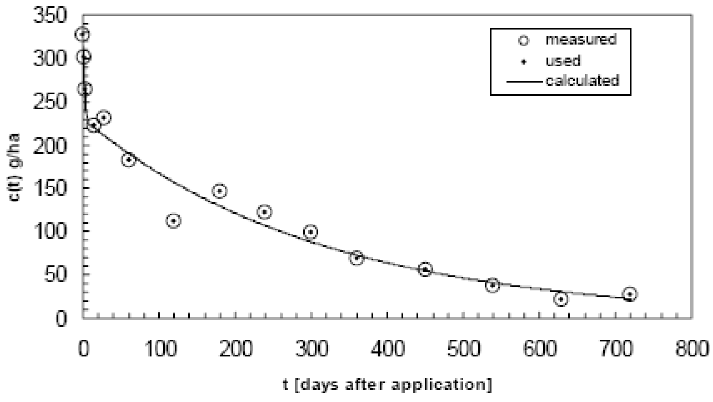


Figure B.8.16 HS graphical output for fluopicolide at Senas Yr 1, free fitting

Figure 5.31: Senas Year 1, Hockey Stick fit, C_0 free fitted



Hockey Stick fit							
	DT_{50}	C_0 free fitted	k_1	k_2	tb	r^2	Error of χ^2 test
	d	g/ha	d^{-1}	d^{-1}	d		%
Senas Year 1	115.1	319	0.0608	0.0032	5.6	0.977	8.7

Figure 5.32: Residual plot for Senas Year 1, Hockey Stick fit, C_0 free fitted

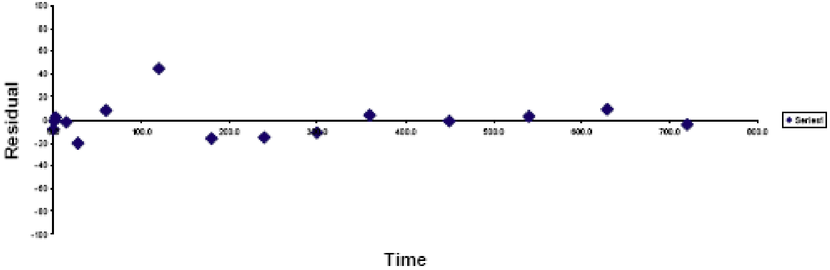
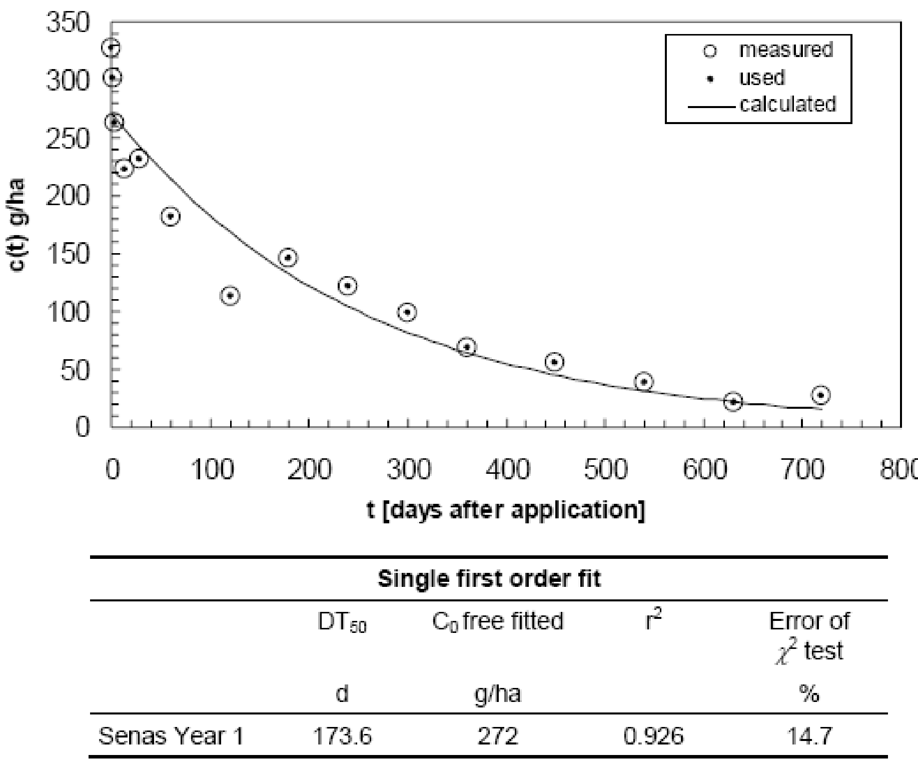


Figure B.8.17 SFO graphical output for fluopicolide at Senas Yr 1, free fitting

Figure 5.33: Senas Year 1, Single First Order fit, C_0 free fitted



Single first order fit				
	DT_{50}	C_0 free fitted	r^2	Error of χ^2 test
	d	g/ha		%
Senas Year 1	173.6	272	0.926	14.7

Figure 5.34: Residual plot for Senas Year 1, SFO fit, C_0 free fitted

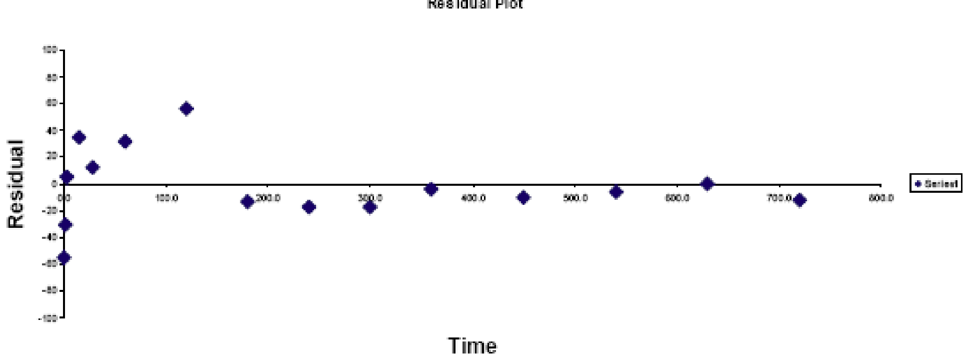
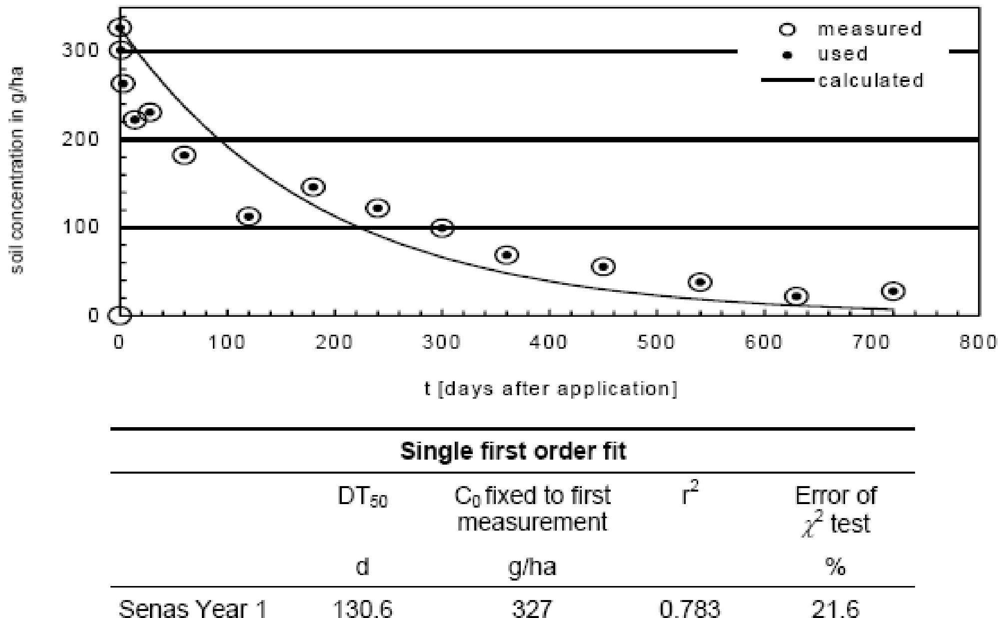


Figure B.8.18 SFO graphical output for fluopicolide at Senas Yr 1, C_0 fixed

Figure 5.35: Senas Year 1, Single First Order fit, C_0 fixed



Single first order fit				
	DT_{50}	C_0 fixed to first measurement	r^2	Error of χ^2 test
	d	g/ha		%
Senas Year 1	130.6	327	0.783	21.6

Figure 5.36: Residual plot for Senas Year 1, SFO fit, C_0 fixed

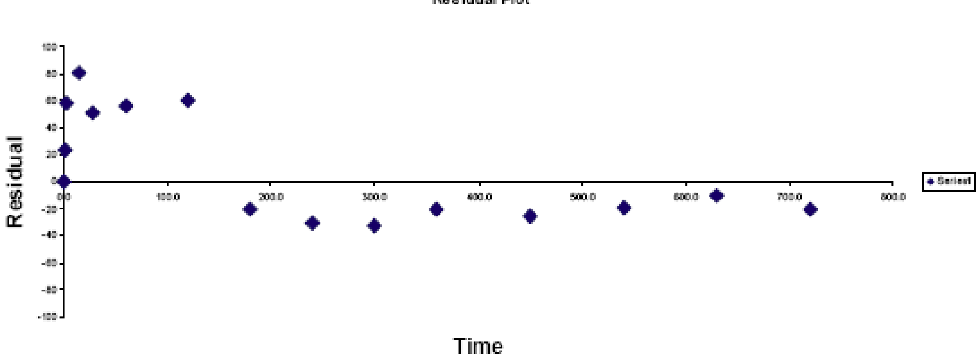
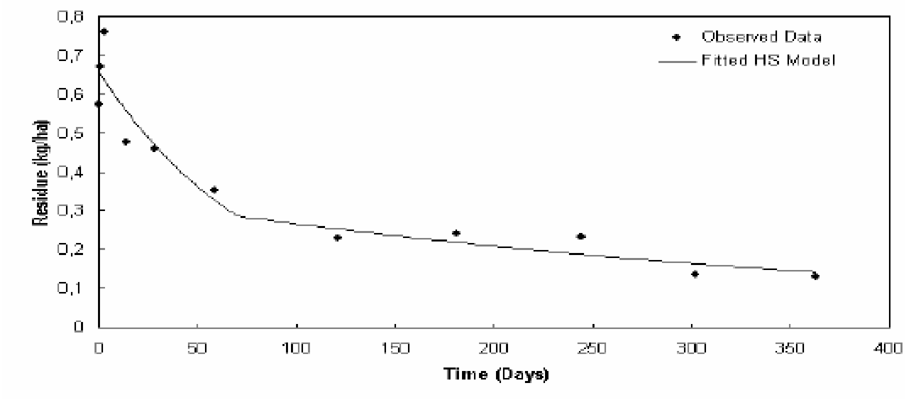


Figure B.8.19 HS graphical output for fluopicolide at Senas Yr 2, free fitting

Figure 5.37: Senas Year 2, Hockey Stick fit, C_0 free fitted



Hockey Stick fit							
	DT_{50}	C_0 free fitted	k_1	k_2	tb	r^2	Error of χ^2 test
	d	g/ha	d^{-1}	d^{-1}	d		%
Senas Year 2	58.4	656	0.0119	0.00242	69.8	0.928	12.7

Figure 5.38: Residual plot for Senas Year 2, Hockey Stick fit, C_0 free fitted

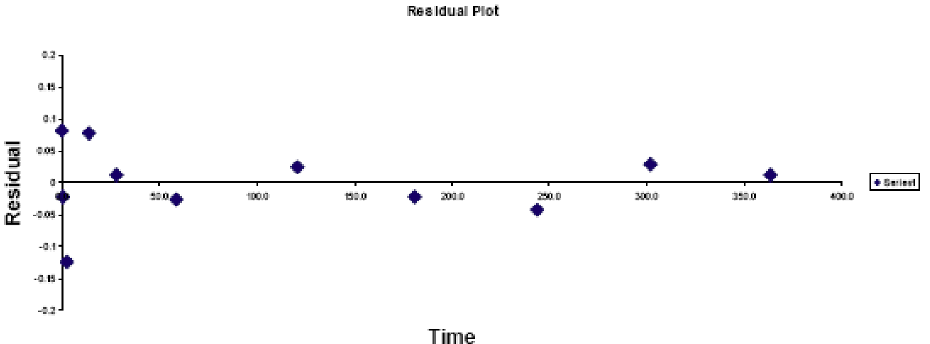
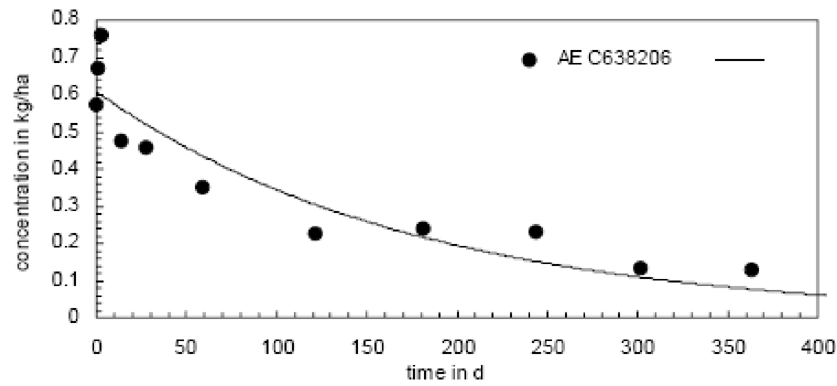


Figure B.8.20 SFO graphical output for fluopicolide at Senas Yr 2, free fitting

Figure 5.39: Senas Year 2, Single First Order fit, C_0 free fitted



Single first order fit				
	DT_{50}	C_0 free fitted	r^2	Error of χ^2 test
	d	g/ha		%
Senas Year 2	121.4	609	0.862	16.0

Figure 5.40: Residual plot for Senas Year 2, SFO fit, C_0 free fitted

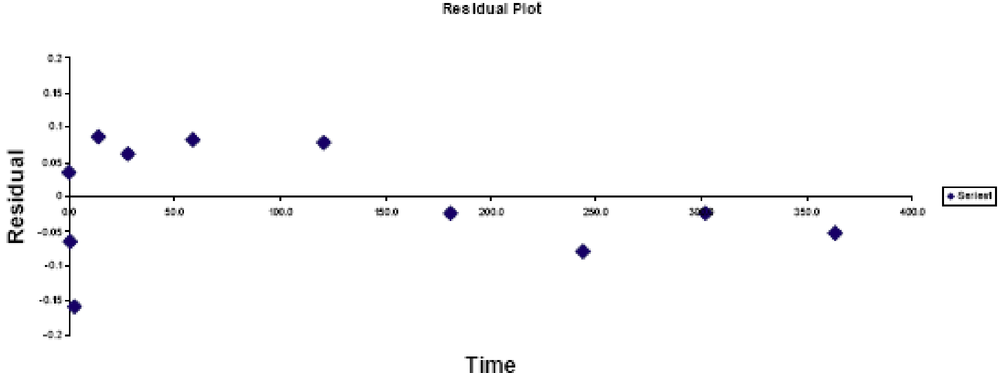
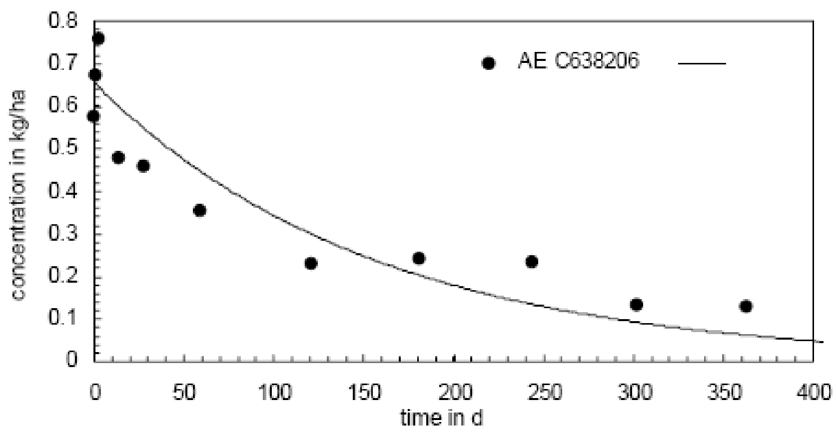


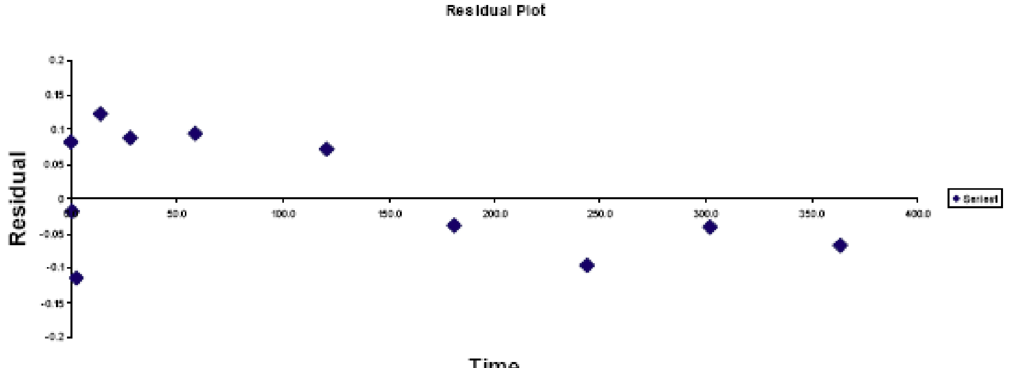
Figure B.8.21 SFO graphical output for fluopicolide at Senas Yr 2, C_0 fixed

Figure 5.41: Senas Year 2, Single First Order fit, C_0 fixed



Single first order fit				
	DT_{50}	C_0 fixed to C_0 of hockey stick	r^2	Error of χ^2 test
	d	g/ha		%
Senas Year 2	106.8	656	0.842	16.4

Figure 5.42: Residual plot for Senas Year 2, SFO fit, C_0 fixed



Appendix 2

Graphical output of kinetic assessment of fluopicolide and soil metabolites residue decline at Huntlosen field dissipation study, rate constant-normalised data, demonstrating impact of fixing initial concentration at a range of values and free optimisation of C_0 (from Kley & Mackenzie 2007b)

NOTE: Care to be taken over interpretation, graphs have different scales

Figure B.8.22 Fluopicolide, M-03 (AE 0608000), M-01 (AE C653711) and M-02 (AE C657188), a.s. C_0 fixed to 400 g a.s./ha

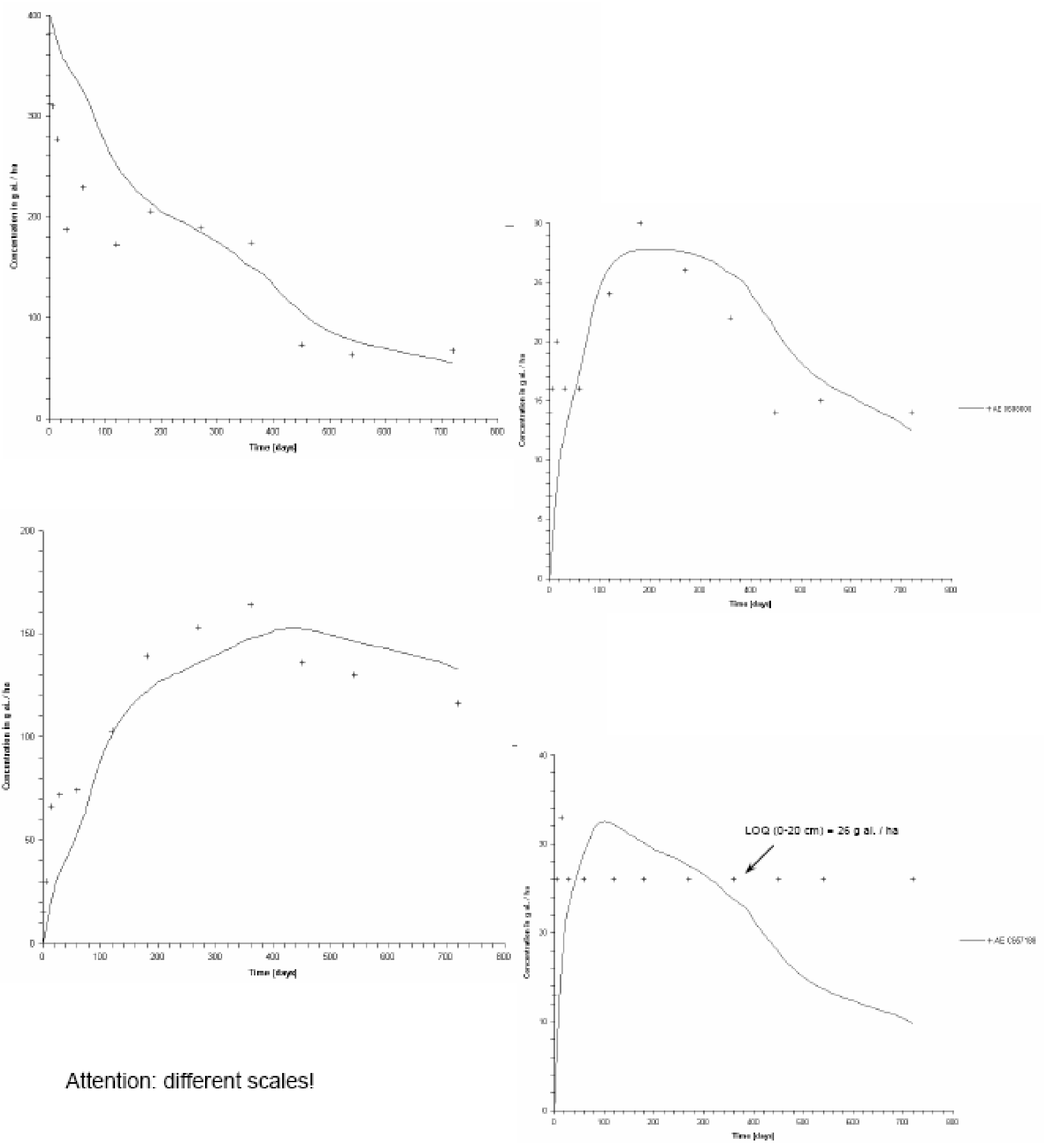


Figure B.8.23 Fluopicolide, M-03 (AE 0608000) and M-01 (AE C653711), a.s. C_0 optimised (resulting value = 321 g/ha)

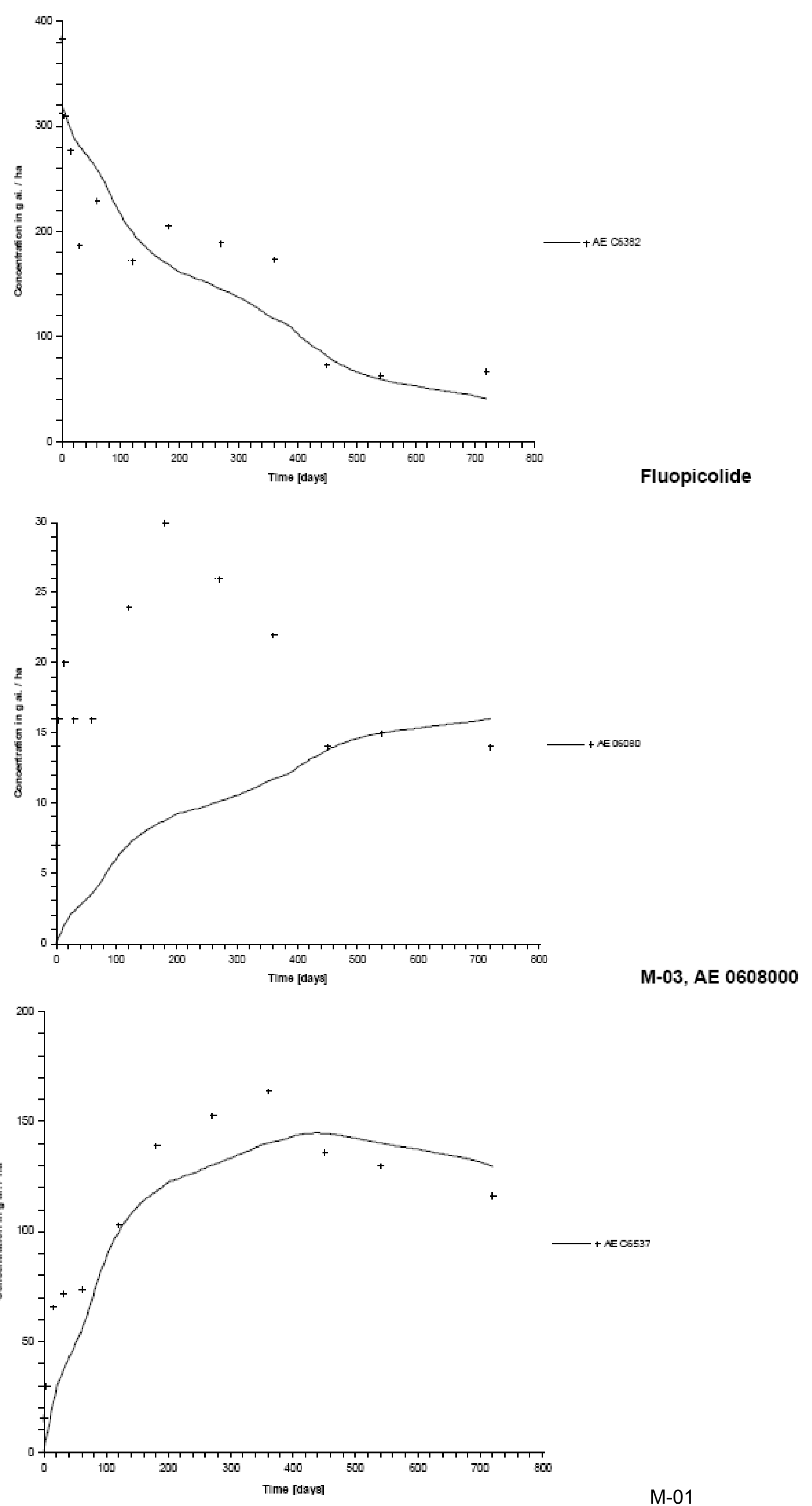


Figure B.8.24 Fluopicolide, M-03 (AE 0608000) and M-01 (AE C653711), a.s. C_0 fixed at 350 g/ha

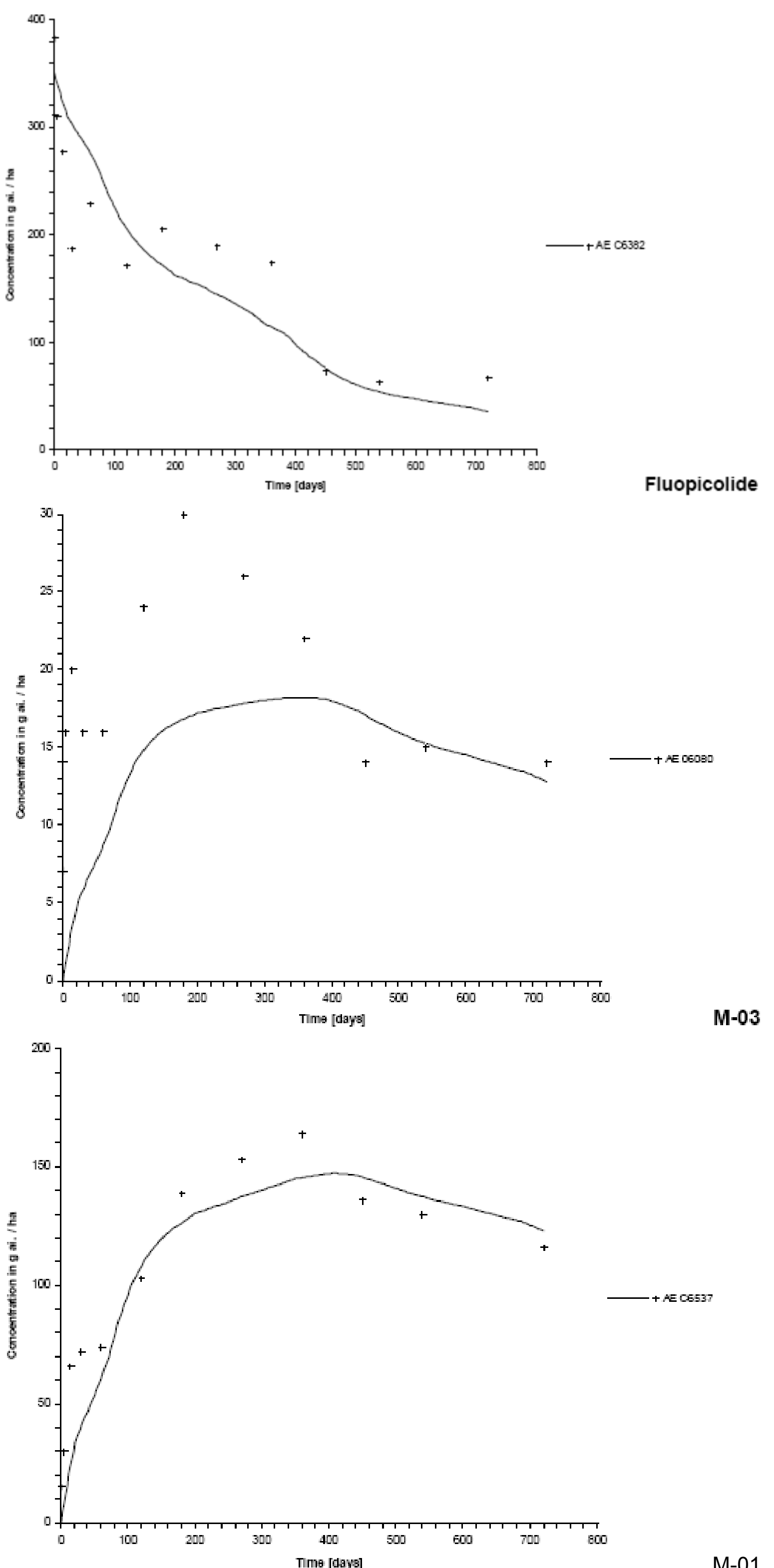


Figure B.8.25 Fluopicolide, M-03 (AE 0608000) and M-01 (AE C653711), a.s. C_0 fixed at 375 g/ha

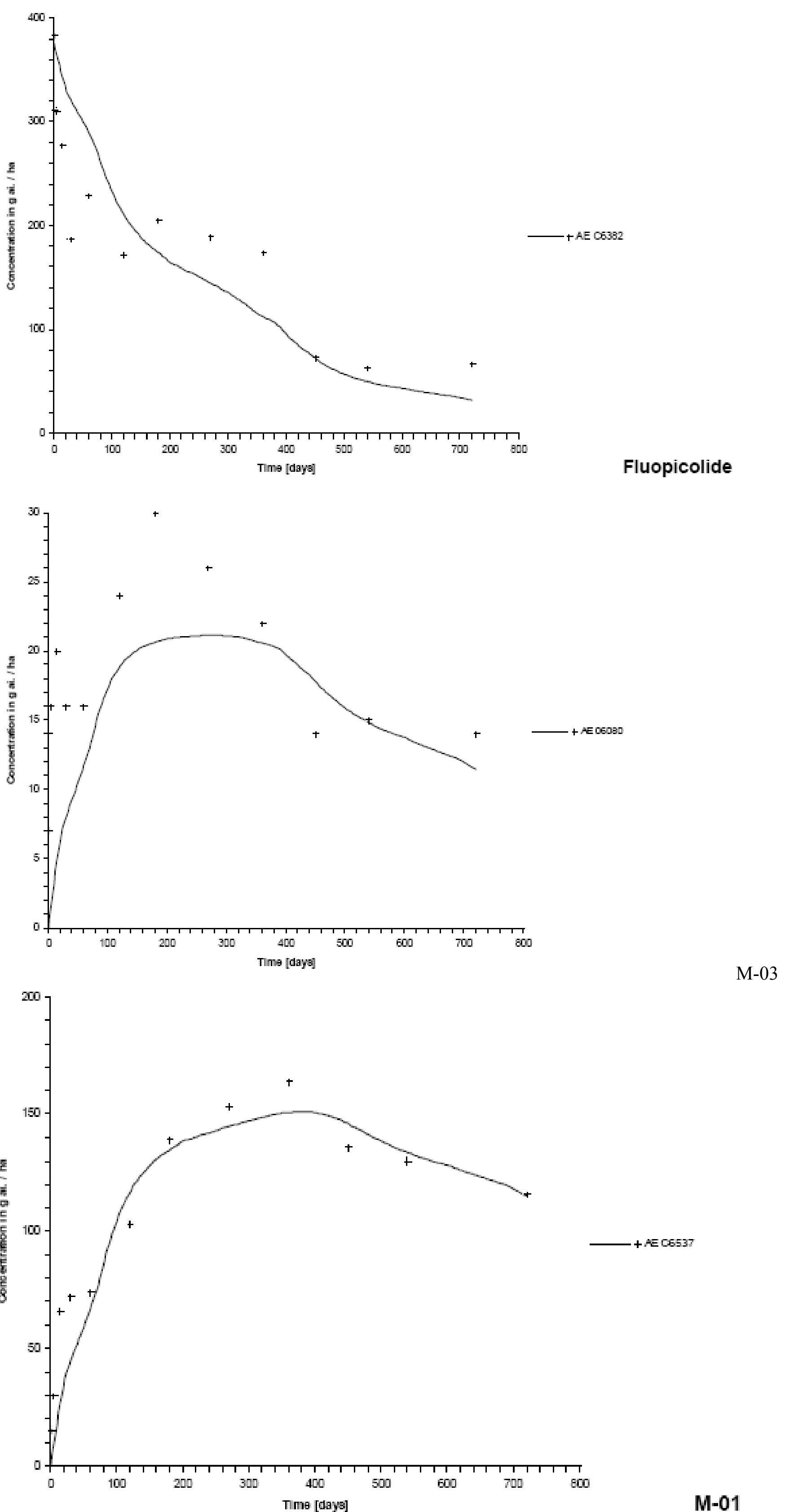


Figure B.8.26 Fluopicolide, M-03 (AE 0608000) and M-01 (AE C653711), a.s. C_0 fixed at 425 g/ha

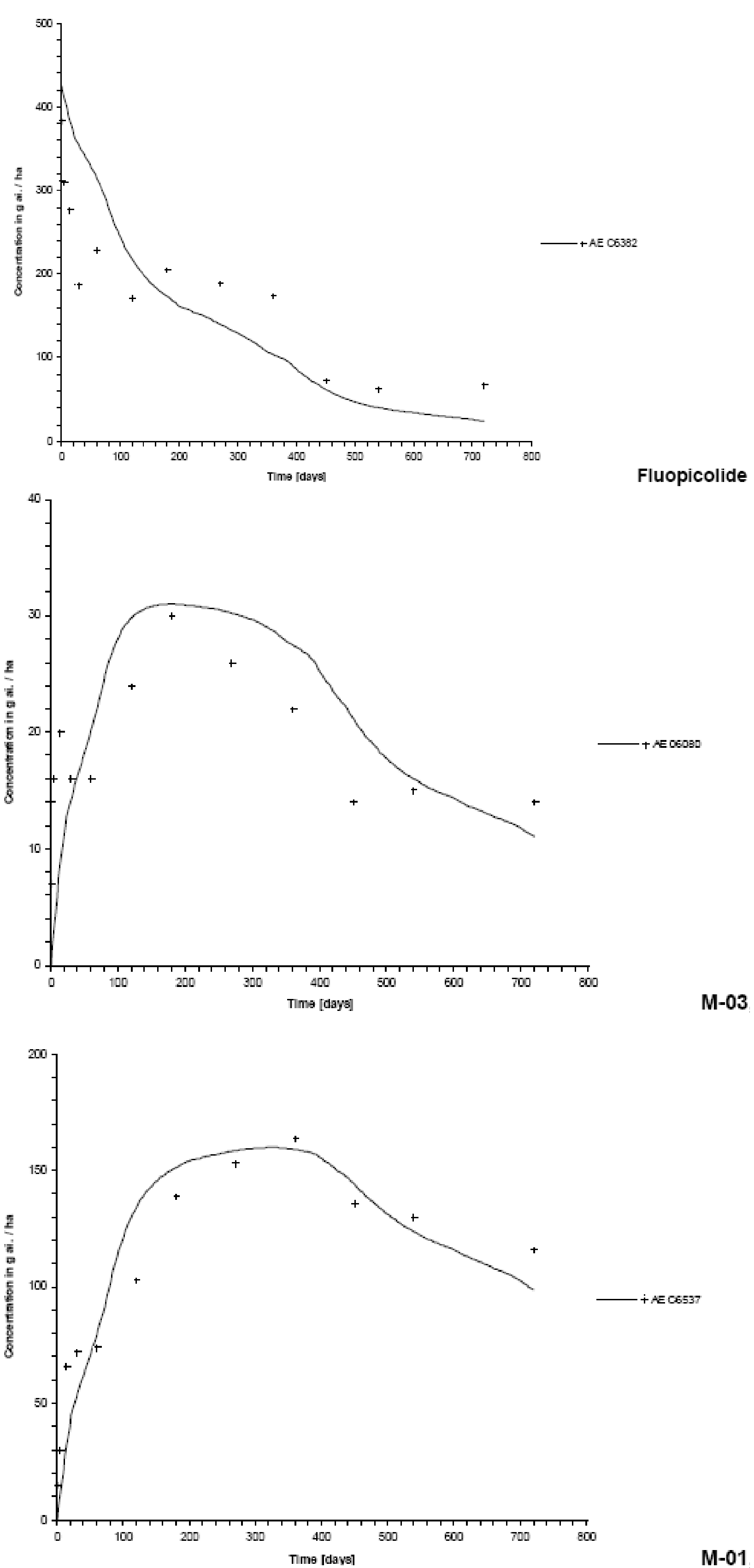
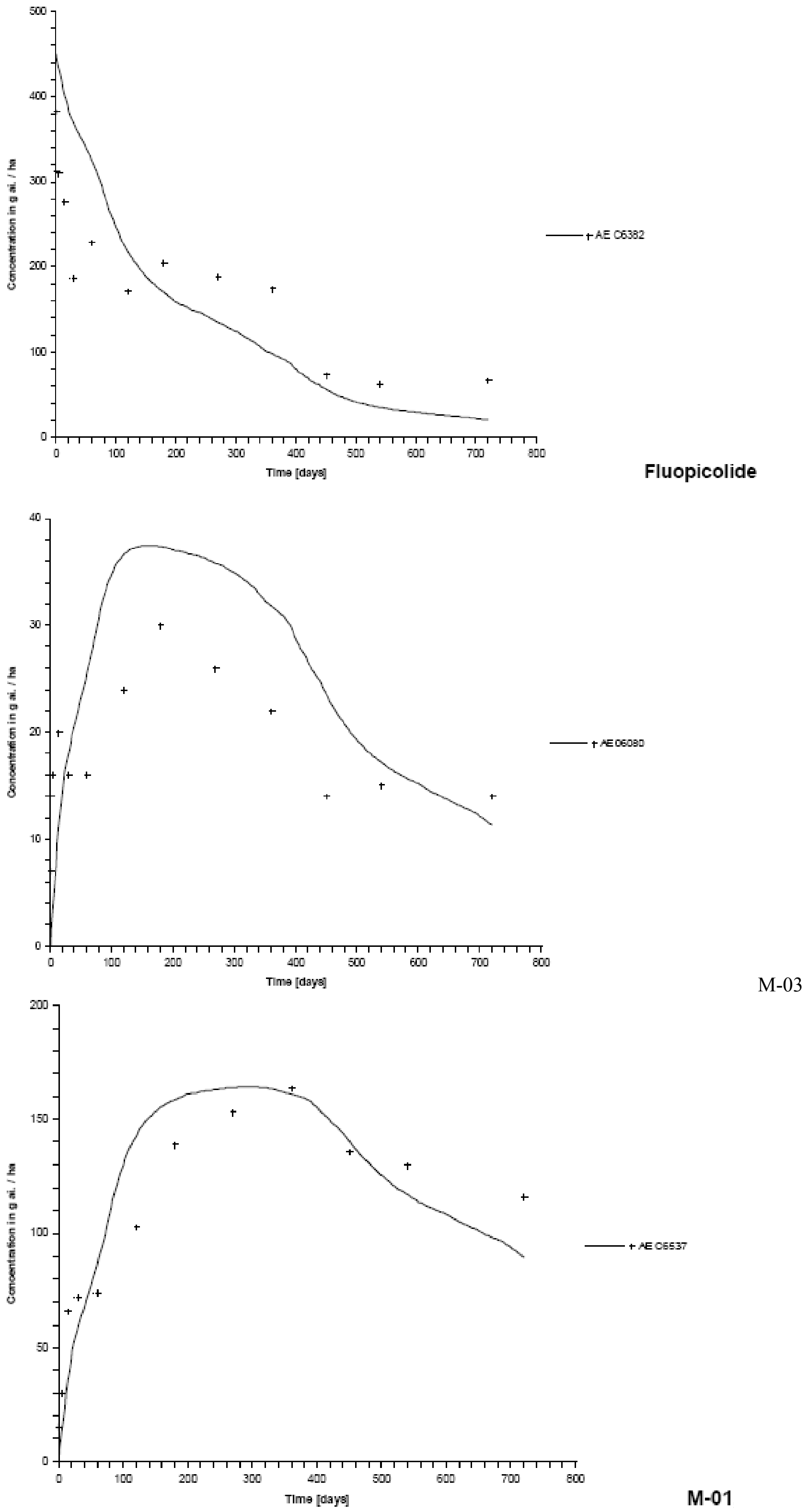


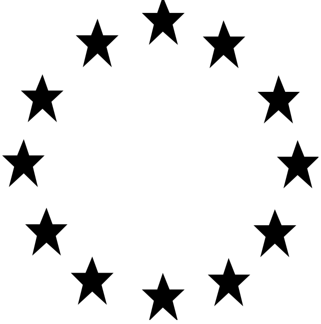
Figure B.8.27 Fluopicolide, M-03 (AE 0608000) and M-01 (AE C653711), a.s. C_0 fixed at 450 g/ha



References:

Annex Point/ Location in Dossier	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Data protect. claimed	Owner
AII IIA 7.1.1 Position Papers	Kley, C; Mackenzie, E	2007	Evaluation of Soil Degradation Parameters for Fluopicolide (AE C638206) for use as Trigger Values Bayer CropScience AG, Report No.: MEF-07/265 Project ID: MEACX083 Date: 2007-11-12 Non GLP, unpublished	Yes	BCS
AII IIA 7.1.1 Position Papers	Kley, C; Mackenzie, E	2007	Evaluation of Soil Degradation Parameters for Fluopicolide AND ITS METABOLITES FROM Laboratory and Field Trials for Modelling Purposes Bayer CropScience AG, Report No.: MEF-07/266 Project ID: MEACX083 Date: 2007-11-12 Non GLP, unpublished	Yes	BCS

Council Directive 91/414/EEC



Fluopicolide (AE C638206)

ADDENDUM 2
TO THE DRAFT ASSESSMENT REPORT PREPARED BY THE UNITED KINGDOM

December 2008

PESTICIDES SAFETY DIRECTORATE
Mallard House, Kings Pool,
3 Peasholme Green,
York YO1 7PX, UK
Website: www.pesticides.gov.uk

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B.6 TOXICOLOGY AND METABOLISM

Open Point 2.3:

Data gap identified at PRAPeR 39:
Notifier to provide further information on M-01 if deemed necessary.

In order to address this data gap the notifier has a new position paper (Leake et al, 2008, report no. M-300114-01-1), title 'The non-relevance of the fluopicolide metabolite M01 (AE C653711): 2,6-dichlorobenzamide (also known as BAM)'. This position paper takes into account:

- o data already submitted with the fluopicolide dossier
- o toxicology data on M-01 (BAM) submitted in the US for dichlobenil which were not submitted in Europe for neither fluopicolide nor dichlobenil but were included into the negative reference list of the dichlobenil dossier.

Therefore this addendum contains the RMS's toxicology assessment of M-01 (BAM) and consists of 3 parts:

1) Evaluation of studies on 2,6-dichlorobenzamide (M-01/BAM) which were noted to have been considered by the EPA but were not available for consideration at the PRAPeR expert meeting and were suggested to at the expert meeting as potentially relevant studies.
2) Overview of the toxicology of M-01 (BAM), including all the available studies considered relevant in the assessment of toxicological relevance and proposals of regulatory reference dose levels.
3) An updated assessment of the relevance of M-01 (BAM) following 'Guidance Document on the Assessment of the Relevenace of Metabolites in Groundwater of Substances Regulated Under Council Directive 91/414/EEC (SANCO/221/2000-rev.10-final, 25 February 2003).

PART ONE: Evaluation of additional studies on 2,6-dichlorobenzamide (M-01/ BAM)

The following studies were noted to be reported in the US EPA's assessment of M-01 (BAM) during PRAPeR 39 (10 – 13 12.2007) and as a consequence of ensuing discussions on relevance for the risk assessment of M-01 (BAM), a data gap was opened for 'the Notifier to provide further information on M-01 if deemed necessary'. The additional data mentioned have been evaluated and considered in the assessment of the toxicity of M-01 (BAM).

1) Two-year dietary toxicity study in dogs

Study	Toxicity studies on 'Prefix' residue 2,6-dichlorobenzmide: Two year oral experiment with dogs
Reference	Wilson, A. B., Thorpe, E. (7/1971)
Date performed	Not stated
Test facility	
Report reference	Laboratory reference: T507531.
Guideline(s)	US EPA 83-1
Deviations from the guideline	Relevant limitations in data presented included absence of individual animal data on body weight development and food intake. The report stated that it is a reissue of the study and included a review conducted in September 1993 to conform with US EPA requirements.
GLP	No, Pre-GLP/no QA statement
Test material	2,6-dichlorobenzmide; batch no.: 133-2-4-104, Purity: 97% w/w
Study acceptable	Yes.

In a study (1971), groups of 4 male and 4 female Beagle dogs received 2,6-dichlorobenzmide (batch no.: 232580250, purity: > 98 %) pre-dissolved in acetone and mixed into the diet at concentrations of 0, 60, 100, 180 or 500 ppm (equal to 0, 1.5, 2.5, 4.5 or 12.5 mg/kg bw/day) for approximately 2 years. All groups were observed daily for general health and behaviour. consumption and growth. Laboratory investigations for haematology and plasma clinical chemistry were performed prior to treatment and at approximately 3 month intervals until study termination. Haematological examination comprised of haemoglobin content, haematocrit, erythrocyte, leucocyte and differential leucocyte counts, prothrombin time and kaolin-cephalin clotting time. Clinical chemistry tests consisted of bromosulphthalein clearance, serum protein, urea, glutamic pyruvic transaminase actvity and plasma alkaline phosphatase activity. All surviving animals were sacrificed after the treatment period and were subjected to detailed gross pathology, organ weight analysis and histopathology.

Statistical analyses of body weight and organ weights were performed using intial body weight as covariate in covariance analysis. Haematology and clinical chemistry measurements were examined using analysis of variance. The Student 't' test was applied to measure the difference between treated and and control groups.

The study was performed pre-GLP requirements and was not quality assured. A 5000 ppm concentrate was initially prepared by mixing a solution of the compound in a minimal quantity of acetone with the appropriate amount of dog food and various dietary concentrations were prepared from the concentrate. The control diet was also premixed with an equal quantity of acetone. The test concentration in the diet was verified at various intervals during the in-life phase of the study and the values for the treatment concentration levels are considered to be within acceptable deviation of the nominal concentration. Homogeneity and stability of the test substance in the diet was not reported. Each animal received 400g of diet moistened with an equal volume of water during the first year which was increased to 600g in the second year.

One deficiency noted for this study by the EPA relates to diet stability and homogeneity data as were other unspecified deficiencies mentioned which were noted

not to affect the acceptability of the study for regulatory purposes. The absence of such data is not unusual for studies from this period because of the absence of clear regulatory guidelines during this period and considering that the test concentrations were verified and found to be acceptable at various time points during the study, the findings of the study are considered acceptable.

General health and behaviour of treated and control animals were comparable throughout the two year period. Body weight gain in females of the 500 ppm dose group was statistically significantly reduced throughout the study and statistically non-significant reduction (*ca*10%) were observed at 180 ppm from week 63 to termination, however the animals were gaining weight at the end of the study and the finding is not considered to be adverse. Two control females and one 60 ppm female were noted by the investigators to be exceptionally large animals but it is noted that the supporting individual animal data was not cited and that the starting mean body weights did not support the explanation. Food consumption data for individual animals was also not provided to permit further analysis and a clear effect on body weight in females is evident at 500ppm. A trend of lower body weights was noted in the 500 ppm males although this did not achieve statistical significance.

Table 1a. Summary of the findings of the body weight development in the 2-year dietary study with BAM in dogs

Parameter		Dose (ppm)				
		0	60	100	180	500
Body weights						
Week 0	M	10.7	10.5	10.7	10.9	10.8
	F	9.8	10.4	9.9	9.9	10.0
Week 2	M	11.1	11.2	11.0	10.8	11.0
	F	10.3	10.3	10.2	10.1	10.0*
Week 6	M	11.9	12.0	11.5	11.4	11.3
	F	11.3	11.1	10.8	10.6	10.2**
Week 15	M	12.7	12.5	11.8	12.1	11.6
	F	11.9	11.6	11.5	11.2	10.4**
Week 54	M	14.4	14.4	13.6	13.3	12.4 (13.9%)
	F	13.7	13.8	12.6	12.4	10.8**
Week 80	M	15.2	15.0	14.4	14.0	13.0 (14.5%)
	F	14.4	14.4	13.2	12.8 (11.2%)	11.3**
Week 104	M	15.5	15.5	15.1	14.5	13.6 (12.3%)
	F	15.0	15.0	13.7	13.4 (10.4%)	11.5**

* $p \leq 0.05$; ** $p \leq 0.01$; (x%) percentage reduction compared with control

Haematology and clinical chemistry values were comparable between controls and treated animals throughout the study and occassional changes did not reveal any treatment or dose relationships

At autopsy, increased relative liver weights were noted in 500 ppm males whilst reduction in absolute and relative liver and kidney weights were observed in females (Table 1b). There was no clear biological relevance for these findings as no dose-response was evident.

Table 1b. Summary of the findings of the organ weight changes in the 2-year dietary study with BAM in dogs

Parameter		Dose (ppm)				
		0	60	100	180	500
Organ weight (g)						
Liver	M	499	531	493	443	544
	F	621	434**	407**	436**	405**
Kidneys	M	69.6	75.3	78.9	70.1	67.0
	F	73.1	62.6	57.4*	58.4*	49.8**
Relative organ weight (g/100g bw)						
Liver	M	3.27	3.44	3.24	3.04	4.01*
	F	4.14	2.93**	2.99**	3.24**	3.49**
Kidneys	M	0.460	0.486	0.523	0.477	0.487
	F	0.483	0.427	0.422	0.437	0.433

* $p \leq 0.05$; ** $p \leq 0.01$;

Gross and histopathological examination of organs and tissues did not reveal any treatment-related changes.

The NOAEL in the 2-year dietary study with BAM in dogs was 180 ppm (4.5 mg/kg bw/day) based on reduction in body weight gain at 500 ppm (12.5 mg/kg bw/day).

Wilson, A. B., Thorpe, E. (1971)

2) Multigeneration study in rats

Study	Results of reproduction study of rats fed diets containing 2,6-dichlorobenzamide (BAM) over three generations
Reference	Hine, C. H., Eisenlord, G., Loquvam, G.S. (10/1971)
Date performed	Not stated
Test facility	[redacted] San Francisco, California.
Report reference	Laboratory reference: Report no. 1.
Guideline(s)	US EPA 83-4
Deviations from the guideline	No significant deviation.
GLP	No, Pre-GLP/no QA statement
Test material	2,6-dichlorobenzmide; batch no.: 195, Purity: 99.5% w/w
Study acceptable	Yes.

In a 3-generation reproductive study, groups of 10 male and 20 female F0 Long Evans rats were administered BAM in the diet at concentrations of 0 (control), 60, 100 and 180 ppm (equal to 0, 4.5, 7.5 or 13.5 mg/kg bw/day) over 3 generations. Each generation of parents was mated twice to produce two litters. F0 Long Evans rats were administered BAM in the diet for 79 days before mating. The rats were 100 days old at the start of mating. Two weeks was allowed for the mating of each female with males rotated once during that time. All pups from the first litters, F1A were discarded at weanling and the parent rats were mated again after 10 days to produce the F1B. Randomly selected pups,

excluding runts from the second litters were maintained on the diets and mated in turn when 100 days old to produce the F2A and F2B litters. Randomly selected F2B were allowed to mate when 100 days old to produce the F3A and F3B litters.

The number of pups in each litter was counted on the day of birth and on the 5th day. Litters greater than 10 were reduced to 10 on the 5th day. On day 21, the weanlings were counted and weighed and either sacrificed or saved for continuation on the diets. Parent rats were weighed, sacrificed and examined grossly when no longer required. Ten male and 10 female F3B weanlings from the control and 180 ppm dose groups and 5 males and 5 females from the 60 and 100 ppm dose groups were selected for autopsy. Individual body weights and brain, liver and kidney weights were recorded. Sections of brain, heart, lung, liver, spleen, kidney and testes were preserved for histological examination.

Statistical analysis was performed of numbers of pups, survival at 21 days, body weights of weanlings at 21 days and weights of parent rats at time of sacrifice and relative organ weights of F3B weanlings. Dunnett’s test was applied for the comparison of treated and control animals for significance of changes. Fertility, gestation, viability and lactation indices were calculated from litter production and mortality and tested for significance using chi-square.

The study was performed pre-GLP regulations. The test substance was analysed and found to contain BAM (99.5%), phenol (0.1%) and iron (0.21%).

The Notifier has observed that the study is of poor quality and presents several deficiencies amongst which include the absence of any individual data, the absence of measurement of several parameters to evaluate the reproductive performance (mating index, pre-coital time, pregnancy rate, length of gestation), and the absence on fetal body weight evolution from birth up to weaning. Given the laboratory that conducted the work is no longer in existence it will not be possible to get further information.

The Rapporteur considers the study is acceptable with the proviso that the limitations mentioned are considered in the assessment. A significant range of core reproductive toxicity parameters were investigated. A new study is considered unnecessary as there are no indications for further concern.

There were no treatment-related signs of toxicity or differences in behaviour and appearance during the study. Hyperexcitability was noted in a few pups of the F1B generation only and is not considered treatment-related.

Fertility was not affected by the treatment and ranged from 85 – 100% throughout the study with no significant intergroup differences (Table 2a).

Table 2a: Fertility index of rat groups in BAM reproductive toxicity study

Generation		Control	60ppm	100 ppm	180 ppm
F1A	Ratio	17/20	18/20	20/20	20/20
	Index	85	90	100	100
F1B	Ratio	20/20	18/20	18/20	19/19
	Index	100	90	90	100
F2A	Ratio	18/20	19/20	20/20	20/20
	Index	90	95	100	95
F2B	Ratio	18/20	20/20	20/20	20/20
	Index	90	100	100	100
F3A	Ratio	19/20	20/20	20/20	20/20
	Index	95	100	100	100
F3B	Ratio	19/20	20/20	19/20	20/20
	Index	95	100	95	100

Fertility index = Number of pregnancies/Number of matings x 100

The average number of pups per litter in the treated and control groups did not exhibit any treatment-related intergroup differences and ranged from 9.3 – 12.2 in the first generation, 8.7 – 10.4 in the second generation and 9.1 – 11.2 in the third generation (Table 2b).

Table 2b. Summary of litter sizes at birth and mean % survival (day 21) for all dose groups in reproductive toxicity study with BAM

Generation	Control		60ppm		100 ppm		180 ppm	
	litter size	% survival	litter size	% survival	litter size	% survival	litter size	% survival
F1A	10.5	72.3	9.9	74.2	10.6	76.1	9.3	69.1
F1B	10.6	63.6	12.2	68.8	11.0	60.7	11.3	75.0
F2A	8.8	90.8	9.5	94.7	8.7	95.1	9.6	95.0
F2B	9.4	89.5	10.2	94.4	10.0	97.7	10.4	86.5
F3A	9.1	80.0	11.2**	80.5	10.6	80.3	10.8*	82.6
F3B	9.8	85.4	11.2	89.8	10.5	83.9	10.2	84.0

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

The investigators submitted that the mean percentage survival of the pups between day 1 and 21 when analyzed by the Dunnett’s test was comparable for all groups and in all generations and did not show any treatment-related differences from controls.

Gestation index were comparable for all dose groups and in all generations (Table 2c)

Table 2c: Gestation index of rat groups in BAM reproductive toxicity study

Generation		Control	60ppm	100 ppm	180 ppm
F1A	Ratio	17/17	18/18	20/20	20/20
	Index	100	100	100	100
F1B	Ratio	20/20	18/18	18/18	19/19
	Index	100	100	100	100
F2A	Ratio	18/18	19/19	20/20	19/19
	Index	100	100	100	100
F2B	Ratio	18/18	20/20	20/20	20/20
	Index	100	100	100	100
F3A	Ratio	19/19	20/20	20/20	20/20
	Index	100	100	100	100
F3B	Ratio	19/19	20/20	20/20	20/20
	Index	100	100	100	100

Gestation index = Number of litters with live pups/Number of pregnancies x 100

Viability index showed occasional statistically significance for the number of pups alive at day 5 when investigated by the chi-test. However, these differences did not show any dose response or treatment-relationship. Although, a treatment-relationship cannot be ruled out for the observation for the F3B 180 ppm dose group, it has to be considered against the absence of any significant effect in 5 litters from the other generations and the total numbers of pups alive in the 180 ppm group (Table 2d)

Commenting on this finding, the Notifier has stated that the study report mentioned a slight decrease in the viability index at 180 ppm in the F3b generation. However, taking into account that: there was no dose-response; there were no consistent effects across generations; the survival of pups from birth to weaning as measured by the lactation index was not affected and that this change was considered to be of very limited significance.

Table 2d: Viability and lactation indices of rat groups in BAM reproductive toxicity study

Generation		Control	60ppm	100 ppm	180 ppm
Viability					
F1A	Ratio	172/178	171/179	190/212	180/180
	Index	96.6	95.5	89.6**	96.8
F1B	Ratio	197/213	198/219	181/198	207/214
	Index	92.5	90.4	91.4	96.7
F2A	Ratio	150/158	177/180	167/174	172/182
	Index	94.9	98.3	96.0	94.5
F2B	Ratio	161/170	202/205	194/200	186/207
	Index	94.7	98.5*	97.0	89.9
F3A	Ratio	164/172	219/224	205/211	194/215
	Index	95.3	97.8	97.2	90.2
F3B	Ratio	178/186	217/223	190/199	175/203
	Index	95.7	97.3	95.5	86.2**
Lactation					
F1A	Index	73.6	74.7	83.3*	66.9
F1B	Index	63.3	70.9	63.8	73.7 *
F2A	Index	96.5	96.4	96.8	98.2
F2B	Index	96.7	95.1	98.9	95.7
F3A	Index	86.8	82.4	84.0	89.9
F3B	Index	93.3	90.2	86.3 *	94.0

Viability index = Number of pups alive at 5 days/Number of pups born x 100
* $p \leq 0.05$; ** $p \leq 0.01$;

Mean body weights of weanlings were significantly lower for the F3A and F3B 180 ppm dose groups and are considered to be biologically relevant (Table 2e).

The Notifier has observed that there were no treatment-related changes in body weight in weanling pups of any generation at any dose level. The Notifier notes that the study report mentioned some slight variations at 180 ppm as evidenced by reduced body weight in weanling pups of the F1b (-15%, $p< 0.05$), F3a (-12%, $p< 0.05$) and F3b (-14%, $p< 0.01$). However, taking into account that: there was no dose-response; there were no consistent effects across generations; the body weight was not calculated from individual pup body weight but expressed at entire litter weight; and there was no data on pup body weight evolution from birth up to weaning. Hence caution should be exercised when drawing conclusions and these slight and inconsistent effects were considered to be of limited significance.

The Rapporteur notes that there is relatively good correlation between the mean body weights of weanlings (Table 2e) and mean maternal weights (Table 2f) to explain the findings in pups at 180 ppm.

Table 2e: Mean body weight of weanlings of rat groups in BAM reproductive toxicity study (g)

Generation	Control	60ppm	100 ppm	180 ppm
F1A	38.7	38.0	37.9	41.0
F1B	38.9	33.4*	35.1	33.1*
F2A	34.4	32.6	32.8	32.7
F2B	38.6	35.5	36.7	36.8
F3A	35.0	32.9	34.4	30.9*
F3B	36.1	33.8	35.3	31.1**

* p = 0.05; ** p = 0.01;

The mean terminal body weight was statistically significantly lower (-6%, $p<0.05$) for the 180 ppm F2B females compared with the concurrent control (Table 2f). However, the change is noted to be small. The Notifier has submitted that taking into account the low magnitude of this effect and the absence of any consistent change in parent animals of previous generations, this change was considered to be of very limited significance.

Table 2f: Mean terminal body weight of parents of rat groups in BAM reproductive toxicity study (g)

Generation		Control	60ppm	100 ppm	180 ppm
F0	M	488	499	488	481
	F	339	328	331	319
F1B	M	488	499	465	461
	F	339	317	323	320
F2B	M	490	487	456	466
	F	325	322	309	305*

* p = 0.05; ** p = 0.01;

Relative liver weights of F3B weanlings were statistically significantly increased in females at dose levels of ≥ 100 ppm and in males at 180 ppm. Considering the significance of the increase at 100 ppm, 1/5 females of the 100 ppm dose group had an absolute liver weight greater than the range of the concurrent controls whilst a second was the same as the largest weight of the control group. For the relative liver weight ratio, 3/5 animals were clearly outside the range for the control group of 10 animals. However, this appeared to be the only finding at this dose level but in the absence of the investigation of histopathology of the liver, a significant treatment-relationship cannot be eliminated. Relative kidney weight was also increased for the F3B 180 ppm females.

Table 2g: Mean organ weights and relative organ weight/body weight ratios of F3B weanlings in BAM reproductive toxicity study

Parameter	Sex	Dose Groups			
		Control	60ppm	100 ppm	180 ppm
Absolute kidney (g)	M	0.434	0.457	0.475	0.450
	F	0.435	0.470	0.467	0.487
Relative Kidney (ratio x 100)	M	1.24	1.31	1.34	1.28
	F	1.26	1.34	1.33	1.41**
Absolute liver (g)	M	1.42	1.55	1.53	1.56
	F	1.46	1.61	1.62	1.62
Relative liver (ratio x 100)	M	4.05	4.44	4.33	4.46*
	F	4.24	4.60	4.62*	4.65**

* p = 0.05; ** p = 0.01;

The NOAEL was for reproductive toxicity was 180 ppm based on the absence of reproductive toxicity at the highest test dose of 180 ppm. The NOAEL for parental toxicity and foetal toxicity was 100 ppm (7.5 mg/kg bw/day) based on effects on mean body weight in offspring and in dams at 180 ppm (13.5 mg/kg bw/day).

Hine, C. H., Eisenlord, G., Loquvam, G.S. (1971)

c) Developmental toxicity study in rabbits with M-01 (BAM)

Study	2,6-dichlorobenzamide: Oral (gavage) teratology study in the rabbit.
Reference	McIntyre M (5/5/1986)
Date performed	13/5/1986 – 5/5/1986
Test facility	[redacted] UK.
Report reference	Laboratory reference: 5308-65/9
Guideline(s)	US EPA 83-3
Deviations from the guideline	No significant deviation.
GLP	Yes/QA statement
Test material	2,6-dichlorobenzmide; batch no.: FUX 001000, Purity: 99.4% w/w
Study acceptable	Yes.

In a study (1987) groups of 16 mated female New Zealand White rabbits were administered orally 2,6-dichlorobenzamide (BAM) by gavage, at dose levels of 0, 10, 30 or 90 mg/kg bw/day (dose volume 2 ml/kg) from day 7 to 19 of gestation inclusive. Control rabbits received 1% gum tragacanth which was used as vehicle for the test substance.

All animals were examined twice daily to detect any which were dead or moribund. All such animals were subjected to gross necropsy. The body weight of each female was recorded on days 0, 7, 13, 19, 23 and 28 of gestation. Food consumption of each female was recorded from days 0-7, 7-13, 13-19, 19-23 and 23-28 of gestation. All surviving females in study were killed on day 28 of gestation and reproductive performance, clinical condition, gross necropsy changes and the outcome of pregnancy were assessed.

The ovaries and uteri were removed and examined and the following data recorded: number of corpora lutea; number and intrauterine position of implantations subdivided into live fetuses, early intrauterine deaths and late intrauterine deaths.

Foetuses were examined for external, visceral and skeletal abnormalities. Foetal abnormalities were recorded as major (rare and/or possibly lethal), minor (deviations from normal that were common at either gross necropsy or skeletal examination) or variants (retarded sternebral or phalangeal ossification, asymmetric insertion of the pelvic girdle or the presence of supernumerary ribs).

Appropriate statistical investigations were performed on the data.

The study was certified to comply with GLP regulations and subjected to a quality assurance audit. Dose levels were reportedly based on two range-finding studies in the rabbit which have not been submitted and are not required for the purpose of this assessment of developmental toxicity. The analysis of the dosing formulations showed that the actual concentrations were close to the nominal values.

Three 90 mg/kg bw/day dose group females were killed following abortion on days 19, 21 or 22 of gestation. Maternal toxicity was observed at 90 mg/kg bw/day and a further two dams were killed for moribundity. The 90 mg/kg bw/day dose group deaths were considered to be treatment-related by the Investigators following body weight loss, thin appearance and reduced food intake in the majority of these animals during the dosing

period. However, no consistent treatment-related macroscopic changes were observed at examination post-mortem.

In the 30 mg/kg bw/day dose group, 2 females were killed for moribundity on days 12 and 14 of gestation. The Investigators noted that although a treatment-relationship could not be entirely excluded, it was considered unlikely as similar incidences of mortality were observed in the concurrent control group and similar mortalities have previously been recorded in this strain of rabbit in these laboratories. No consistent, treatment-related findings were recorded at necropsy in these animals.

However, it is noted that the pattern of reduced food consumption, thin appearance (reported for at least one of the two deaths at 30 mg/kg bw/day) and fur staining prior to death is consistent for deaths and moribundity in all dose groups. Animals which aborted also showed lower body weights reduced food intake and or fur staining and there appears to be no clear basis for the exclusion of a treatment-relationship for the 30 mg/kg bw/day dose group other than on a comparison of incidences. The summary of body weights and food consumption across dose groups suggests that toxicity resulting in effects on body weight and food consumption was only apparent in the top dose group (Table 3.1).

One control female was killed following abortion on day 20 of gestation and a further female was killed for moribundity following deterioration in physical condition on day 24 of gestation. Both control animals showed reduced food consumption before termination. One 10 mg/kg bw/day dose group female was killed following abortion on day 23 of gestation.

Table 3a: Summary of mean food intake and body weight in development toxicity study in rabbits

Parameter	Group dose level (mg/kg bw/day)			
	0	10	30	90
Mean food intakes g/day				
days 0-7	184	183	190	190
days 7-13	189	200	192	94***
days 13-19	196	199	198	102***
days 19-23	177	181	186	203
Mean total food intake (g/period)				
days 0-28	4980	5015	5053	4266
Mean body weight (kg)				
day 7	3.74	3.65	3.73	3.80
day 19	4.02	3.95	4.01	3.72
day 28	4.17	4.04	4.11	3.99
% body weight change				
day 7-19	7.5	8.2	7.5	-2.1***

* ** $p < 0.001$

There was a marked decrease in group mean body weight of the 90 mg/kg bw/day dose females during the early part of the dosing period, with 9 out of the 11 surviving females losing weight between days 7 and 13 of gestation. Body weight gain of these animals from day 13 of gestation up to the end of the dosing period was reduced compared to that of the control (Table 3.1). The body weight gain throughout gestation of the females in 10 and 30 mg/kg bw/day dose groups was comparable to that of the controls.

There was a marked reduction in food intake of the 90 mg/kg bw/day dose group animals throughout the dosing period. Some compensatory increase in food consumption was observed after cessation of dosing on day 19 of gestation.
The majority of the 90 mg/kg bw/day dose group females were observed to have a thin appearance and fur staining. These observations continued even after cessation of dosing on day 19 of gestation.

The clinical condition of the females in the 10 and 30 mg/kg bw/day dose groups was comparable to that of the controls and was restricted to those changes which are not uncommon in this strain of rabbit in these laboratories.

There were no treatment-related maternal macroscopic findings at, examination post-mortem on day 28 of gestation. Pregnancy incidence was between 93.8 and 100% in all groups (Table 3.2).

The mean number of corpora lutea and implantations and the extent of pre-implantation loss showed some intergroup variations but no dose-related trends were apparent. The values for these parameters were noted by the investigators to be similar to the expected background control range (not supplied).

Table 3b: Summary of reproductive and foetal findings in development toxicity study in rabbits

Parameter	Group dose level (mg/kg bw/day)			
	0	10	30	90
No. of pregnant females	16/16	16/16	16/16	15/16
No. of pregnant females at 28 days	14	15	14	11
Mean no. of corpora lutea/female	10.9	9.7	10.1	10.3
Mean no. of implantations/female	10.4	9.0	8.4	9.5
% pre-implantation loss	4.6	7.5	16.3	8.0
Mean number of early intrauterine deaths/female	1.3	0.5	0.1	0.5
Mean number of late intrauterine deaths/female	0.4	0.2	0.4	0.3
% post-implantation loss	16.6	7.4	6.8	8.7
Mean number of foetuses per female	8.6	8.3	7.9	8.6
% of implantations	83.4	92.6	93.2	91.3
Mean foetal weight (g)	36.0	36.4	36.8	33.9
External and visceral defects				
Number showing major defects	0	2	0	0
% of foetuses examined	0	1.6	0	0
Number showing minor defects	11	16	17	14
% of foetuses examined	9.1	12.8	15.5	14.7
Number showing variants	21	23	11	26
% of foetuses examined	17.4	18.4	10	27.4
Skeletal defects				
Number showing major defects	2	7	2	2
% of foetuses examined	1.7	5.6	1.8	2.1
Number showing minor defects	34	43	44	37
% of foetuses examined	28.1	34.4	40.0	38.9
Number showing variants	101	101	91	78
% of foetuses examined	83.5	80.8	82.7	82.1
Total no. of major defects	2	7	2	2
% of foetuses examined	1.7	5.6	1.8	2.1

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Post-implantation loss and subsequent litter size were not adversely affected by treatment.

Mean foetal weight was slightly but not statistically significantly (p>0.05) reduced in the 90 mg/kg bw/day dose group.

The overall incidence of foetuses with major malformations was not adversely affected by treatment. An increase in the number of malformed foetuses was observed in group 2 but these findings were isolated and not observed at higher dose levels.

The overall type and incidence of minor external and visceral defects and variants showed some intergroup variations but no consistent treatment related trends were apparent (Table 3b).

Ossification parameters were comparable to the background controls and not considered to be adversely affected by treatment.

The NOAEL for maternal toxicity was 30 mg/kg bw/day based on maternal deaths and increased incidence of abortions most likely a consequence of body weight loss and for foetotoxicity 30 mg/kg bw/day based on statistically non-significant reduction in foetal birth weights. BAM was not teratogenic in the developmental toxicity study in rabbits and the NOAEL for developmental toxicity was 90 mg/kg bw/day, the highest test dose.

McIntyre M (1986)

d) 90-day dietary study in dog

Study	The study of the oral toxicity of 'Prefix' residue 2,6-dichlorobenzmide: 13 week exposure to dogs
Reference	Walker, A.I.T. (02/1967)
Date performed	Not stated
Test facility	
Report reference	Laboratory reference: T507531/1.
Guideline(s)	Not stated
Deviations from the guideline	Relevant limitations in data presented included absence of individual animal data on body weight development and food intake and in clinical chemistry data. Ascariasis was present in all animals at all dose levels with the exception of 2/6 control females and 1/4 100 ppm males
GLP	No, Pre-GLP/no QA statement
Test material	2,6-dichlorobenzmide; batch no.: 133/2/4/104, Purity: 97% w/w
Study acceptable	No. Supplementary information considered unreliable for regulatory decision making. Interpretation of findings severely limited by ascariasis in most animals

In a study (1967), groups of 4 male and 4 female Beagle dogs (control group consisted of 6 males and 6 females) received 2,6-dichlorobenzamide (batch no.: 133/2/4/104, purity: > 98 %) in the diet at concentrations of 100, 300 or 2000 ppm (equal to 0, 7.5, 22.5, 4.5 or 50 mg/kg bw/day) for 13 weeks. All groups were observed for general

health food consumption and body weight gain. Laboratory investigations for haematology and plasma clinical chemistry investigations were performed throughout the study. Liver function tests were performed during the treatment period on control and top dose animals.

Haematological examination included of haemoglobin content, packed cell volume, erythrocyte, leucocyte and differential leucocyte counts. Clinical chemistry tests included of bromosulphthalein clearance, serum protein, urea, glutamic pyruvic transaminase actvity and plasma alkaline phosphatase activity.

All surviving animals were sacrificed after the treatment period and were subjected to gross pathology, organ weight measurements and histopathology of a wide range of organs and tissues was performed.

Statistical analyses of terminal body weight and organ weights were performed using intial body weight as covariate in covariance analysis. Haematology and clinical chemistry measurements were examined using analysis of variance.

The study was performed pre-GLP requirements and was not quality assured. Homogeneity and stability of the test substance in the diet was not reported. Verification of the test concentration in the diet was not reported or performed. Diets for the dose groups were prepared from concentrates after pre-dissolving the test sample in acetone. Concentrates were well mixed until considered homogenous with a mixer and the solvent was allowed to evaporate. The control diet was also pre-mixed with an equal quantity of acetone. Diets were prepared every 4 weeks.

In a range-finding acute toxicity study, 2 male dogs were administered orally 500 mg/kg bw BAM in a capsule. One male dog received 100 mg/kg bw dose in the same manner . The dogs were observed for 72 h, killed and autopsied. Within 1h of dosing , the 500 mg/kg bw animals showed a lack of coordination in their gait which progressed to inability to rise within 2 h. However, within 24 h, complete recovery was reported without any further information about the intervening period. No effects were seen at 100 mg/kg bw. Post-mortem examination did not reveal any treatment-related findings.

In the 13-week study, observations on health and food intake did not reveal any treatment-related findings. In the 2000 ppm dose group, loss of condition, thin dull lifeless coat and loss of hair was observed from week 4 onwards but food consumption was not affected.

The mean body weights of the 2,000 ppm dose group were lower than those of the other treatments. This decrease in body weight was observed in males by the third week and in females by the fifth week of treatment.
Haematology in males showed no evidence of any treatment-related effect The increased haemoglobin in the 2,000 ppm dose group after 13 weeks exposure was due to one animal and was not considered of any toxicological significance. ln females, a decreased packed cell volume in the 2,000 ppm group was observed during the 13 weeks of exposure.

Clinical chemistry parameters in males did not show any treatment-relationship. However, in females the only apparent treatment-related change was increase in alkaline phosphatase in the 2000 ppm dose group. Liver function test measuring bromosulphthalein clearance rate did not show any significant treatment-related differences.

Urinalysis showed all samples to be normal and no evidence of intergroup differences were observed.

In the 2,000 ppm group, the terminal body weight of both sexes was decreased and the liver weight of the females increased, while in the 300 ppm dose group females both liver and left kidney weights were statistically significantly increased but the biological significance was unclear.

Microscopic examination of tissues taken at autopsy were reported to be normal with no evidence of structural changes. Findings suggested by the investigators to be related to parasitic infection included granulomas of the lungs, liver and kidneys with incidences unrelated to dose, interstitial nephritis and pneumonitis described as resulting from "mild spontaneous disease present in animals". These observations emphasise the limitations of this study for toxicological risk assessment

The NOAEL in the 13-week dietary study in dogs was 300 ppm (22.5 mg/kg bw/day) based on clinical signs in males and females including thin appearance, dull coat, and hair loss, decreased body weight gain in males and females, and increased liver weight and serum alkaline phosphatase concentrations in females only at 2000 ppm (150 mg/kg bw/day)

Table 4a: Summary of terminal body weight and some organ weight and clinical chemistry findings in the 13-week oral study in dogs

Parameter	Group dose level (ppm)			
	0	100	300	2000
Terminal body weights (kg)				
M	10.5	10.6	10.5	9.9*
F	9.7	9.5	9.5	8.9**
Organ weight				
Liver weight (g)				
M	416	375	434	465
F	328	326	417*	442**
Brain weight (g)				
M	77	77	74	74
F	74	71	71	67
Right/Left testes weight (g)				
M	7.8/7.7	7.7/8.0	7.6/7.7	+6.1/+5.8
Right/Left kidney weight (g)				
M	28.5/28.0	24.7/25.5	27.6/26.5	+23.2/+23.2
F	21.5/22.2	21.4/21.6	26.3/27.5*	24.0/24.7
Clinical chemistry				
Alkaline phosphatase activity (i.u.)				
M	91	112	96	99
F	82	102	111	136**

+ Mean of 3 animals
* $p < 0.05$; ** $p < 0.01$

Walker, A.I.T. (1967)

Part 2: OVERVIEW OF THE METABOLISM AND TOXICITY DATA ON THE METABOLITE M-01 (BAM)

The Notifier has submitted a range of bridging studies and an argued case for the equivalence in toxicity of BAM and Fluopicolide considering all relevant studies available in the original DAR and additional studies summarised in this Addendum at Appendix 2 (Leake et al, 2008, report no. M-300114-01-1, title 'The non-relevance of the fluopicolide metabolite M01 (AE C653711): 2,6-dichlorobenzamide (also known as BAM))'. The Rapporteur has assessed the case for the equivalence in toxicity of BAM and Fluopicolide.

Full details of these studies, as presented in the DAR, can be found in Appendix 2.

ADME

Following single oral administration of [14C]-M-01 to the male and female rat at the rates of 10 and 150 mg/kg most of the administered radioactivity was eliminated in the urine (ca 82 %dose) although the rate of elimination was relatively slow. Lower levels (ca 13 % dose) were eliminated via the faeces. The highest concentrations in tissues were seen in the kidney (ca 0.57 µg equiv./g) and liver (ca 0.44 µg equiv./g) for the 10mg/kg dose group and in the skin & fur (3.8 to 5.0 µg equiv./g), kidneys (2.8 to 3.0 µg equiv./g) and liver (2.1 to 2.3 µg equiv./g) for the 150 mg/kg dose group. Tissue concentrations therefore increased by approximately five-fold for a fifteen-fold increase in dose rate. Overall, multiple dosing (14 daily doses at 10 mg/kg) did not have any significant impact on the absorption, distribution, metabolism and elimination compared to results after single oral dosing. Thus, the results in this study showed that the routes and the rates of excretion were maintained despite the multiple dosing, which meant that most of the radioactivity was eliminated via the urinary route. The distribution pattern in the tissues was also similar between single and multiple dosing with the highest mean concentrations observed in the skin & fur (3.0 µg equiv./g), kidney (1.9 µg equiv./g) and liver (1.3 µg equiv./g). Bioretention or accumulation was therefore not indicated. The routes of biotransformation were similar between dose levels and sexes with hydrolysis of the amide group to form AE C416656, hydroxylation to form hydroxy-BAM (M-04) and subsequent conjugation with either glucuronic acid or sulphate, and the loss of a chlorine atom following glutathione conjugation. Further metabolism of the glutathione group to the mercapturic acid or S-methyl metabolites was observed.

ACUTE TOXICITY

M-01 was shown to be of relatively low acute oral toxicity. In a study by the acute toxic class method, the acute oral LD50 of M-01 was 2000 mg/kg bw in males and 500 mg/kg bw in females. According to the OECD 423 guideline, M-01 should be classified as harmful if swallowed. In an older, pre-GLP but acceptable study, the LD50 values with 95% confidence levels were calculated after a 14-day observation period to be 1470 (951–2270) and 2330 (1430–3780) mg/kg bw for male and female rats, respectively. In the older study, toxicity to females was less than in males. M-01 qualifies for an Xn classification according to the current European directive. It is notable that this metabolite is of greater acute oral toxicity than the parent, fluopicolide (LD50 > 5000 mg/kg bw), but not particularly hazardous in absolute terms. However,

this difference in toxicity between Fluopicolide and BAM for acute toxicity is not considered relevant under the groundwater metabolite assessment guidance because BAM is not classified as TOXIC nor is Fluopicolide.

MUTAGENICITY/GENOTOXOCITY

The genotoxicity profile of M-01 was assessed in three *in vitro* and one *in vivo* assays and no evidence of genotoxicity was observed in any assays. The *in vitro* studies were the bacterial gene mutation assay in bacterial cells, V79/HPRT gene locus assay, and unscheduled DNA synthesis assay and the mouse micronucleus assay *in vivo*. BAM is not considered to be a genotoxic compound.

SUBACUTE/SUBCHRONIC TOXICITY

In a 13-week toxicity study performed in CD rats with M-01 at doses up to 2300 ppm, reduced body weight gains and food consumption was observed at dose levels of ≥ 600 ppm (49 mg/kg bw/day) but no target organ toxicity was observed. The NOAEL of M-01 was 180 ppm (equivalent to 14 mg/kg bw/day) in males and females based on decreased body weight gain (M), food intake and clinical signs (M&F). In a 90-day study in dogs, the NOAEL was 300 ppm (equivalent 22.5 mg/kg bw/day) based on clinical signs (thin appearance, dull coat, hair loss) and increased liver weight and serum alkaline phosphatase concentrations (F) at dose levels of 2000 ppm (equivalent 150 mg/kg bw/day). However, this study is noted to be unreliable for regulatory purposes considering significant infestation with ascariasis. In a 2-year dietary study in dogs, the NOAEL was 4.5 mg/kg bw/day based on decreased body weight and body weight gain at the higher dose level of 12.5 mg/kg bw/day

CARCINOGENICITY

In a carcinogenicity study performed in CD rats with M-01 at doses up to 500 ppm, the liver as the principal target organ with a slightly increased incidence (of non statistical significance) of hepatocellular adenoma in females at 500 ppm. No carcinogenic effect was seen after a 2-year treatment with M-01. The NOAEL was (180 ppm) 5.7 mg/kg bw/day in males and 8.6 mg/kg bw/day in females. **It was agreed at PRAPeR 39 (10–13 12.2007) that neither for fluopicolide nor for metabolite M01 should be classified for carcinogenicity.**

REPRODUCTION/DEVELOPMENTAL TOXICITY

In a multigeneration study, the NOAEL for reproductive toxicity was 180 ppm based on the absence of reproductive toxicity at the highest test dose of 180 ppm. The NOAEL for parental toxicity and foetal toxicity was 100 ppm (7.5 mg/kg bw/day) based on effects on mean body weight in offspring and in dams at 180 ppm (13.5 mg/kg bw/day.

In a developmental toxicity study in rabbits, the NOAEL for maternal toxicity was 30 mg/kg bw/day based on maternal deaths and increased incidence of abortions most likely a consequence of body weight loss at 90 mg/kg bw/day and for foetotoxicity 30 mg/kg bw/day based on statistically non-significant reduction in foetal birth weights at

90 mg/kg bw/day. BAM was not teratogenic in the developmental toxicity study in rabbits and the NOAEL for developmental toxicity was 90 mg/kg bw/day, the highest test dose.

In conclusion, these data show that the toxicological profile of the metabolite M-01 is similar to that of fluopicolide. The Notifier has provided a discussion of the non relevance of M-01 (see Appendix 3).

Overall summary of studies on BAM

Study Type	Year/ Study design	Results	Reference
Acute oral rat (gavage) *	**1967/ along lines of OECD 401**	LD_{50} = 1470 mg/kg [951-2270] (M) and 2330 mg/kg [1430-3780](F)	Kemp, A.
Acute oral rat (gavage) *	**2003** Acute toxic class method.	$LD_{50} \geq$ 2000 mg/kg (M) and $LD_{50} \geq$500 mg/kg (F)	Schuengel, M.
90-day oral rat (dietary) *	**1967** 0, 50, 180, 600, or 2300 ppm (equal to 0, 4, 14, 49, or 172 mg/kg/day)	NOAEL = 180 ppm = 14 mg/kg bw/day LOAEL = 49 mg/kg bw/day based on decreased body weight gain (M), food intake and clinical signs (M&F)	Boschman, T. et al.
90-day oral dog (dietary) *	**1967** 0, 100, 300, or 2000 ppm (equal to 0, 7.5, 22.5, or 150 mg/kg/day)	NOAEL = 300 ppm = 22.5 mg/kg bw/day LOAEL = 2000 ppm = 150 mg/kg bw/day based on clinical signs (thin appearance, dull coat, hair loss) and increased liver weight and serum alkaline phosphatase concentrations (F) and clinical signs (thin appearance, dull coat, hair loss) (M) NB:	Walker, A.I.T.
2-year oral rat (dietary) *	**1967** 0, 60, 100, 180, or 500 ppm [equal to 0/0, 2.2/2.8, 3.6/4.7, 6.5/8.5 or 19/25 mg/kg/day (M/F)]	NOAEL = 180 ppm = 6.5 mg/kg bw/day (M) and 8.6 mg/kg bw/day (F) LOAEL = 500 ppm = 17.6 mg/kg bw/day (M) and 21.3 mg/kg bw/day (F) based on decreased body weights and histological liver changes in females	Wheldon, G.H.
	1996	Re-assessment of Liver lesions/tumours	Connick, H.,Crome,S.J. and Gopinath, C.
	1996	Homogeneity/Stability data addendum to report	Johnson, S.F.
	2006	Re-Assessment of liver lesions/tumours – complimentary statistical analysis	Pallen, C.
	2007	Expert opinion on the carcinogenic potential of BAM (2,6-dichlorobenzamide)	Gopinath, C.
	2008	Letter from the conducting laboratory on the carcinogenic potential of BAM (2,6-dichlorobenzamide	Pilling, A.
2-year oral dog (dietary) **	**1971** 0, 60, 100, 180, or 500 ppm (equal to 0, 1.5, 2.5, 4.5, or 12.5 mg/kg/day)	NOAEL = 4.5 mg/kg bw/day LOAEL = 12.5 mg/kg bw/day based on decreased body weight and body weight gain	Wilson, A.B. and Thorpe, E.

3-generation reproduction rat study (dietary) **	**1971** 0, 60, 100, or 180 ppm (equivalent 0, 4.5, 7.5, or 13.5 mg/kg/day)	Parental NOAEL ppm = 7.5 mg/kg bw/day Parental LOAEL was 180 ppm based on reduced body weight and change in relative organ weights. Reproductive NOAEL = 180 ppm (13.5 mg/kg bw/day) based on absence of reproductive effects at 180 ppm Offspring NOAEL = 100 ppm (7.5 mg/kg bw/day) Offspring LOAEL = 180 ppm (13.5 mg/kg bw/day based on reduced offspring body weights .	Hine, C.H., Eisenlord, G. and Loquvam, G.S.
Developmental toxicity oral rabbit (gavage) **	**1986** 0, 10, 30, or 90 mg/kg/day	Maternal NOAEL = 30 mg/kg bw/day Maternal LOAEL= 90 mg/kg bw/day based on increased incidences of clinical signs and decreased body weight gain and food consumption during dosing Developmental NOAEL = 30 mg/kg bw/day Developmental LOAEL = 90 mg/kg bw/day	McIntyre, M.

* = Studies submitted as part of the flupicolide dossier
** = Studies not submitted as part of the fluopicolide EU dossier

B.6.1.4.1 Assessment of Relevance of Groundwater metabolites

In the environmental fate and behaviour assessments Sections B8.9 and B8.10, of the original DAR, a need for an assessment of the relevance of the metabolites M-01, M-05, M-10, M-11, M-12, M-13 and M-14 was identified. These metabolites were either predicted to occur in groundwater at >0.1 µg/l or were found in lysimeter leachate at an annual average concentration >0.1 µg/l.

Further FOCUS groundwater modelling was submitted by the applicant and this was evaluated by the RMS and presented in Section B.8.6.2 of Addendum 1 (November 2007). Following consideration of this FOCUS groundwater modelling, the following metabolites were predicted to have potential to exceed 0.1 µg/l in groundwater: M-01, M-03 (acidic soils), M-05, M-10, M-11, M-12 and M-13 (NB. M-14 was not predicted >0.1 µg/l in the new modelling, but it was >0.1 µg/l in the lysimeter leachate).

Following these findings, a full relevance of metabolites in groundwater assessment following EU Guidance Document - SANCO/221/200-rev 10, 25 February 2003 was presented Addendum 1 (November 2007) for all those metabolites that exceed 0.1 µg/l. This was discussed at PRAPeR 39 (10-13 12.2007) and it was concluded that the only metabolite for which non-relevance was not fully demonstrated was M-01 (BAM). The concern being that that some studies on 2,6-dichlorobenzamide (M-01/BAM) had been considered by the EPA but were not available for consideration at the PRAPeR expert meeting. It was suggested that these could be potentially relevant studies. Hence, as discussed above, a new data gap was identified for the 'Notifier to provide further information on M-01 if deemed necessary'

In order to address this data gap the notifier has a new position paper (Leake et al, 2008, report no. M-300114-01-1), title 'The non-relevance of the fluopicolide metabolite M01 (AE C653711): 2,6-dichlorobenzamide (also known as BAM)'. This position paper is attached at Appendix 3 of this addendum.

The RMS assessment of M-01 is also presented below and follows the step-wise approaches as outlined in the Guidance Document.

STEP 1: EXCLUSION OF DEGRADATION PRODUCTS OF NO CONCERN

All of the metabolites observed in the soil metabolism and lysimeter studies contain either the pyridine ring or the phenyl ring and therefore are not automatically of no concern. In addition there was insufficient information available on their possible natural occurrence and/or of their toxicological or ecotoxicological properties prior to initiating the testing program (See Appendix 1 for chemical structures).

STEP 2: QUANTIFICATION OF POTENTIAL GROUNDWATER CONTAMINATION

As summarised in the original DAR a comprehensive range of studies have been conducted under laboratory, outdoor and field conditions to quantify the potential concentrations in groundwater. Further FOCUS groundwater modelling was also presented in Addendum 1 (November 2007). However, at PRAPeR 37 (3-6.12.2007) the applicant was also requested to submit first Tier standard FOCUS PEARL modelling due to the inclusion of time dependent soil adsorption processes for fluopicolide. The meeting was not content with this particular approach and thus requested repeated modelling using standard input parameters as used in the PELMO modelling. The Applicant repeated the FOCUS PEARL modelling originally conducted and reported in the Addendum 1 (November 2007). The only substantive change is that the soil DT50 and Koc for fluopicolide have been amended to reflect the standard first tier input parameters (i.e. DT50 138.8 days, Koc 32.1 l/kg). Kinetic adsorption assumptions were not used. All other assumptions remained the same from the previous modelling and are detailed in the previous addendum. Full details are presented in detail below at B.8.1 (Data Requirement 4.3).

In comparison with previous assessments, the new modelling only increases the concentrations of two metabolites, M-01 and M-02. M-02 is below 0.1 µg/l and thus does not require assessment. M-01 has increased marginally compared to previous assessments and does not exceed 10 µg/l, a value stated in the Guidance Document on the Relevance of Metabolites in Groundwater, and viewed as an important trigger value.

	Highest concentrations in original DAR	**Highest concentrations in 2007 addendum**	**Highest concentrations in 2008 addendum**
M-01	4.614 (H)	6.733 (H)	**6.743** (H)
M-02	0.033 (P)	0.038 (P)	**0.041** (P)

STEP 3: HAZARD ASSESSMENT -- IDENTIFICATION OF RELEVANT METABOLITES

Progressing to step 3 requires the assessment to be conducted in three stages:

- Stage 1: screening for biological activity
- Stage 2: screening for genotoxicity
- Stage 3: screening for toxicity

STEP 3, Stage 1: screening for biological activity

One of the key stages in the assessment of potential relevance of a metabolite is the determination of biological activity. Many small molecules with molecular weights below 200 can be found to occur naturally in soil as a result of organic matter decomposition. The key distinguishing feature of the metabolites formed from plant protection products is the potential to have biological activity and therefore retain the properties of the xenobiotic.

As stated in the Guidance Document Sanco/221/2000, rev. 10, 25 Feb 2003, the goal is to identify metabolites which have comparable target activity as the parent active ingredient. It also states that efficacy testing should be focused on the question of comparing the activity against the biological target. Included in this assessment is the structure-activity relationship and the necessary functional groups to give the fungicidal activity that is present in the parent fluopicolide (AE C638206) molecule.

The metabolites M-01 (AE C653711), M-02 (AE C657188), M-05 (AE 1344122), M-10 (AE 1344123), M-14 (AE 1388273) and M-15 (AE 1413903) were therefore tested for their fungicidal activity in comparison with the parent AE C638206 (Latorse, M.P., Flahout, J. 2004, C038369) (see Appendix 3 (B.10.7.5, Addendum 1 (November 2007)). The six metabolites did not show any biological activity in comparative tests with the parent fluopicolide, which showed biological effects in the range of 80 -100%.

It is known from the biological screens that both the pyridine and phenyl ring parts of the molecule are required for fungicidal activity therefore the metabolites without both these rings would be predicted to have no fungicidal activity. It is also known that adding functional groups, especially polar ones, to the phenyl ring causes loss of fungicidal activity. Therefore the addition of SO_3H in the case of M-15 or SO_3H and OH in the case of M-16 would result in the loss of fungicidal activity. Of the remaining metabolites that triggered a consideration of biological activity only M-03, M-11, M-12 (mixture of 2 isomers) and M-13 were not tested for fungicidal activity. Three (M-11, M-12 and M-13) are all single pyridine ring structures and are unlikely to have any significant fungicidal activity. M-03 is a structurally-related transient hydroxylated-derivative of fluopicolide and is an unstable intermediate prior to cleavage of fluopicolide to M-01 and M-02. It is very unstable in water and at environmental pH will rapidly degrade to M-01 and M-02 and the RMS considers it inconceivable that significant exposure to M-03 will occur via groundwater.

The RMS concludes that all metabolites theoretically occurring in groundwater >0.1µg/L will not retain or express biological activity of the parent, fluopicolide.

STEP 3, Stage 2: screening for genotoxicity

In the guidance document Sanco/221/2000 rev.l0, there is a requirement that metabolites that have shown some potential to be mobile and are not biologically active should be screened for their genotoxic activity in a series of three in vitro genotoxicity studies. These three study types are the Ames test, gene mutation test with mammalian cells and the chromosome aberration test. The guidance document also states that equivocal results in *in-vitro* studies should be substantiated by *in vivo* experiments.

Metabolite M-01
The genotoxicity profile of M-01 was assessed in three in vitro and one in vivo assays and no evidence of genotoxicity was observed in any assays. The *in vitro* studies were the bacterial gene mutation assay in bacterial cells, V79/HPRT gene locus assay, and unscheduled DNA synthesis (UDS) assay and for *in vivo,* the mouse micronucleus assay. Overall, M-01 is not considered a genotoxic compound. Although no *in vitro* chromosomal aberration study has been performed, the liver UDS assay is considered an acceptable equivalent given the investigation of genotoxic potential in the liver whilst the in vivo assay adequately investigates potential for clastogenicity of M-01.

STEP 3, Stage 3: screening for toxicity

Stage 3 of Step 3 is aimed at the question of whether a metabolite has certain toxicological properties, which - from a regulatory perspective - qualify for considering it "relevant". A metabolite is considered "relevant" if its toxicological properties lead to a classification as toxic or very toxic (T or T+) according to Directive 67/548/EEC. Therefore, in addition to genotoxicity testing, further toxicity testing has been conducted to determine whether the metabolite has certain toxicological properties which from a regulatory perspective would qualify it to be classified as relevant. These studies include metabolism studies to understand the adsorption, distribution, metabolism and elimination from the body.

Metabolite M-01
Following single oral administration of [14C]-M-01 to the male and female rat at the rates of 10 and 150 mg/kg most of the administered radioactivity was eliminated in the urine (ca 82 %dose) although the rate of elimination was relatively slow. Lower levels (ca 13 %dose) were eliminated via the faeces. The highest concentrations in tissues were seen in the kidney (ca 0.57 µg equiv./g) and liver (ca 0.44 µg equiv./g) for the 10mg/kg dose group and in the skin & fur (3.8 to 5.0 µg equiv./g), kidneys (2.8 to 3.0 µg equiv./g) and liver (2.1 to 2.3 µg equiv./g) for the 150 mg/kg dose group. Tissue concentrations therefore increased by approximately five-fold for a fifteen-fold increase in dose rate. Overall, multiple dosing (14 daily doses at 10 mg/kg) did not have any significant impact in the absorption, distribution, metabolism and elimination compared to results after single oral dosing. Thus, the results in this study showed that the routes and the rates of excretion were maintained despite the multiple dosing, which meant that most of the radioactivity was eliminated via the urinary route. The distribution pattern in the tissues was also similar between single and multiple dosing with the highest mean concentrations observed in the skin & fur (3.0 µg equiv./g), kidney (1.9 µg equiv./g) and liver (1.3 µg equiv./g). Bioretention or accumulation was therefore not indicated. The routes of biotransformation was similar between dose

levels and sexes with hydrolysis of the amide group to form AE C416656, hydroxylation to form hydroxy-BAM (M-04) and subsequent conjugation with either glucuronic acid or sulphate, and the loss of a chlorine atom following glutathione conjugation. Further metabolism of the glutathione group to the mercapturic acid or S-methyl metabolites was observed.

M-01 was shown to be of relatively low acute oral toxicity, however the available data indicate that it is of greater acute oral toxicity than fluopicolide (LD50 >5000 mg/kg bw). The LD50 of M-01 was found to be >2000 mg/kg bw in males and >500 mg/kg bw in females in a modern study using the acute toxic class method (OECD 423). However, in an older non-GLP study (performed to OECD 401) LD50 values of 1470 (951–2270) and 2330 (1430–3780) mg/kg bw were calculated for male and female rats respectively. The findings of the two studies are therefore inconsistent, but taken together do indicate that M-01 is of greater acute oral toxicity than fluopicolide, but is not particularly hazardous in absolute terms. M-01 is not classified as toxic and at the maximum levels predicted to occur in groundwater it is not considered relevant for acute toxicity.

In a 13-week toxicity study performed in CD rats with M-01 at doses up to 2300 ppm, reduced body weight gains and food consumption was observed at dose levels of ≥ 600 ppm but no target organ toxicity was observed. The NOAEL of M-01 was 180 ppm (equivalent to 14 mg/kg bw/day) in both males and females.
In comparison, the NOAEL for the fluopicolide 90-day rat study was 100 ppm (equivalent to 7.4 or 8.4 mg/kg bw/d in males and females respectively), based on treatment-related haematological (reduced haemoglobin and haematocrit in male rats), clinical chemistry (increased cholesterol) and urinalysis findings (increased urine volume and specific gravity in females); organ weight changes (increased relative liver and kidney weights in males and relative spleen weight in females) and histopathological changes in the liver and kidneys at the LOAEL of 1400 ppm (equivalent to 109 or 119 mg/kg bw/d in males and females respectively). Findings show that the short-term toxicity of fluopicolide and M-01 is comparable, and therefore that further (long-term) studies with M-01 are not required. In a 2-year dog study with M-01 (BAM), the NOAEL was 4.5 mg/kg bw/day based on effects on body weight gain at the higher dose of 12.5 mg/kg bw/day.

The Notifier has, however, provided a 2-year rat chronic toxicity study performed with M-01. The results of this study indicate that the liver is the target organ of toxicity; a NOAEL of 180 ppm (equivalent to 5.7 and 8.6 mg/kg bw/d in males and females respectively) can be determined. The NOAEL in this study is therefore comparable to the NOAEL of 200 ppm from the rat chronic toxicity/carcinogenicity study performed with fluopicolide, indicating similar long-term toxicity. In the M-01 study, a slight (but not statistically significant) increase in the incidence of hepatocellular adenoma was seen in males at the top dose level of 500 ppm; a dose level considered exceeding the MTD. No evidence of carcinogenicity was seen in this study.

In conclusion, these data show that the toxicological profile of the metabolite M-01 is similar to that of fluopicolide. The relevant NOAEL for the risk assessment of M-01 (BAM) obtained in the 2-year dietary study in dogs does not differ substantial for that derived from long-term toxicity and carcinogenicity in rats and mice for Fluopicolide.

When dose spacing between the NOAELs and LOAELs is taken in to account there is some degree of overlap.

It can be concluded that M-01 (BAM), does not require classification as toxic (T) or very toxic (T+). In addition since the parent is not classified as a reproductive toxicant and is not carcinogenic, there is in principle no reason to require reproductive or carcinogenic testing. Nonetheless, data on carcinogenicity and reproductive toxicity of BAM are available and the assessment reveals that BAM is not classifiable as a carcinogen or reproductive toxicant. It has been confirmed that BAM is not genotoxic. Therefore from a toxicological perspective BAM passes the assessment of Stages 2 and 3 and is considered as a non-relevant metabolite under the rules for the assessment of relevance of groundwater metabolites.

STEP 4: EXPOSURE ASSESSMENT - THRESHOLD OF CONCERN APPROACH

For those metabolites for which the exposure assessment shows they are below the threshold of concern which is given in the Guidance Document as 0.75 µg/L they can be determined to be non relevant at Step 4. However, M-01 is identified as requiring a refined risk assessment as the highest concentration in the DAR or addenda evaluations is predicted to be above 0.75 µg/l.

Based on the proposed ADI for fluopicolide, 0.08 mg/kg bw, a health based drinking water limit of 240µg/L can be proposed. This is based on a 60kg person consuming 2L of water per day and allocating 10% of the ADI to drinking water. Predicted levels of the fluopicolide metabolites are all <5% of the health based drinking water limit for fluopicolide. Therefore the metabolites are not considered to present a concern to human health.

STEP 5: REFINED RISK ASSESSMENTS FOR THE REMAINING METABOLITES

The metabolite M-01has been found to lie in the concentration range between 0.75 µg/L and 10 µg/L. Therefore, a refined risk assessment is presented below:

M-01 was negative in three *in vitro* genotoxicity assays and in an *in vivo* assay for micronuclei induction. M-01 is more acutely toxic than fluopicolide, but in 90 day and 2-year studies in the rat the toxicity of both compounds is considered equivalent, taking account of dose spacing and relative molecular weights. For the dog, the overall NOAEL was 4.5 mg/kg bw/day based on the M-01 had no biological (fungicidal) activity. This NOAEL is of the same order of magnitude as that determined for the rats if allowance is made for dose spacing. It is proposed that an ADI for BAM of 0.045 mg/kg bw/day based on the NOAEL of 4.5 mg/kg bw/day in the 2-year dog study can be set as a basis for the risk assessment of exposures via drinking water and via diet.

For exposure via drinking water, in accordance with Council Directive 97/57/EC, exposure to BAM through the drinking water should account for not more than 10% of

the ADI. If it is assumed that the average daily consumption of water amounts to 2 litre per person of 60 kilogram, a health based drinking water limit of ((60x0.045)/10)/2 mg/l, i.e. 0.14 mg/l (140µg/l) can be established.

Predicted exposures to M-01 from fluopicolide use including drinking water are based on the worst case scenario less that 6% of the proposed ADI for BAM based on the NOAEL in the 2-year dog study

Predicted Dietary exposure to M-01is also expected to be low and would not add significantly to consumer exposure via sources other than drinking water. Therefore M-01(BAM) is not considered to present a concern to human health. (see Table 5 for worst case estimate of dietary exposure and exposure via drinking water).

Table 5: NEDIs of BAM

Commodity	Residue (mg/kg)	NEDI for Adult (mg/kg bw/day)	NEDI for Infant (mg/kg bw/day)	NEDI for Toddlers (mg/kg bw/day)	NEDI for Children Years 4-6 (mg/kg bw/day)	NEDI for Children Years 7-10 (mg/kg bw/day)	NEDI for Children Years 11-14 (mg/kg bw/day)	NEDI for Children Years 15-18 (mg/kg bw/day)	NEDI for Vegeta-rian (mg/kg bw/day)	NEDI for Elderly (Own home) (mg/kg bw/day)	NEDI for Elderly (Reside ntial) (mg/kg bw/day)
Potato	0.01	0.00004	0.00011	0.00009	0.00008	0.00007	0.00005	0.00005	0.00004	0.00003	0.00003
Grape-table	0.02	0.00003	0.00003	0.00009	0.00004	0.00005	0.00002	0.00001	0.00004	0.00003	0.00001
Wine*	0.01*	0.00006	L/C	L/C	L/C	L/C	0.00001	0.00002	0.00006	0.00004	0.00001
Cabbage	0.01	0.00001	0.00002	0.00002	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
Wheat	0.01	0.00004	0.00003	0.00008	0.00009	0.00007	0.00005	0.00004	0.00004	0.00003	0.00003
Barley	0.01	L/C	L/C	L/C	L/C	0.00001	L/C	L/C	L/C	L/C	L/C
Oats	0.01	L/C	0.00002	0.00001	0.00001	L/C	L/C	0.00001	0.00001	0.00001	0.00001
Water**	0.01#	0.00026	0.0023	0.0014	0.00098	0.00065	0.00042	0.00031	0.0003	0.00028	0.00032

*the STMR from the grapes trials was 0.02, applying a processing factor of 0.5 gives a residue of 0.01 mg/kg in wine

**Water consumption taken to be 2 litres for each consumer group

L/C = Low Consumption

Rye – no consumption data were available, however based on the WHO intake figures, consumption of rye would be considerably lower than wheat.
= 10µg/l (worse case than highest concentration of 6.743 µg/l)

The NEDIs for grape, wine, potato, cabbage, wheat, barley and oats (and drinking water) are all below (less than 6%) the ADI of 0.045 mg/kg bw/day.

The total NEDIs from the combined consumption of all raw commodities have been calculated using the Rees/Day model and are presented below:

Consumer groups	Total NEDI (mg/kg bw/day)
ADULT	0.00039
INFANT	0.0025
TODDLER	0.0016
CHILDREN (Years 4-6)	0.0012
CHILDREN (Years 7-10)	0.00079
CHILDREN (Years 11-14)	0.00053
CHILDREN (Years 15-18)	0.00043
VEGETARIAN	0.00039
ELDERLY (FREE LIVING)	0.00037
ELDERLY (INSTITUTIONAL)	0.00039

The total NEDIs for adults, children, toddlers, infants, vegetarians and the elderly are all well below (less than 6%) the ADI of 0.045 mg/kg bw/day.

CONCLUSION

To conclude, the M-01 metabolite meets the criteria of the guidance document and is considered to be toxicologically non-relevant in groundwater. In addition, the ecotoxocological assessment (see Section B.9.2, Addendum 1 (November 2007)) also concludes that M-01 can be considered environmentally 'non-relevant'.

B.6.16 Additional References Relied On:

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Data protect. claimed	Owner
AII 5.8.1.1 /18	Leake, C. R.; Mackenzie, E.; Kley, C.; Mallyon, B.; Renault, D.; Pross, S.; Theurig, M.	2008	The non-relevance of the fluopicolide metabolite M01 (AE C653711): 2,6-dichlorobenzamide (also known as BAM) BCS, Report No.: M-300114-01-1, Edition Number: M-300114-01-1 Date: 2008-04-11 Non GLP, unpublished	Yes	BCS
AII 5.8.1.1 /19	Pilling, A.	2008	Effects of BAM (2,6-dichlorobenzamide) in dietary administration to rats for 2 years (3980/71/138; CA-515-1) [redacted] United Kingdom Bayer CropScience, Report No.: M-299662-01-1, Edition Number: M-299662-01-1 Date: 2008-03-11 Non GLP, unpublished	Yes	BCS
AII 5.8.1.1 /20	Walker, A. I. T.	1967	The study of the oral toxicity of the "prefix" residue 2,6-dichlorobenzamide: 13 week exposure to dogs [redacted] United Kingdom BCS, Chemtura, Report No.: M-311805-01-1, Edition Number: M-311805-01-1 Date: 1967-02-28 Non GLP, unpublished	Yes	BCS, Chem-tura

AII 5.8.1.1 /21	Wilson, A. B.; Thorpe, E.	1971	Toxicity studies on the Prefix residue 2,6-dichlorobenzamide: two year oral experiment with dogs [redacted] United Kingdom Chemtura, Report No.: M-301179-01-1, Edition Number: M-301179-01-1 Date: 1971-09-30 Non GLP, unpublished	Yes	BCS, Chem-tura
AII 5.8.1.1 /22	McIntyre, M.	1993	2,6-dichlorobenzamide: oral (gavage) teratology study in the rabbit [redacted] United Kindom Chemtura, Report No.: CA-2615, Edition Number: M-301030-01-1 Date: 1993-11-02 Non GLP, unpublished	Yes	BCS, Chem-tura
AII 5.8.1.1 /23	Hine, C. H.; Eisenlord, G.; Loquvam, G. S.	1993	Results of reproduction study of rats fed diets containing 2,6-dichlorobenzamide (BAM) over three generations [redacted] Chemtura, Report No.: CA-512-3, Edition Number: M-301025-01-1 Date: 1993-09-02 Non GLP, unpublished	Yes	BCS, Chem-tura

B.8 ENVIRONMENTAL FATE AND BEHAVIOUR

B.8.1 Route and rate of degradation in soil

Data requirement 4.1
"Calculation of $DT50_{photolysis}$ for adequate latitudes in Europe."

To recap, questions were originally raised on the significance of soil photolysis and the Notifier was requested to provide estimates of soil photolysis at other latitudes, e.g. 40ºN and 45ºN. The Notifier submitted a very complex assessment, described in a previously submitted addendum which was considered at the PRAPeR 37 meeting. Experts at PRAPeR 37 were not convinced by the Notifiers arguments, particularly noting the apparent contradictions that light energy in the laboratory soil photolysis studies were claimed to be equivalent to those at the location in Scotland, but also that these light levels were higher than those expected in other Southern EU locations; a summary of the discussions can be found in the report of the PRAPeR 37 meeting. This prompted the re-setting of the data requirement.

The Notifier has responded by submitting a position paper on soil photolysis.

RMS evaluation of new information:

The major part of the Notifier's case rests on comparison of light energy output in the soil photolysis studies reported in the DAR with published data on environmental light levels in Europe. Key to this comparison is the need (according to the Notifier) to adjust the measured light levels in the soil photolysis studies to account for differences in the wavelengths over which measurements were taken. Light energy in the soil photolysis studies was measured over the wavelength range 290-800 nm, however, measured natural light levels were measured over a range of 300 – 3000 nm, and thus the two sets of information cannot be compared directly.

To correct for this difference in measured wavelength ranges, the Notifier cited published information on the distribution of light intensity in a standard natural light spectrum (Commission Internationale de L'Eclairage, International Commission on Illumination, Publication No. 20, TC 2.2, 1972; RMS notes that this publication is now withdrawn and replaced by a new version, TC 2.17, published in 1989). Within the range of 280 – 3000 nm, it was stated that wavelengths 280 – 400 nm accounted for 6.1% of intensity and the range 400 – 800 nm accounted for 51.8% of intensity. Thus, the range 280 – 800 nm accounts for 57.9% of intensity. Using this value, the light energy output of the lamps used in the soil photolysis tests was extrapolated to the wider range of 300 – 3000 nm. The average hourly light energy emitted by the photolysis equipment was 456 W/m^2 in the range 280 – 800 nm, and the Notifier calculated that this was equivalent to an output of 787.6 W/m^2 in the range 300 – 3000 nm.

In addition, whilst the light energy in this study was expressed as W/m^2 for an hour (W.h/m^2), published values of sunlight energy tend to be expressed in MJ/m^2, thus an additional conversion is required.

1 kilowatt hour = 3.6 MJ

Light energy, 300–3000 nm range=787.6 W.h/m2 = 0.7876 kW.h/m2 = 2.83 MJ/m^2

Over the course of 24 hours continuous illumination, this is 68 MJ/m^2

The Notifier cited global radiation values from two sources, the 'Handbook of Material Weathering, 2nd Edition' (Wypych, 1995, Chemtec Publishing) and the FOCUS groundwater scenarios report. These are shown below in Table B.8.1.

Table B.8.1 Examples for Global Sunlight Irradiation (300-3000 nm)

Location	Latitude	Period/Month	Global radiation for 1 day (MJ*m^{-2})
Chateaudun	48.08 N	June	18.6
Kremsmunster	48.03 N	June	18.5
Piacenza	44.92 N	June	22.4
Porto	41.23 N	June	22.6
Thiva	38.32 N	June	23.8
Seville	37.42 N	June	25.8
Dundee (UK)	56.26 N	June	17
London (UK)	51.31 N	June	16
Vienna (Austria)	48.14N	June	19
Zurich (CH)	47.23 N	June	18
Athens (Greece)	38.03 N	June	20
Tunis (Tunisia)	36.80 N	June	27

It should be recalled that the light energy output in the photolysis studies was considered to be equivalent to that in Scotland on a sunny summers day. Notifier calls this into question due to comparison with published data shown in Table B.8.1 above. In addition, solar irradiation data for Tranent, Scotland (the location of the soil photolysis studies) was obtained from the MARS database for the 10 year period 1992 – 2002. The average irradiation for June during this 10 year period was approx. 17 MJ/m^2, however, the maximum was approx 32 MJ/m^2. Judging from the 10 year dataset, this value appears to be exceptional, which suggests that whilst the natural measured conditions described for Tranent might have been equivalent to the Suntest equipment output, this would have been a highly unusual event and not typical of summer sunlight conditions at this location. The Notifier also sought the opinion of the manufacturer of the light sensor used in this study. The manufacturer has stated that the specific sensor cited is only suitable for use in 'Suntest' equipment, and is not suitable as an outdoor light sensor.

The RMS considers that this is a reasonable argument, but may call into question any other studies which have relied upon the same argument by the original study authors that output of the 'Suntest' equipment was equivalent to natural light levels at Tranent,

Scotland. From the data submitted, this claim would appear to have been based on a day of exceptional solar irradiation for that location.

In order to calculate environmental soil photolysis DT50 values, the Notifier used the global irradiation values in Table B.8.1, the mean light energy output in each study and both Notifier and RMS DT50 values as presented in the DAR in Tables B.8.60 and B.8.63.

The following shows an example of environmental soil photolysis DT50 calculation:

Mean measured light intensity at 300-800 nm = 442W*m^{-2} (Report 18768, Kiers & Lowrie, 2001).

Calculated light intensity at 300-3000 nm = 442W*m^{-2}/57.9% * 100% = 763 W*m^{-2} where 57.9% of the light intensity of 300-3000 nm falls within the wavelengths 300-800 nm (see section 2.1.1 for details on the distribution of light intensity for a standard spectrum).

Radiation (300-3000 nm) of a Suntest Unit in 1 hour (1 hour = 3600 s)

442W*m^{-2}/57.9% * 100% * 3600s =	2,748,187 W*s*m^{-2}
(W*s = J)	2,748,187 J*m^{-2}
(M = 1,000,000)	2.75 MJ*m^{-2} per hour

Global Radiation (300-3000 nm) for London, UK, Latitude 51.31 N in June = 16 MJ*m^{-2} per day (Chemtec, 1995)

Number of hours in suntest equivalent to I day at London = 16 MJ* m^{-2} / 2.75 MJ* m^{-2} = 5.82 hours

Environmental DT50 (based on experimental DT50 = 86 days, RMS calculation in DAR)
= ((24 hours x 86 days) / 5.82 hours) = 355 days.

Environmental DT50 (based on experimental DT50 = 48 days, Notifier calculation in DAR) = ((24 hours x 48 days) / 5.82 hours) = 198 days.

The following figures give details for calculations based on the Kiers and Lowrie 2001a study (Figure B.8.1) and the Mackie 1999a study (Figure B.8.2).

Figure B.8.1 Calculation of environmental soil photolysis DT50 values from Kiers and Lowrie 2001a soil photolysis study

Calculation of Environmental Days Based on Light Intensity of an Artificial Light Source

Light intensity of a Suntest unit measured at 300-800 nm (radiometer):
Mean during the main test (App. 7): 442 W*m^{-2}

Light intensity of an artificial light source (calc. for 300-3000 nm):
58% of the light intensity 300-3000 nm falls in the range of 300-800 nm *
(442 W*m^{-2} / 57.9% * 100%) 763 W*m^{-2}

Radiation (300-3000 nm) of a Suntest unit during 1 hour (1h = 3600 s): 2,748,187 W*s*m^{-2}
(W*s = J) 2,748,187 J*m^{-2}
(M = 1,000,000) 2.75 MJ*m^{-2}

Examples for Global Radiation (300-3000 nm):

Location	Latitude	Period/ Month	Global radiation 1 day (MJ*m^{-2})
Châteaudun	48.08 N	June	18.6
Kremsmünster	48.03 N	June	18.5
Piacenza	44.92 N	June	22.4
Porto	41.23 N	June	22.6
Thiva	38.32 N	June	23.8
Sevilla	37.42 N	June	25.8
Dundee (UK)	56.26 N	June	17
London (UK)	51.31 N	June	16
Vienna (Austria)	48.14 N	June	19
Zürich (CH)	47.23 N	June	18
Athens, Greece (EU)	38.03 N	June	20
Tunis (Tunesia)	36.80 N	June	27

Irradiation Time (Suntest Unit) Transferred to Environmental Days at Specimen Locations

Location	Suntest (hours)	Environm. (days)	DT50 Fluopicolide [environmental days] *	DT50 Fluopicolide [environmental days] **
Châteaudun	6.76	1	170	305
Kremsmünster	6.73	1	171	307
Piacenza	8.15	1	141	253
Porto	8.22	1	140	251
Thiva	8.66	1	133	238
Sevilla	9.39	1	123	220
Dundee (UK)	6.19	1	186	334
London (UK)	5.82	1	198	355
Vienna (Austria)	6.91	1	167	299
Zürich (CH)	6.55	1	176	315
Athens, Greece (EU)	7.28	1	158	284
Tunis (Tunesia)	9.82	1	117	210
			* Notifier calc. 48 days	** RMS calc. in the DAR 86 days

Figure B.8.2 Calculation of environmental soil photolysis DT50 values from Mackie 1999a soil photolysis study

Calculation of Environmental Days Based on Light Intensity of an Artificial Light Source

Light intensity of a Suntest unit measured at 300-800 nm (radiometer):
Mean during the main test (App. 7): 452 W*m^{-2}

Light intensity of an artificial light source (calc. for 300-3000 nm):
58% of the light intensity 300-3000 nm falls in the range of 300-800 nm *
(452 W*m^{-2} / 57.9% * 100%) 781 W*m^{-2}

Radiation (300-3000 nm) of a Suntest unit during 1 hour (1h = 3600 s): 2,810,363 W*s*m^{-2}
(W*s = J) 2,810,363 J*m^{-2}
(M = 1,000,000) 2.81 MJ*m^{-2}

Examples for Global Radiation (300-3000 nm):

Location	Latitude	Period/ Month	Global radiation 1 day (MJ*m^{-2})
Châteaudun	48.08 N	June	18.6
Kremsmünster	48.03 N	June	18.5
Piacenza	44.92 N	June	22.4
Porto	41.23 N	June	22.6
Thiva	38.32 N	June	23.8
Sevilla	37.42 N	June	25.8
Dundee (UK)	56.26 N	June	17
London (UK)	51.31 N	June	16
Vienna (Austria)	48.14 N	June	19
Zürich (CH)	47.23 N	June	18
Athens, Greece (EU)	38.03 N	June	20
Tunis (Tunesia)	36.80 N	June	27

Irradiation Time (Suntest Unit) Transferred to Environmental Days at Specimen Locations

Location	Suntest (hours)	Environm. (June days)	DT50 Fluopicolide [environmental days] *	DT50 Fluopicolide [environmental days] **
Châteaudun	6.61	1	123	131
Kremsmünster	6.58	1	124	131
Piacenza	7.97	1	102	108
Porto	8.04	1	101	107
Thiva	8.47	1	96	102
Sevilla	9.18	1	89	94
Dundee (UK)	6.05	1	135	143
London (UK)	5.69	1	143	152
Vienna (Austria)	6.76	1	121	128
Zürich (CH)	6.40	1	127	135
Athens, Greece (EU)	7.12	1	115	121
Tunis (Tunesia)	9.61	1	85	90
			* Notifier calc. 34 days	** RMS calc. in the DAR 36 days

Based on the values above, the Notifier concludes that irrespective of location, degradation of fluopicolide is only slightly enhanced by soil photolytic processes.

The RMS considers that the basis of the comparison of experimental light energy with natural light levels is acceptable; it seems appropriate to the RMS that the experimental light energy should be corrected for a wider wavelength range when levels of natural light energy quoted are from the wider range of wavelengths. Additionally, the RMS

considers the resultant environmental soil photolytic DT50 to be reasonable based on average conditions.

(Hellpointer and Stupp, 2008)

Open point 4.21

"RMS to include in the LoEP the values from HS fitting presented in the addendum."

Hockey Stick values as presented in the addendum of November 2007 (Open Points 4.8 and 4.1 only) have now been incorporated into the List of End Points.

Open point 4.22

"RMS to include the non normalised SFO DT50 values for parent used for their calculation of the accumulated PECsoil in the/a table in the LoEP."

These values have now been incorporated into the List of End Points. The RMS also wishes to state that in Volume 3, Section B.8.1.5(a), the RMS calculated DT50 for fluopicolide of 133 days for the Rodelsee site is incorrect and should read **253 days (r^2 = 0.818) for 0-20cm depth**. The RMS apologises for this mistake.

Open point 4.23

"RMS to either recalculate the PEC soil for M01 and M02 or include a note what is the agreed value for formation percentage of M01and M02 in field."

Following the PRAPeR 37 meeting, it is clarified that the maximum observed formation levels of metabolites are:

Table B.8.2 Maximum observed formation of fluopicolide metabolites M01 and M02 in field dissipation studies

	% molar basis (adjusted for molecular weight)	% wt/wt	Study location
M01	24.1	11.9	Senas (1999)
M02	16.3	9.6	Senas (1999)

Formation % from the Senas 2000 field site shown in Table B.8.145 of the DAR should be excluded from consideration. The RMS PECsoil calculations for M01 in vines have been amended in the List of End Points to reflect the revised observed formation rates. Amendment of PECsoil for M02 is not needed as this has already been conducted with the correct values as shown in Table B.8.2 above.

Data requirement 4.3

"The applicant is requested to submit a first Tier standard FOCUS PEARL modelling.

However the data requirement may be re-classified as point of clarification by the applicant since the information required is limited to standard modelling recalculation

using agreed input parameters. Alternatively the calculation may be provided directly by the RMS."

To recap, in response to MS and EFSA comments, the Notifier had submitted new FOCUS PELMO and FOCUS PEARL modelling. PEARL modelling was a particular object of scrutiny by PRAPeR 37 due to the inclusion of time dependent soil adsorption processes for fluopicolide. The meeting was not content with this particular approach and thus requested repeated modelling using standard input parameters as used in the PELMO modelling. New modelling will have potential implications for not only predicted a.s. concentrations in groundwater, but also on metabolite concentrations.

The Notifier has responded by submitted two FOCUS PEARL modelling studies, one to address PECgw under the potato GAP, and one to address the vines GAP. It has been noted that the reports include reference to PEARL modelling with and without time dependent sorption processes, and PELMO modelling. Given that the data requirement was set only for standard PEARL modelling, PEARL modelling with time dependent sorption processes and PELMO modelling have not been reported here.

RMS evaluation of new information:

The Notifier repeated the FOCUS PEARL modelling originally conducted and reported in the Addendum 1, November 2007. The only substantive change is that the soil DT50 and Koc for fluopicolide have been amended to reflect the standard first tier input parameters (i.e. DT50 138.8 days, Koc 32.1 l/kg). Kinetic adsorption assumptions were not used. All other assumptions remained the same from the previous modelling and are detailed in the previous addendum.

Table B.8.3 Predicted 80th percentile average groundwater concentrations of fluopicolide and metabolites in **potatoes** at 1 m depth (4 • 100 g/ha, 2 • 50 + 2 • 80 % int., 5 d interval, application **every year**; FOCUS PEARL, no sorption kinetic)

Scenario				Annual	PEC$_{gw}$	in µg/L				
	Fluo-picolide	M-03, AE 0608000	M-01, AE C653711	M-02, AE C657188	M-05, AE 1344122	M-14, AE 1388273	M-11, P2a	M-12, P2b	M-13, P3	M-10, AE 1344123
Châteaudun	0.106	< 0.001	5.371	0.007	0.504	0.024	0.207	0.138	0.068	0.271
Hamburg [a]	0.119	0.477	6.743	0.033	0.749	0.035	0.408	0.272	0.202	0.492
Jokioinen [a]	0.003	0.183	5.684	0.011	0.518	0.023	0.502	0.335	0.272	0.486
Krems-münster	0.094	< 0.001	4.992	0.006	0.461	0.022	0.227	0.151	0.082	0.270
Okehamp-ton [a]	0.123	0.463	5.460	0.030	0.625	0.029	0.246	0.164	0.114	0.343
Piacenza	0.575	< 0.001	4.957	0.034	0.572	0.026	0.164	0.110	0.081	0.275
Porto [a]	< 0.001	0.014	1.641	< 0.001	0.092	0.004	0.120	0.080	0.047	0.096
Sevilla	0.003	< 0.001	3.611	< 0.001	0.186	0.008	0.090	0.060	0.025	0.099
Thiva	0.073	< 0.001	4.205	0.005	0.299	0.014	0.110	0.073	0.025	0.125

[a] acidic soil, corresponding metabolism pathway used

Table B.8.4 Predicted 80th percentile average groundwater concentrations of fluopicolide and metabolites in **potatoes** at 1 m depth (4 • 100 g/ha, 2 • 50 + 2 • 80 % int., 5 d interval, application **every two years**; FOCUS PEARL, no sorption kinetic)

Scenario	Annual PEC_{gw} in µg/L									
	Fluo-picolide	M-03, AE 0608000	M-01, AE C653711	M-02, AE C657188	M-05, AE 1344122	M-14, AE 1388273	M-11, P2a	M-12, P2b	M-13, P3	M-10, AE 1344123
Châteaudun	0.038	< 0.001	2.557	0.003	0.228	0.011	0.103	0.069	0.032	0.126
Hamburg [a]	0.044	0.231	3.236	0.012	0.333	0.015	0.189	0.126	0.096	0.233
Jokioinen [a]	0.001	0.081	2.645	0.004	0.225	0.010	0.239	0.159	0.127	0.217
Krems-münster	0.034	< 0.001	2.394	0.002	0.212	0.010	0.114	0.076	0.040	0.125
Okehamp-ton [a]	0.046	0.219	2.598	0.013	0.289	0.013	0.117	0.078	0.058	0.168
Piacenza	**0.249**	< 0.001	2.321	0.014	0.293	0.013	0.078	0.052	0.040	0.134
Porto [a]	< 0.001	0.006	0.727	< 0.001	0.035	0.001	0.061	0.041	0.023	0.043
Sevilla	0.001	< 0.001	1.629	< 0.001	0.081	0.004	0.049	0.033	0.015	0.052
Thiva	0.025	< 0.001	2.047	0.002	0.132	0.006	0.056	0.037	0.011	0.059

[a] acid soil, corresponding metabolism pathway used

Table B.8.5 Predicted 80th percentile average groundwater concentrations of fluopicolide and metabolites in **potatoes** at 1 m depth (4 • 100 g/ha, 2 • 50 + 2 • 80 % int., 5 d interval, application **every three years**; FOCUS PEARL, no sorption kinetic)

Scenario	Annual PEC_{gw} in µg/L									
	Fluo-picolide	M-03, AE 0608000	M-01, AE C653711	M-02, AE C657188	M-05, AE 1344122	M-14, AE 1388273	M-11, P2a	M-12, P2b	M-13, P3	M-10, AE 1344123
Châteaudun	0.022	< 0.001	1.697	0.001	0.147	0.007	0.069	0.046	0.022	0.086
Hamburg [a]	0.024	0.143	2.133	0.007	0.224	0.010	0.118	0.079	0.064	0.151
Jokioinen [a]	0.001	0.049	1.634	0.002	0.132	0.006	0.145	0.097	0.084	0.134
Krems-münster	0.018	< 0.001	1.610	0.001	0.134	0.006	0.079	0.053	0.025	0.081
Okehamp-ton [a]	0.027	0.141	1.731	0.008	0.185	0.008	0.077	0.051	0.038	0.108
Piacenza	**0.151**	< 0.001	1.610	0.009	0.195	0.009	0.053	0.036	0.027	0.091
Porto [a]	< 0.001	0.003	0.454	< 0.001	0.020	0.001	0.039	0.026	0.015	0.025
Sevilla	< 0.001	< 0.001	0.699	< 0.001	0.051	0.002	0.035	0.023	0.009	0.033
Thiva	0.016	< 0.001	1.367	0.001	0.088	0.004	0.040	0.027	0.008	0.039

[a] acidic soil, corresponding metabolism pathway used

Table B.8.6 Predicted 80th percentile average groundwater concentrations of fluopicolide and metabolites in **vines** at 1 m depth (3 • 133 g/ha, 60 + 2 • 70 % int., 10 d interval, every year; FOCUS PEARL, no sorption kinetic)

Scenario	Annual PEC$_{gw}$ in µg/L									
	Fluo-picolide	M-03, AE 0608000	M-01, AE C653711	M-02, AE C657188	M-05, AE 1344122	M-14, AE 1388273	M-11, P2a	M-12, P2b	M-13, P3	M-10, AE 1344123
Châteaudun	0.231	0.001	5.172	0.017	0.580	0.027	0.216	0.144	0.089	0.307
Hamburg [a]	0.146	0.517	6.075	0.035	0.723	0.033	0.348	0.232	0.184	0.446
Krems-münster	0.123	< 0.001	4.599	0.008	0.463	0.022	0.215	0.143	0.083	0.267
Piacenza	**0.678**	< 0.001	4.549	0.041	0.604	0.027	0.166	0.110	0.086	0.283
Porto [a]	< 0.001	0.017	1.626	0.001	0.091	0.004	0.121	0.081	0.045	0.096
Sevilla	0.124	< 0.001	3.876	0.012	0.386	0.017	0.141	0.094	0.049	0.192
Thiva	0.220	< 0.001	4.056	0.034	0.395	0.018	0.132	0.088	0.040	0.180

[a] acidic soil, corresponding metabolism pathway used

(Kley & Ellerich, 2008a & b)

In comparison with the previous assessments in the 2007 addendum, fluopicolide concentrations have increased using the first tier PEARL modelling. For use on vines, the 80th percentile annual average concentration at 1m depth exceeds 0.1 µg/l at 6 out of 7 scenarios. Only the Porto scenario passes. In the previous assessments, only 2 out of 7 scenarios failed. For use on potatoes, with the most extreme rotational regime of use every year (which the RMS considers to be extreme), 4 out of 9 scenarios fail (Chateaudun, Hamburg, Okehampton and Piacenza), whilst with more realistic use regimes of application every other year or every third year, one scenario (Piacenza) fails. It is clear that, if the new modelling is deemed acceptable on peer review and leads to Annex I listing, that for National authorisations, MS will have to pay attention to the risk of groundwater contamination posed by use of fluopicolide, particularly from use on vines.

The situation is less clear for metabolites, some increasing in concentration and some decreasing in concentration compared to previous assessments. A comparison of results from this assessment and previous assessments is shown in the following table.

Table B.8.7 Comparison of highest metabolite groundwater PEC values from original DAR, 2007 addendum and this addendum for regulatory decision-making (µg/l)

	Highest concentrations in original DAR	Highest concentrations in 2007 addendum	Highest concentrations in 2008 addendum
M-03	0.381 (H)	**0.525** (H)	0.517 (H)
M-01	4.614 (H)	6.733 (H)	**6.743** (H)
M-02	0.033 (P)	0.038 (P)	**0.041** (P)
M-05	**0.90** (L)	0.715 (H)	0.749 (H)
M-14	**0.19** (L)	0.033 (H)	0.035 (H)
M-11	0.55 (L)	**0.813** (J)	0.502 (J)
M-12	0.36 (L)	**0.542** (J)	0.335 (J)
M-13	0.160 (H)	**0.369** (J)	0.272 (J)
M-10	**0.83** (L)	0.586 (H)	0.492 (J)

Values in bold are the highest values

In comparison with previous assessments, the new modelling only increases the concentrations of two metabolites, M-01 and M-02. M-02 is below 0.1 µg/l and thus does not require assessment. M-01 has increased marginally compared to previous assessments and does not exceed 10 µg/l, a value stated in the Guidance Document on the Relevance of Metabolites in Groundwater, and viewed as an important trigger value. This new modelling does not change the quantity of any metabolite such that it triggers additional non-relevance testing.

Data requirement 4.4

*"Data requirement (*for additional groundwater modelling*) fulfilled for PELMO. For PEARL: see data requirement 4.3."*

Please see date requirement 4.3 above for the results of FOCUS PEARL modelling using agreed input parameters.

Open Point 4.18

"RMS to indicate in the LoEP box "relevant metabolites" in soil the max. amount of M02 (with respect to applied fluopicolide) found in field studies (at this stage this value is 21.3 %)."

For clarification of the maximum amount of M02 in field studies, please see Open Point 4.23 above; the maximum amount is 16.3% on molar basis. The List of End Points has been amended.

Open Point 4.24

"RMS to amend the list of end points according to the discussions during the PRAPeR 37 meeting."

The amendments required to the List of End Points have been implemented.

B.8.10 Additional References Relied On:

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Data protect. claimed	Owner
AIIIa 9.1.3 /03	Kley, C.; Ellerich, C.	2008	Predicted environmental concentrations in groundwater (PECgw) for fluopicolide and its metabolites calculated with FOCUS PEARL and FOCUS PELMO - Use in vines in Europe BCS, Report No.: MEF-08/155, Edition Number: M-299231-01-1 Date: 2008-03-13 Non GLP, unpublished	Yes	BCS
AIIIb 9.1.3 /03	Kley, C.; Ellerich, C.	2008	Predicted environmental concentrations in groundwater (PECgw) for fluopicolide and its metabolites calculated with FOCUS PEARL and FOCUS PELMO - Use in potatoes in Europe BCS, Report No.: MEF-08/154, Edition Number: M-299223-01-1 Date: 2008-03-13 Non GLP, unpublished	Yes	BCS
DOC K b /09	Hellpointner, E.; Stupp, H. P.	2008	Statement (Version 2) - The light intensity measured during the studies on phototransformation of fluopicolide on soil surfaces and the transfer of experimental results to environmental phototransformation half-lives BCS, Report No.: M-300764-02-1, Edition Number: M-300764-02-1 Date: 2008-09-22 Non GLP, unpublished	Yes	BCS

B.9 ECOTOXICOLOGY

New open point 5.13:
RMS to include a note in the LoEP for the long-term risk assessment for herbivours mammals with the explanations, that the current risk assessment of mammals covers only one out of three applications in vineyards during early growth stages (up to BBCH 57).

New open point 5.14:
RMS to revise LoEP with correct short-term bird endpoint.

The RMS has amended the List of Endpoints as requested.

Open point 5.5
RMS to include the information and argumentation regarding the ecotoxicological relevance of GW metabolites presented in column 3 in an addendum for the sake of completeness.

We agree that since the TER for M05 is >18519 (vine) and >58824 (potato) for algae and this metabolite is the one of highest concentration in the FOCUSgw modelling, apart from M01, the risk from M10, M11, M12 and M13 to aquatic organisms can be considered to be low. The information presented is however of value for the assessment of "pesticidal activity".

No discussion in an experts meeting is required.
See reporting table 5(27).

The RMS has reassessed the aquatic risk posed by groundwater metabolites formed >0.1ug/L using revised PECgw values (see Addendum 2, 2008) and included a table in the LOEPs. No risk to aquatic organisms is indicated. Other conclusions with respect to biological activity of the metabolites and the overall absence of relevance of fluopicolide GW metabolites from an ecotoxicological perspective remain as presented in Addendum 1, 2007.

Open point 5.6
RMS to correct the list of endpoint with exact %-age effect on fecundity instead of <50%. Note that highest conc. with effects <50% for A. rhopalosiphi was 2 L/ha
See reporting table 5(38).

The RMS has amended the List of Endpoints as requested.

Open point 5.7
RMS to update the list of endpoints for earthworms. It is still not clear if the values for the formulation are based on a.s. or formulation concentrations. Furthermore, values should be given as mg/kg DS.

Corrected calculations should be included in a corrigendum.
See also the comment from the applicant on the reporting table to be discussed in an experts meeting.
See reporting table 5(39).

The soil macroorganism LOEPs has been revised and are expressed as mg/kg d.wt. soil and to clarify where correction for logPow and soil organic matter is appropriate. Some TERs have been also been amended (see Open pt. 5.8). No change in low risk conclusion.

Data gap5.1 - identified at PRAPeR 38:
'Notifier to address the ecotoxicological relevance of toluene in the technical material'.

The RMS has considered the case proposed the Notifier (Pross, 2008). Ecotoxicological testing was undertaken using fluopicolide technical material (batches OP2050046, OP2050190, OP2350005, R001737, OP20500045) containing 0.1-0.4% w/w toluene (AEF125577) (see DAR Vol 4,Table C.1). Therefore the ecotoxicological risk assessment for technical fluopicolide essentially encompasses the risk from toluene in technical material (max. <0.5%w/w pilot plant; <0.3%w/w manufacturing plant – Volume 4, Addendum 2, C 2.2). Furthermore, the ecotoxicological profile of "pure" toluene shows it not to be more toxic than fluopicolide technical. A risk assessment using worse case toluene PECsoil (0.0009 mg/kg) and PECsw (0.000046 mg/L) initial values based on theoretical toluene content in fluopicolide PECs generate respective TERs of 16667, 16087 and 76087 with worse toxic toluene endpoints for worm(28dNOEC=15 mg/kg d.wt soil), Daphnia (96hEC50=3.5 mg/L) and Ceriodaphnia (7dNOEC=0.74 mg/L). The TERs clearly exceed relevant Annex VI EU 91/414 thresholds indicating low risk. Toluene also has low bioaccumulation potential (BCF=90).

Thus all evidence indicates that environmental toluene derived from fluopicolide technical use in PPPs will not cause concern from an ecotoxicological perspective.

B.9.13 Additional References Relied On:

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Data protect. claimed	Owner
DOC K b /10	Pross, S.	2008	Ecotoxicological relevance of toluene as impurity in Fluopicolide technical material BCS, Report No.: M-300968-01-1, Edition Number: M-300968-01-1 Date: 2008-04-23 Non GLP, unpublished	Yes	BCS

APPENDIX 1

Summary of the significant metabolites of fluopicolide identified in studies in animals, plants and the environment

M-Code number (Company code number)	Other identifiers	Structure	Formula	Presence in metabolism studies
AE C638206	Fluopicolide (parent)	F_3C, Cl, Cl, NH, N, O, Cl	2,6-dichloro-N-{[3-chloro-5-(trifluoromethyl)-2-pyridinyl]methyl}benzamide $C_{14}H_8Cl_3F_3N_2O$ MW = 383.59	
M-01 (AEC653711)	BAM	Cl, H_2N, O, Cl	2,6-dichlorobenzamide $C_7H_5Cl_2NO$ MW = 190.0	rat liver, laying hen, crop, soil, lysimeter leachate, rotational crop
M-02 (AEC657188)	PCA UMET/2	F_3C, Cl, N, OH, O	3-chloro-5-trifluoromethyl-pyridine-2-carboxylic acid $C_7H_3ClF_3NO_2$ MW = 225.6	rat, crop, rotational crop, soil, water
M-05 (AE 1344122)	P1x RPA433497	F_3C, $SOCH_3$, N, COOH	3-methylsulfinyl-5-trifluoro-methylpyridine-2-carboxylic acid $C_8H_6F_3NO_3S$ MW = 253	rotational crop, lysimeter leachate,
M-04 (AEC657378)	3-hydroxy BAM	Cl, H_2N, OH, O, Cl	2,6-dichloro-3-hydroxy-benzamide $C_7H_5Cl_2NO_2$ MW = 206	rotational crop rat (BAM ADME study)

List of metabolites continued

Company code number	Other identifiers	Structure	Formula	Presence in metabolism studies
M-06 (AEC643890)	3-OH 206 MET IV MET.F/16 FMET/38 UMET/51 FMET/8 UMET/44 UMET/53 FMET/33	F3C, Cl, Cl, NH, N, OH, O, Cl	2,6-dichloro-N-[(3-chloro-5-trifluoromethylpyridin-2-yl) methyl]-3-hydroxybenzamide $C_{14}H_8Cl_3F_3N_2O_2$ MW = 399	laying hen, lactating cow crop, confined rotational crop, rat
M-07 (AE 0712556)	4-OH 206 UMET/54 UMET/26	F3C, Cl, Cl, OH, NH, N, O, Cl	2,6-dichloro-N-[(3-chloro-5-trifluoromethylpyridin-2-yl) methyl]-4-hydroxybenzamide $C_{14}H_8Cl_3F_3N_2O_2$ MW = 399	laying hen, lactating cow rat
M-08 (AEC653598)		F3C, Cl, NH2, N, O	3-chloro-5-trifluoromethyl pyridine-2-carboxamide $C_7H_4ClF_3N_2O$ MW = 224.57	confined rotational crop
M-09 (AE B102859)		F3C, Cl, N, OH	3-chloro-2-hydroxy-5-trifluoromethylpyridine $C_6H_3ClF_3NO$ MW = 197.54	confined rotational crop
M-03 (AE060800)	RPA427967	F3C, Cl, Cl, H N, N, OH, O, Cl	4-N-[3-chloro-5-trifluoro-methylpyridin-2-yl) (hydroxyl)methyl]-2,6-dichlorobenzamide $C_{14}H_8Cl_3F_3N_2O_2$ MW = 399.58	soil
M-10 (AE 1344123)	P4 RPA433965	F3C, SO3H, N, COOH	3-sulfo-5-trifluoromethyl pyridine-2-carboxylic acid $C_7H_4F_3NO_5S$ MW = 271.17	lysimeter leachate, soil (PCA soil degradation study)

List of metabolites continued

Company code number	Other identifiers	Structure	Formula	Presence in metabolism studies
M-11 M-12	P2 Mixture of 2 isomers (P2a and P2b)	F_3C, SO_3H, HO, N, COOH	isomers x-hydroxy -y-sulfo-5-trifluoromethylpyridine-2-carboxylic acid $C_7H_4F_3NO_6S$ MW = 287.17	lysimeter leachate, soil (PCA soil degradation study)
M-13	P3	F_3C, Cl, HO, N, COOH proposal not confirmed	$C_7H_3ClF_3NO_3$ MW = 241.3	lysimeter leachate,
M-14 (AE 1388273)	P7 RPA433986	F_3C, SO_2CH_3, N, OH	3-mesyl-5-(trifluoromethyl) pyridin-2-ol $C_7H_6F_3NO_3S$ MW = 241.19	lysimeter leachate, soil (PCA soil degradation study)
M-15 (AE 1413903)	P8	F_3C, Cl, Cl, SO_3H, H, N, N, O, Cl	3,5-dichloro-4-[3-chloro-5-trifluoromethylpyridine-2-yl-methyl) carbamoyl]benzene sulfonic acid $C_{14}H_8Cl_3F_3N_2O_4S$ MW = 463.65	lysimeter leachate,
M-16	P9 UMET/40 FMET/23	F_3C, Cl, Cl, H, N, N, OH, O, SO_3H	3-chloro-2-[({3-chloro-5-trifluoromethylpyridine-2-ylmethyl}amino) carbonyl]-6-hydroxybenzene sulfonic acid $C_{14}H_9Cl_2F_3N_2O_5S$ MW = 444	lysimeter leachate, rat
M-17	Metabolite 1	F_3, Cl, Cl, H, N, N, S, O, O, CH_3, O, Cl	2,6-dichloro-N-{[3-chloro-5-(trifluoromethyl)pyridin-2-yl]methyl}-3-(methylsulfonyl)benzamide $C_{15}H_{10}Cl_3F_3N_2O_3S$ MW = 462	laying hen

List of metabolites continued

Company code number	Other identifiers	Structure	Formula	Presence in metabolism studies
M-18	HS (hydroxy sulphate of fluopicolide) UMET/45 UMET/47	F_3C Cl Cl H N N OSO$_3$H O Cl or F_3C Cl Cl OSO$_3$H H N N O Cl	2,4-dichloro-3-[({[3-chloro-5-(trifluoromethyl)pyridin-2-yl]methyl}amino)carbonyl] phenyl hydrogen sulfate or 3,5-dichloro-4-[({[3-chloro-5-(trifluoromethyl)pyridin-2-yl]methyl}amino)carbonyl] phenyl hydrogen sulfate $C_{14}H_7Cl_3F_3N_2O_5S$ MW = 477	laying hen lactating cow rat
M-19	DHS (dihydroxy sulphate of fluopicolide) UMET/23 UMET/39 UMET/46 UMET/49	F_3C Cl Cl OH H N N OSO$_3$H O Cl	3,5-dichloro-4-[({[3-chloro-5-(trifluoromethyl)pyridin-2-yl]methyl}amino)carbonyl] hydroxyphenyl hydrogen sulfate $C_{14}H_7Cl_3F_3N_2O_6S$ MW = 493	laying hen lactating cow rat

APPENDIX 2

DETAILED STUDIES AS REPORTED IN ORIGINAL DAR

6.8.1.1 Metabolism and Toxicity studies on M-01

a) Single high dose, oral route, A.D.M.E. in rats (phenyl radiolabel)

Study	[Phenyl-U-^{14}C] AE C653711 (BAM): Single Oral High Dose ADME. study in the rat
Reference	Gutierrez, L., 29/7/2003b
Date performed	7/10/2002 – 5/6/2003
Test facility	[redacted] France.
Report reference	Laboratory ref SA 02327/ Notifier reference no. Dossier ref: C035247
Guideline(s)	OECD *414 (1981)*; USEPA : OPPTS 870.7485 (*1998*) JMAFF, 12 Nohsan N°8147,2000
Deviations from the guideline	No significant deviation
GLP	Yes and QA
Test material	Batch no., SEL/1059, radiopurity 99%); non-radiolabelled M-01 (Batch Number R001724, purity 97.0%
Study acceptable	Yes

In a study (2003) groups of 4 male and 4 female Sprague-Dawley CD rats were administered by gavage a nominal single oral high dose of 150 mg/kg bw [phenyl-U-^{14}C]-M-01 (radiopurity 99%, specific activity 2.246 GBq/mM) as a suspension in 0.75% (w/w) aqueous methyl cellulose (Nominal Radioactive dose 125µCi/kg). Sampling of urine was at 6, 24, 48, 72, 96, 120, 144, 168h; cage wash at 24, 48, 72, 96, 120, 144, 168; and faeces 24, 48, 72, 96, 120, 144, 168.

All animals were exsanguinated whilst under Imalgene-500 anaesthesia 168 hours after administration, when most of the administered dose had been eliminated. After sacrifice the following tissues and organs were collected: liver, kidneys, heart, lungs, brain, cardiac blood, spleen, pancreas, muscle, abdominal fat, ovaries (for females), testes (for males), stomach plus contents, intestine plus content, bone and marrow, adrenals, the skin & fur, uterus (for females), eyes, Harderian glands and thyroids were removed from each animal after exsanguination. The residual carcass was also retained for analysis. Plasma was prepared from cardiac blood samples by centrifugation (approx. 2000 x G for 10 minutes).

Cl, H_2N, O, Cl, *

*= position of radiolabel

The amounts of radioactivity in the various samples were determined by liquid scintillation counting. Samples were counted for 10 minutes or for 2 sigma % in an appropriate scintillation cocktail using a Packard 1900 TR counter with on-line

computing facilities in which quenching effects were determined using an external standard and spectral quench parameter (tSIE) method. Efficiency correlation curves were prepared for each scintillation cocktail and were regularly checked by the use of [^{14}C]-n-hexadecane standards. The scintillation counter was recalibrated when a deviation of greater than 2 % was observed when counting quality control standards. Results were automatically transmitted to an NT server *via* the internal Ethernet network from whence they were imported directly into a Debra database. The limit of detection was taken to be twice the background values for blank samples in appropriate scintillation cocktails.

Urine and faecal samples contained almost the entire amount of the recovered radioactivity and therefore, the metabolism study was performed with these two categories of samples. Based on the amounts of excreted radioactivity in these samples, it was decided to investigate the time intervals of 0-6h, 6-24h, 24-48h, 48-72h, 72-96h, 96-120h for male and female urine. The faecal samples analysed were the time intervals of 24-48h and 48-72h for male and female faecal samples.

The metabolite profile was investigated by a radio-HPLC technique using two gradient elution systems and a reverse phase LUNA C18 HPLC column. Metabolite identification was performed by comparison of retention times with known standards and by LC/MS and LC/MS/MS.

The study was certified to be GLP compliant and was conducted in accordance with USEPA OPPTS 8707485 (1998) guidelines. It is considered to be acceptable.

The mean recovery over a seven-day period post-administration was found to be 92.21 % in male rats and 98.18 % in female rats (Table 6.122).

Table 6.122 Summary of the distribution of radiolabel in tissues and excreta after a following Single Oral dose of 150 mg/kg bw [^{14}C]-M-01 (% administered dose)

Sample	150 mg/kg bw			
	Males		Females	
	Mean	SD	Mean	SD
Urine	69.28	1.74	78.14	1.74
Cage wash	9.33	3.53	6.20	1.58
Faeces	12.44	3.50	12.63	1.11
Tissues	1.17	0.27	1.21	0.21
Total	**92.21**	**1.46**	**98.18**	**1.42**

SD = standard deviation

The major route of excretion was found to be the urine for both sexes with the mean percentage of the administered dose eliminated (urine plus cage washes) during the 7-day sampling period being 69.28 % in male rats and 78.14 % in female rats. The mean proportions of the administered radioactivity eliminated via the faecal route were 12.44 % in male rats and 12.63 % in female rats.

The rate of elimination was slow with 96 hours being necessary for the excretion of at least 90 % of the radioactivity eliminated in the urine. The routes and rates of elimination were very similar between male and female rats (Table 6.123).

Table 6.123 Elimination of Radioactivity following Administration of [^{14}C]-M-01 at the rate of 150 mg/kg bw (% administered radioactivity)

Sample	Sampling Period	150 mg/kg bw			
		Males		Females	
		Mean	SD	Mean	SD
Urine	0-6	3.12	0.27	4.09	1.08
	6-24	23.88	2.26	23.81	1.08
	24 -48	48.80	3.14	51.14	2.53
	48 -72	60.13	2.12	65.77	2.53
	72-96	65.02	2.20	72.84	2.61
	96-120	67.60	2.10	76.20	1.93
	120-144	68.75	1.86	77.55	1.77
	144-168	69.28	1.74	78.14	1.74
Faeces	0-24	2.20	1.74	0.77	0.54
	24 -48	8.00	3.25	7.48	1.06
	48 -72	10.44	3.24	10.40	1.40
	72-96	11.32	3.49	11.53	1.17
	96-120	11.83	3.46	12.24	1.16
	120-144	12.29	3.48	12.52	1.13
	144-168	12.44	3.50	12.63	1.11
Cage Wash	0-168	9.33	3.53	6.20	1.58
Total Eliminated		91.04	1.29	96.97	1.47

SD = Standard Deviation

The results showed that following a 7-day period post-administration the levels of radioactivity distributed in tissues represented a sum total of 1.17 % of the administered dose in male rats and 1.21 % in female rats. The highest tissue concentrations in male and female rats were found in skin & fur with mean values of 3.783 and 5.081 µg equivalents/g respectively.

In male rats, the second most significant residues were seen in liver and kidney with mean values of 2.083 and 2.987 µg equivalents/g respectively. The levels of radioactivity in adrenals, Harderian glands and intestine & contents of male rats presented mean values of 1.585, 1.107 and 1.042 µg equivalents/g respectively. The remaining tissue concentrations, in male rats, were found to be below 1.000 µg equivalents/g.

A similar distribution pattern was seen in female rats, where the mean levels of radioactivity in liver and kidney were 2.257 and 2.791 µg equivalents/g respectively. The levels of radioactivity in adrenals, Harderian glands and intestine & contents of female rats presented mean values of 1.604, 1.335 and 1.101 µg equivalents/g respectively. The remaining tissue concentrations, in female rats, were also found to be below 1.000 µg equivalents/g.

Table 6.124 Distribution of [14C]-M-01 in the Tissues of the Rat following a Single Oral Dose at the rate of 150 mg/kg bw (µg M-01 equivalents/g tissue)

Tissues	150 mg/kg bw			
	Males		Females	
	Mean	SD	Mean	SD
Cardiac blood	0.66	0.217	0.791	0.251
Intestine & contents	1.042	0.454	1.101	0.303
Harderian Gland	1.107	0.263	1.335	0.321
Residual Carcass	0.663	0.159	0.701	0.125
Skin & Fur	3.783	0.791	5.081	0.959
Cardiac Plasma	0.558	0.215	0.587	0.227
Eyes	0.517	0.196	0.663	0.232
Brain	0.601	0.232	0.616	0.275
Fat	0.4	0.133	0.376	0.099
Heart	0.742	0.228	0.755	0.266
Lungs	0.751	0.245	0.85	0.27
Spleen	0.8	0.245	0.83	0.233
Liver	2.083	0.689	2.257	0.413
Kidneys	2.987	0.713	2.791	0.645
Stomach & contents	0.508	0.235	0.621	0.228
Thyroids	n.d.	n.a.	n.d.	n.a.
Testes	0.658	0.256	n.a.	n.a.
Ovaries	n.d.	n.a.	0.904	0.267
Pancreas	0.671	0.237	0.718	0.237
Adrenal	1.585	0.414	1.604	0.426
Uterus	n.a.	n.a.	0.596	0.22
Muscle	0.675	0.207	0.695	0.22
Bone & Marrow	0.391	0.151	0.386	0.157

n.d. = not detected, n.a. = not applicable,

The unchanged parent compound, M-01, was one of the major components in urine and faeces that added to a sum total of 13 % in males and 24.60 % in females (ca. 7.65 % in male urine, ca. 17.85 % in female urine, ca. 5.35 % in male faeces and ca. 6.75 % in female faeces). Several different metabolic pathways were postulated for the biotransformation of the M-01. One of them was the hydrolysis of the test product leading to dichlorobenzoic acid (AE C416656) that accounted for 0.64 % dose in male rats and 0.65 % in female rats. The hydrolysis of the aryl amide functional group was also seen in metabolites USHD/4 (O-glucuronide conjugate) and USHD/10 (sulphate conjugate) that were transformed in carboxylic acid derivative intermediates, which were subsequently decarboxylated leading to USHD/5 (O-glucuronide conjugate of dichlorophenyl) and USHD/19 (O-sulphate conjugate of dichlorophenyl) respectively.

The principal metabolic pathway led to USHD/9, USHD/16 and FSHD/4 that were identified to be a mercapturic acid conjugate of hydroxy-chlorobenzamide. The major metabolite eliminated in the urine was USHD/9 that accounted for a sum total of ca. 20.85 % dose in male urine and 17.85 % in female urine (Table 6.81). The metabolite USHD/16 is an isomer of USHD/9 that was present in urine samples at lower levels, 0.45 % and 0.56 % for male and female rats respectively. The faecal metabolite FSHD/4 was the same mercapturic acid conjugate, USHD/9 thus, both of them were eluted with the same retention times. There were two potential pathways that could

have led to the mercapturic acid conjugate of hydroxy-chlorobenzamide, the first one was the aromatic dehalogenation of M-01 that was subsequently followed by the action of glutathione S-transferase leading to a glutathione conjugate intermediate. The GS-intermediate would have been further biotransformed (losing glutamic acid and glycine) leading to a cysteine conjugate that was subsequently hydroxylated in the aromatic ring, resulting in USHD/6. The metabolite USHD/6 was the cysteine conjugate of hydroxy-chlorobenzamide that was subsequently N-acetylated leading to its derivative mercapturic acid conjugate. We have postulated that the aromatic position of the hydroxyl group and/or the S-cysteine group could have led to isomers (USHD/9 and USHD/16). The second proposed metabolic pathway leading to USHD/9, USHD/16 and FSHD/4 was the aromatic hydroxylation on the test product, which was subsequently dehalogenated and followed by the action of GSH enzymes, loss of glutamic acid and glycine. See the metabolic pathway described below for further details of the two biotransformation pathways leading to the major metabolite (mercapturic acid conjugate).

There were at least two different hydroxy derivative metabolites of M-01, one of them was USHD/11 that was co-eluted with M-04, which was found in urine samples. The other one was an isomer of position (FSHD/7) that was seen in faeces. The hydroxyl metabolite was a potential intermediate leading to USHD/9, USHD/16 and FSHD/4, as described above, but was also the intermediate leading to O-glucuronide and O-sulphate conjugates, by the action of glucuronidase and sulphatase enzymes. A different glucuronide conjugate was USHD/8 that was obtained by the action of N-glucuronide enzymes. The metabolite USHD/8 added to a sum total of 0.53 % in male urine and 0.43 % in female urine. The metabolite USHD/6 accounted for *ca.* 4.52 % in male rats and *ca.* 10.76 % in female rats. USHD/6 was further metabolised by the action of glucuronide enzymes leading to USHD/3 that was identified to be the O-glucuronide conjugate derived from USHD/6. The metabolite USHD/3 accounted for a sum total of *ca.* 4.97 % in male urine and *ca.* 3.78 % in female urine. A different biotransformation of USHD/6 was obtained by the action of S-dealkylation enzymes (aryl cysteine lost) leading to thiomethyl hydroxy-chlorobenzamide (FSHD/8) that was subsequently metabolised by the action of sulphatase enzymes leading to O-sulphate conjugate of thiomethyl-chlorobenzamide (USHD/10). The thiomethyl hydroxy-chlorobenzamide (FSHD/8) could also have been obtained by hydroxylation of the thiomethyl intermediate (FSHD/10).

The distribution of identified metabolites in urine and faeces in presented in the following table (Table 6.81).

Table 6.125 Summary of Identified Urine and Faecal Metabolites following a Single Oral Dose of 150 mg/kg b/w [14C]-M-01.

Radioactive peaks	Structural Identification (Key funtional groups)	% Dose	
		♂	♀
USHD/3	cysteine and O-glucuronide conjugate of chlorobenzamide	4.97	3.78
USHD/4	O-glucuronide conjugate of dichlorobenzamide	1.46	2.17
USHD/6	cysteine conjugate of hydroxy-chlorobenzamide	4.52	10.76
USHD/7	dichlorobenzoic acid (AE C416656)	0.64	0.65
USHD/8	N-glucuronide conjugate of dichlorobenzamide	0.53	0.43
USHD/9	mercapturic acid conjugate of hydroxy-chlorobenzamide	20.85	17.85
FSHD/4		0.38	0.26
USHD/10	O-sulfate conjugate of dichlorobenzamide O-sulfate conjugate of thiomethyl-chlorobenzamide	13.83	12.45
USHD/11	3-hydroxy-chlorobenzamide (M-04)	7.32	2.65
FSHD/7	hydroxy-chlorobenzamide	0.24	0.14
USHD/15	O-glucuronide conjugate of dichlorophenyl	0.64	0.75
USHD/16	mercapturic acid conjugate of hydroxy-chlorobenzamide	0.45	0.56
FSHD/8	thiomethyl-hydroxychlorobenzamide	0.39	0.61
FSHD/10	thiomethyl-chlorobenzamide	0.18	0.09
USHD/18	2,6-dichlorobenzamide (M-01)	7.65	17.85
FSHD/11		5.35	6.75
USHD/19	O-sulfate conjugate of dichlorophenyl	0.70	0.81

Most of the administered radioactivity was eliminated in the urine, where the rate of elimination was slow, whilst only low levels were eliminated via the faeces. The high levels of oral bioavailability were seen in both male and female rats. Overall, the quantification of radioactive residues in tissues showed low residual levels with sum total mean values of 1.17 % and 1.21 % for male and female rats respectively. The highest concentrations in tissues were seen in skin & fur, liver and kidney, where the mean values ranged between 2.10 and 5.10 µg equivalents/g. The unchanged parent compound was seen in urine and faecal samples from male and female rats. The major metabolite was USHD/9 that represented ca. 20.85 % dose and 17.85 % dose in male and female urine respectively and was identified to be a mercapturic acid conjugate of hydroxy-chlorobenzamide. Metabolite USHD/9 was obtained from a complex metabolic pathway that included the activities of GSH transferase and peptidase enzymes leading to a cysteinyl conjugate that was subsequently N-acetylated to obtain USHD/9. The biotransformation of M-01 also included the activities of dealkyl-S-cysteine, O-glucuronidase, O-sulphatase enzymes and N-glucuronidase enzymes. The proposed metabolic pathway is shown on the following page in Figure 5.8.1.1-1.

Figure 6.6. Proposed Metabolic Pathway for [^{14}C]-M-01 following a Single Oral Dose at the rate of 150 mg/kg bw

hydroxylation (isomers)

AE C657378

N-glucuronidation

N-glucuronide

AE C653711

hydrolysis

AE C416656

glutathione S-transferase

glucuronidation

sulfation

O-glucuronide conjugate

sulfate conjugate

hydrolysis (ary carboxamide) +decarboxilation

hydrolysis (ary carboxamide) +decarboxilation

y-glutamyltranspeptidase (glutamic acid lost)

cysteinyl glycinase (glycine lost)

O-glucuronide conjugate

sulfate conjugate

S-dealkylation (aryl cysteine) + S-methylation

cysteine conjugate

hydroxylation

glutathione S-transferase

y-glutamyltranspeptidase + cysteinyl glycinase

N-acetyl transferase (acetylation of cysteine)

S-dealkylation (aryl cysteine) + S-methylation

cysteine conjugate

mercapturic acid conjugate

hydroxy tiomethyl

glucuronidation

sulfation

*[intermediates]

sulfate conjugate

cysteine O-glucuronide conjugate

(Gutierrez, 2003b)

b) Single oral low dose A.D.M.E. study in rats (phenyl radiolabel)

Study	[Phenyl-U-^{14}C]-AE C653711 (BAM): Single Oral Low Dose ADME. study in the rat
Reference	Gutierrez, L., 29/07/2003
Date performed	13/5/2002 – 15/5/2003
Test facility	[redacted] France.
Report reference	Laboratory ref SA 02156/ Notifier reference no. Dossier ref: C035245
Guideline(s)	USEPA : OPPTS 870.7485 (1998) JMAF 12 Nohsan N°8147 (2000) ; EU (=EEC) 87/302, part B
Deviations from the guideline	No significant deviation
GLP	Yes and QA
Test material	Batch no., SEL/1059, radiopurity 99%); non-radiolabelled M-01 Batch Number R001724, purity 97.0%
Study acceptable	Yes

In a study (2003), groups of 4 male and 4 female Sprague-Dawley CD rats were administered by gavage a nominal single oral low dose of 10 mg/kg bw [phenyl-U-^{14}C]-M-01 (Batch no., SEL/1059; radiopurity 99 %; specific activity 2.246 GBq/mM) as a suspension in 0.75 % (w/w) aqueous methyl cellulose (Nominal Radioactive dose 125µCi/kg). Sampling of urine was over 0-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144; cage wash over 0-24, 24-48, 48-72, 72-96, 96-120, 120-144; faeces over 0-24, 24-48, 48-72, 72-96, 96-120, 120-144; and carbon dioxide over 0-24, 6-24, and 24-48. Cage washes were performed with distilled water at all time points plus an additional acetonitrile rinse at 168 hours

All animals were exsanguinated whilst under Imalgene-500 anaesthesia 144 hours after administration, when most of the administered dose had been eliminated. After sacrifice the following tissues and organs were collected: liver, kidneys, heart, lungs, brain, cardiac blood, spleen, pancreas, muscle, abdominal fat, ovaries (for females), testes (for males), stomach plus contents, intestine plus content, bone and marrow, adrenals, the skin & fur, uterus (for females), eyes, Harderian glands and thyroids were removed from each animal after exsanguination. The residual carcass was also retained for analysis. Plasma was prepared from cardiac blood samples by centrifugation (approx. 2000 x G for 10 minutes).

The amounts of radioactivity in the various samples were determined by liquid scintillation counting. Samples were counted for 10 minutes or for 2 sigma % in an appropriate scintillation cocktail using a Packard 1900 TR counter. Quenching effects were determined using an external standard and spectral quench parameter (tSIE) method. Efficiency correlation curves were prepared for each scintillation cocktail and were regularly checked by the use of [14C]-n-hexadecane standards. The scintillation counter was recalibrated when a deviation of greater than 2 % was observed when counting quality control standards. The limit of detection was taken to be twice the background values for blank samples in appropriate scintillation cocktails.

Urine and faecal samples contained almost the entire amount of the recovered radioactivity and therefore, the metabolism study was performed with these two categories of samples. Based on the amounts of excreted radioactivity in these samples, it was decided to investigate the time intervals of 0-6h, 6-24h, 24-48h and 48-

72h, for male and female urine. The 0-24 h time period was analysed for the faecal samples for both sexes.

The metabolite profile was investigated by a radio-HPLC technique using three gradient elution systems and a reverse phase LUNA C18 HPLC column. Metabolite identification was performed by comparison of retention times with known standards and by LC/MS and LC/MS/MS.

The study was certified to be GLP compliant and was conducted in accordance with USEPA OPPTS 870.7485 (1998) guideline for metabolism studies. It is considered to acceptable.

Male and Female rats of the Sprague Dawley strain were orally administered with radiolabelled M-01 at a nominal dose rate of 10 mg/kg body weight (single oral low dose). The mean recovery over a six-day period post-administration was found to be 96.55 % in male rats and 97.97 % in female rats (Table 6.126).

Table 6.126 Recovery of Radioactivity following Single Oral Administration of [14C]-M-01 at the rate of 10 mg/kg bw (% administered dose).

Sample	10 mg/kg bw			
	Males		Females	
	Mean	SD	Mean	SD
Urine	66.43	6.10	70.85	5.42
Cage wash	14.39	5.77	13.38	5.04
Faeces	13.53	1.48	12.03	1.75
CO_2 traps	n.d.	n.a.	n.d.	n.a.
Tissues	2.21	0.16	1.71	0.10
Total	**96.55**	**1.30**	**97.97**	**1.64**

SD = standard deviation

The mean percentage of the administered dose eliminated via the urinary route (urine plus cage washes) during the 6-day elimination period was found to be 80.82 % in male rats and 84.23 % in female rats. The mean proportions of the administered radioactivity eliminated via the faecal route were 13.53 % in male rats and 12.03 % in female rats. The levels of radioactivity eliminated by exhalation were negligible for both male and female rats. Therefore, the results showed that most of the administered radioactivity was eliminated in the urine, whilst only low levels were eliminated via the faeces. The estimated minimum levels of absorption were measured as the total radioactivity in urine, cage washes plus tissues. The results showed that at least 83.03 % of the administered dose was absorbed by male rats and similar results were seen in female rats with 85.94 % of the administered dose been absorbed. Therefore, high oral bioavailability was seen in both male and female rats.

The rate of elimination was slow; hence 96 hours were necessary for the excretion of ca 95 % of the total radioactivity excreted in urine. The routes and rates of elimination were very similar between male and female rats (Table 6.127).

Table 6.127 Elimination of Radioactivity following Administration of [14C]-M-01 at the rate of 10 mg/kg bw (% administered dose)

Sample	Sampling Period (h)	10 mg/kg bw			
		Males		Females	
		Mean	SD	Mean	SD
Urine	0-6	3.44	0.467	3.563	1.272
	6-24	36.03	3.393	38.723	5.081
	24 -48	53.397	4.036	59.476	5.765
	48 -72	60.607	5.146	66.64	5.332
	72-96	64.173	5.574	69.218	5.581
	96-120	65.685	5.953	70.345	5.438
	120-144	66.427	6.103	70.846	5.416
Faeces	0-24	6.711	1.641	5.823	1.21
	24 -48	10.809	1.599	9.483	1.44
	48 -72	12.336	1.587	10.994	1.78
	72-96	13.04	1.526	11.602	1.719
	96-120	13.355	1.501	11.846	1.747
	120-144	13.517	1.481	12.026	1.749
Cage Wash	0-168	14.39	5.773	13.383	5.042
Total Eliminated		94.334	1.393	96.255	1.734

SD = Standard Deviation

The results showed that following a 6-day period post-administration there were low levels of radioactivity distributed in tissues. The sum total of percentage administered dose distributed in tissues was 2.21 % in male rats and 1.71 % in female rats. The highest tissue residues in male rats were found in liver (mean: 0.439 µg equivalents/g) and kidney (0.566 µg equivalents/g). Similar concentrations were seen in female tissues (Table 6.128). The highest levels of radioactivity in female rats where seen in liver (mean: 0.445 µg equivalents/g) and kidney (mean: 0.556 µg equivalents/g). The mean levels of radioactivity in the Harderian glands were 0.350 µg equivalents/g in males and 0.329 µg equivalents/g in females. The mean levels of radioactivity in adrenals were 0.262 µg equivalents/g in males and 0.274 µg equivalents/g in females. The mean levels of radioactivity in skin & fur were 0.350 µg equivalents/g in males and 0.321 µg equivalents/g in females. The mean levels of radioactivity in heart were 0.161 µg equivalents/g in males and 0.149 µg equivalents/g in females. The mean radioactive residues in carcass were 0.115 µg equivalents/g in males and 0.113 µg equivalents/g in females. Overall, the quantification of radioactive residues in tissues showed low levels for both male and female rats, the mean values ranged below 0.600 µg equivalents/g.

Table 6.128 Concentration of [14C]-M-01 in the Tissues of the Rat following a Single Oral Dose at the rate of 10 mg/kg b/w (µg M-01 equivalents/g tissue)

Tissues	10 mg/kg bw			
	Males		Females	
	Mean	SD	Mean	SD
Cardiac blood	0.057	0.014	0.043	0.004
Intestine & contents	0.103	0.022	0.076	0.012
Harder's Gland	0.350	0.030	0.329	0.021
Residual Carcass	0.115	0.018	0.113	0.015
Skin & Fur	0.350	0.022	0.321	0.071
Cardiac Plasma	0.050	0.014	0.034	0.004
Eyes	0.058	0.012	0.039	0.008
Brain	0.073	0.013	0.049	0.007
Fat	0.042	0.007	0.029	0.006
Heart	0.161	0.027	0.149	0.008
Lungs	0.099	0.020	0.085	0.015
Spleen	0.075	0.017	0.054	0.007
Liver	0.439	0.041	0.445	0.081
Kidneys	0.566	0.066	0.556	0.057
Stomach & contents	0.054	0.009	0.057	0.031
Thyroids	0.154	0.049	n.a.	n.a.
Ovaries	n.a.	n.a.	0.054	0.007
Testes	0.074	0.015	n.a.	n.a.
Pancreas	0.098	0.019	0.083	0.011
Uterus	n.a.	n.a.	0.041	0.005
Adrenal	0.262	0.089	0.274	0.028
Muscle	0.098	0.011	0.088	0.005
Bone & Marrow	0.038	0.010	0.030	0.006

n.d. = not detected, n.a. = not applicable.

The unchanged parent compound, M-01, was seen to be one of the major components in urine (USLD/13) and faeces (FSLD/6) that accounted for a sum total of 14 % of the administered dose for both male and female rats, (ca. 10.3 % in male urine, ca. 11.2 % in female urine, ca. 3.6 % in male faeces and ca. 3.3 % in female faeces). Three different metabolic pathways were postulated for the biotransformation of the M-01. One of them was the hydrolysis of the test product leading to dichlorobenzoic acid (AE C416656).

The principal metabolic pathway led to USLD/6 that was the major metabolite in urine samples (Table 6.85). The sum total of USLD/6 in urine samples was ca. 26.24 % in male urine and 25.37 % in female urine. The metabolite USLD/6 was identified as being the mercapturic acid conjugate of hydroxy-chlorobenzamide. There were two potential pathways that could lead to USLD/6, the first one was the aromatic dehalogenation of M-01 that was followed by the action of glutathione S-transferase leading to a glutathione conjugate intermediate. The GS-intermediate was further biotransformed with the loss of glutamic acid and glycine leading to cysteine conjugate that was subsequently hydroxylated in the aromatic ring, leading to USLD/4. The metabolite USLD/4 was the cysteine conjugate of hydroxy-chlorobenzamide that was subsequently N-acetylated leading to its derivative USLD/6 (mercapturic acid conjugate).

The second proposed metabolic pathway leading to USLD/6 was the aromatic hydroxylation on the test product leading to USLD/4 followed by dehalogenation, followed by the action of GSH enzymes, loss of glutamic acid and glycine. See the metabolic pathway described below for further details of the two biotransformation pathways leading to the major metabolite (USLD/6). The hydroxy derivative metabolite of the test product was named (intermediate-1) that was subsequently biotransformed by the action of glucuronidase and sulphatase enzymes leading to O-glucuronide and O-sulphate conjugates. The metabolite USLD/4 accounted for a sum total of ca. 1.87 % in male rats and ca. 9.10 % in female rats (Table 6.85). USLD/4 was further metabolised by the action of glucuronide enzymes leading to USLD/2 that was identified to be the O-glucuronide conjugate derived from USLD/4. The metabolite USLD/2 accounted for a sum total of ca. 6.35 % in male urine and ca. 6.57 % in female urine. A different biotransformation of USLD/4 was obtained by the action of S-dealkylation enzymes (aryl cysteine loss) leading to thiomethyl hydroxy-chlorobenzamide (intermediate-3) that was seen in High Dose A.D.M.E. Study (section 5.8.1.1.1.). The intermediate-3 was subsequently metabolised by the action of sulphatase enzymes leading to O-sulphate conjugate of thiomethyl-chlorobenzamide (USLD/8). The metabolite USLD/8 added to a sum total of ca. 6.21 % in male urine and 6.16 % in female urine that in fact represents two different O-sulphate conjugates, the second one was the O-sulphate conjugate of dichlorobenzamide. The following table presents a summary of the identified metabolites and their quantification.

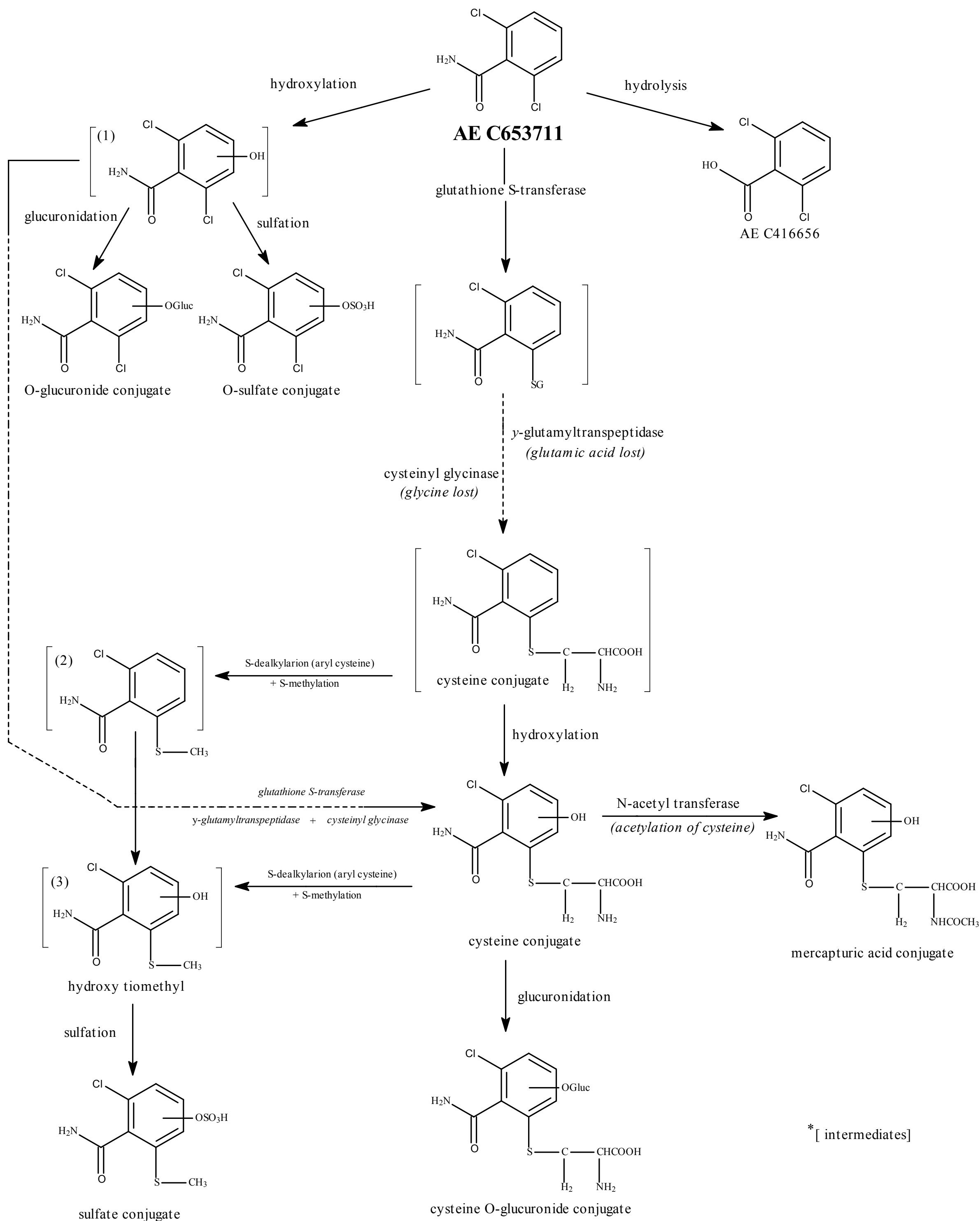
Table 6.129 Summary of Identified Urine and Faecal Metabolites following a Single Oral Dose of [14C]-M-01 at the rate of 10 mg/kg bw

Radioactive peaks	Append. G Refer.	Structural Identification (Key funtional groups)	% Dose	
			♂	♀
USLD/2	F2	cysteine and O-glucuronide conjugate of chlorobenzamide	6.35	6.57
USLD/4	F4 F5	cysteine conjugate of hydroxy-chlorobenzamide	1.87	9.10
USLD/5	F6	O-glucuronide conjugate of dichlorobenzamide	0.02	0.05
	F8	dichlorobenzoic acid		
USLD/6	F9	mercapturic acid conjugate of hydroxy-chlorobenzamide	26.24	25.37
USLD/8	F10	O-sulfate conjugate of dichlorobenzamide O-sulfate conjugate of thiomethyl-chlorobenzamide	6.21	6.16
USLD/13	--	2,6-dichlorobenzamide (M-01)	10.33	11.23
FSLD/6	--	2,6-dichlorobenzamide (M-01)	3.61	3.30

The results showed that most of the administered radioactivity was eliminated in the urine, where the rate of elimination was slow. Only low levels were eliminated via the faeces. The high levels of oral bioavailability were seen in both male and female rats. Overall, the quantification of radioactive residues in tissues showed low concentrations for both male and female rats, where the mean values were all below 0.600 µg equivalents/g. The unchanged parent compound was seen in urine and faecal samples from male and female rats. The major metabolite was USLD/6 that represented ca.

26.24 % and 25.37 % in male and female urine respectively and was identified to be a mercapturic acid conjugate of hydroxy-chlorobenzamide. Metabolite USLD/6 was obtained from a complex metabolic pathway that included the activities of GSH transferase and peptidase enzymes leading to a cysteinyl conjugate that was subsequently N-acetylated to obtain USLD/6. The biotransformation of M-01 also included the activities of dealkyl-S-cysteine, O-glucuronidase and O-sulphatase enzymes. The proposed metabolic pathway is shown on the following page in Figure 6.7.

Figure 6.7 Proposed Metabolic Pathway for [14C]-M-01 following a Single Oral Dose at the rate of 10 mg/kg bw



intermediates (1), (2) & (3) were seen in the High Dose study

c) **Repeat low dose, oral route, A.D.M.E. in rats (phenyl radiolabel)**

Study	[[Phenyl-U-^{14}C]-AE C653711 (BAM): Repeat Oral Low Dose ADME. study in the rat
Reference	Gutierrez, L., 25/08/2003
Date performed	27/1/2003 – 26/6/2003
Test facility	[redacted] France.
Report reference	Laboratory ref SA 03018/ Notifier reference no. Dossier ref: C035920
Guideline(s)	USEPA : OPPTS 870.7485 (1998) JMAF 12 Nohsan N°8147 (2000) ; EU (=EEC) 87/302, part B
Deviations from the guideline	No significant deviation
GLP	Yes and QA
Test material	Batch no., SEL/1059, radiopurity 99%); non-radiolabelled M-01 (Batch Number R001724, purity 97.0%
Study acceptable	Yes

In a study (2003), groups of 5 male and 5 female Sprague-Dawley CD rats were administered by gavage a nominal single oral low dose of 10 mg/kg bw [phenyl-U-^{14}C]-M-01 (Batch no., SEL/1059; radiopurity 99 %; specific activity 2.246 GBq/mM) as a suspension in 0.75 % (w/w) aqueous methyl cellulose (Nominal Radioactive dose 125µCi/kg). Sampling of urine, cage wash and faeces was daily for 19 days, 5 days after the final dose. Cage washes were performed with distilled water at all time points plus an additional acetonitrile rinse at 168 hours after the final dose.

All animals were exsanguinated whilst under Imalgene-500 anaesthesia 144 hours after administration, when most of the administered dose had been eliminated. After sacrifice the following tissues and organs were collected: liver, kidneys, heart, lungs, brain, cardiac blood, spleen, pancreas, muscle, abdominal fat, ovaries (for females), testes (for males), stomach plus contents, intestine plus content, bone and marrow, adrenals, the skin & fur, uterus (for females), eyes, Harderian glands and thyroids were removed from each animal after exsanguination. The residual carcass was also retained for analysis. Plasma was prepared from cardiac blood samples by centrifugation (approx. 2000 x G for 10 minutes).

The amounts of radioactivity in the various samples were determined by liquid scintillation counting. Samples were counted for 10 minutes or for 2 sigma % in an appropriate scintillation cocktail using a Packard 1900 TR counter. Quenching effects were determined using an external standard and spectral quench parameter (tSIE) method. Efficiency correlation curves were prepared for each scintillation cocktail and were regularly checked by the use of [14C]-n-hexadecane standards. The scintillation counter was recalibrated when a deviation of greater than 2 % was observed when counting quality control standards. The limit of detection was taken to be twice the background values for blank samples in appropriate scintillation cocktails.

Representative Urine and faecal samples collected after single dosing (Day 1) and multiple dosing (Day 14) were selected to compare the chromatographic profiles of excreta samples before and after multiple dosing.

The pooled urine samples were concentrated by evaporation under nitrogen and the resulting mean dpm/g determined by LSC. The samples were concentrated under a nitrogen stream to reach an approximate radioactivity level allowing 100 000 dpm to be injected within a maximum of *ca.* 100 µl and were subsequently analysed by radio-HPLC.

The male and female faecal samples were centrifuged at 3500 rpm for 20 minutes and the aqueous supernatants were decanted in separated vessels that were labelled as "Extract 1". The remaining pellets (non-extracted faecal residues) were extracted with acetonitrile solvent. The acetonitrile faecal extracts were centrifuged at 3500 rpm for 20 minutes and the supernatants were retained and labelled as "Extract 2". Following the first acetonitrile extraction the remaining pellets were extracted a second time with acetonitrile. The second acetonitrile faecal extracts were centrifuged at 3500 rpm for 20 minutes and the supernatants were retained and labelled as "Extract 3". The extracts numbered 1, 2 and 3 were pooled and concentrated under a gentle stream of nitrogen allowing 100 000 dpm to be injected within a maximum of *ca.* 100 µl for HPLC analysis. The concentrated samples were radioassayed by LSC to determine the percentage of recovered radioactivity in the final samples and subsequently analysed by radio-HLC.

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The metabolite profile was investigated by a radio-HPLC technique using three gradient elution systems and a reverse phase LUNA C18 HPLC column. Metabolite identification was performed by comparison of retention times with known standards and by LC/MS and LC/MS/MS.

The study was certified to be GLP compliant and was conducted in accordance with USEPA OPPTS 870.7485 (1998) guideline for metabolism studies. It is considered to be acceptable.

Male and female Sprague Dawley strain rats received repeated daily oral doses of 10 mg/kg bw radiolabelled M-01 for a 14 days. The mean recovery over the 14 day administration period and a 6 day period post-administration was found to be 96.53 % ± 1.34 % in male rats and 98.63 % ± 3.25 % in female rats. The mean total recovery for male and female rats was calculated to be 97.58 % ± 2.59 %. The total mean cumulative elimination of radioactivity in urine, six-days after multiple dosing, was 53.42 % ± 7.03 in males and 68.88 % ± 11.59 in females. The majority of the radioactivity eliminated in urine was excreted within the first 96 hours after the 14-day daily treatments and was in total 52.60 % in males and 68.55 % in females. Significant levels were also seen in cage washes that were included as part of the urinary excretion. The total elimination *via* the urinary route (urine plus cage washes) was calculated to be *ca*. 76.67 % dose in male rats and *ca*. 82.41 % dose in female rats. The results showed that most of the administered radioactivity was found to be excreted *via* the urine, whilst lower levels were seen in faeces for both male and female rats. The mean total elimination of radioactivity *via* the faeces was 18.80 % ± 2.76 % dose in males and 16.24 % ± 4.87 % in females. The majority of the radioactivity eliminated in faeces was already excreted by 72 hours post 14 daily administrations, in both male and female rats. The results showed that after multiple dosing most of the administered radioactivity was eliminated *via* the urinary route in both male and female rats, which indicates a potential for high oral bioavailability.

The minimum amount of dose absorbed after multiple dosing was calculated to be *ca.* 77.7 % in males and *ca.* 83.0 % in females, both values were obtained as the sum total of percentage dose eliminated *via* the urinary route plus percentage dose in tissues.

Table 6.130 Recovery of Radioactivity following 14 Days of Repeated Oral Administration of [14C]-M-01 at the rate of 10 mg/kg bw (% administered dose).

Parameter	Repeat Dose: 10 mg/kg			
	Day 14 (24 hours post last dose)			
	Males		Females	
	Mean	SD	Mean	SD
Urine	47.45	6.03	64.08	11.14
Faeces	16.95	2.72	14.96	4.38
Cage wash	21.26	4.80	12.45	5.85
Sub total	**85.67**	**0.83**	**91.50**	**2.96**
Day 19 (sacrifice)				
Urine	53.42	7.03	68.88	11.59
Faeces	18.80	2.76	16.24	4.87
Cage wash	23.25	4.85	13.53	6.01
Tissues	1.07	0.17	0.59	0.10
Total	**96.53**	**1.34**	**98.63**	**3.25**

SD = standard deviation

The routes and rates of elimination were very similar between male and female rats. The highest tissue residues were seen in skin & fur with mean values of 3.169 and 2.846 µg equivalents/g for male and female rats respectively (Table 6.131). Liver and kidney presented mean values of 1.672 and 2.713 µg equivalents/g for male rats. The radioactive residues found in liver and kidney of female rats presented mean values of 0.829 and 1.075 µg equivalents/g. The adrenals presented mean values of 1.379 and 0.410 µg equivalents/g for male and female rats respectively. All other tissues presented mean values that were below 1.000 µg equivalents/g for both male and female rats. Overall, the residue concentration was higher in male rats compared to female rats for all tissues analysed. The radioactive residues in ovaries and uterus were also seen to be lower than in testes.

Six days after multiple dosing, the unchanged parent compound was seen to be one of the major components in urine (URLD/18) and faeces (FRLD/11) that accounted for a mean cumulative total of ca. 19 % dose for both male and female rats (Table 6.132). The principal metabolic pathway led to URLD/9 that was the major metabolite in urine samples. The mean cumulative sum total of URLD/9 added to *ca.* 15.48 % in male urine and 16.00 % in female urine. The metabolite URLD/9 was identified to be the mercapturic acid conjugate of hydroxy-chlorobenzamide. There were two potential pathways that could lead to URLD/9. The first one was the aromatic dehalogenation of M-01 that was followed by the action of glutathione S-transferase leading to a glutathione conjugate intermediate. The GS-intermediate was further biotransformed with the loss of glutamic acid and glycine leading to a cysteine conjugate (intermediate 5) that was subsequently hydroxylated in the aromatic ring, leading to URLD/6.

Table 6.131 Concentration of Radioactivity in the Tissues of the Rat 6 days after the last of 14 daily repeated administrations with 10 mg/kg bw [14C]-M-01 (µg M-01 equivalents/g tissue)

Tissues	10 mg/kg			
	Males		Females	
	Mean	SD	Mean	SD
Adrenal	1.379	0.311	0.410	0.062
Bone & Marrow	0.334	0.067	0.100	0.019
Brain	0.578	0.147	0.107	0.021
Carcass	0.466	0.099	0.182	0.012
Cardiac blood	0.588	0.141	0.273	0.065
Cardiac Plasma	0.497	0.133	0.093	0.020
Eyes	0.402	0.067	0.087	0.013
Fat	0.267	0.043	0.077	0.009
Harderian Gland	0.901	0.139	0.409	0.034
Heart	0.659	0.139	0.230	0.019
Intestine & contents	0.357	0.042	0.122	0.010
Kidneys	2.713	0.730	1.075	0.140
Liver	1.672	0.334	0.829	0.088
Lungs	0.656	0.103	0.199	0.026
Muscle	0.500	0.069	0.184	0.018
Ovaries	n.a.	n.a.	0.231	0.043
Pancreas	0.493	0.113	0.116	0.049
Skin & Fur	3.169	0.673	2.846	0.738
Spleen	0.674	0.160	0.177	0.026
Stomach & contents	0.583	0.135	0.270	0.047
Testes	0.566	0.085	n.a.	n.a.
Thyroids	0.738	0.183	0.288	0.172
Uterus	n.a.	n.a.	0.111	0.060

n.d. = not detected, n.a. = not applicable

The metabolite URLD/6 was the cysteine conjugate of hydroxy-chlorobenzamide that was subsequently N-acetylated leading to its derivative URLD/9 (mercapturic acid conjugate).

The second proposed metabolic pathway to obtain URLD/9 was the aromatic hydroxylation of the test product leading to a hydroxyl metabolite (intermediate 1) that was subsequently dehalogenated, followed by the action of GSH enzymes, loss of glutamic acid and glycine to obtain URLD/6, which was subsequently N-acetylated to yield URLD/9. The metabolic pathway Figure 6.8 provides further details of the two biotransformation pathways leading to the mercapturic acid conjugate.

The metabolite URLD/6 accounted for a total of ca. 4.20 % in male rats and ca. 12.36 % in female rats. URLD/6 was further metabolised by the action of glucuronide enzymes leading to URLD/3 that was identified to be a cysteine and O-glucuronide conjugate. The metabolite URLD/3 accounted for a sum total of ca. 3.36 % in male urine and ca. 5.19 % in female urine. A different biotransformation pathway of URLD/6 was obtained by the action of S-dealkylation enzymes (aryl cysteine lost) leading to thiomethyl hydroxy-chlorobenzamide (intermediate 3). The intermediate 3 was subsequently metabolised by the action of sulphatase enzymes leading to the O-

sulphate conjugate of thiomethyl-chlorobenzamide, which was named URLD/10. The metabolite URLD/10 amounted to a sum total of ca. 5.47 % in male urine and 5.44 % in female urine. The spectral data of URLD/10 showed the presence of a shoulder peak that was identified to be the O-sulphate conjugate derived from the hydroxy-dichlorobenzamide metabolite. The metabolic pathway was completed with the identification of two more products that resulted from the decarboxylation of derived metabolites; one of them was the O-glucuronide conjugate of dichlorobenzamide (URLD/15) and the other was the O-sulphate conjugate of dichlorobenzamide (URLD/19). The presence of intermediates 1, 2, 3 and 4 were confirmed from samples of the single oral high dose study (section 6.8.1.1.a). The following table presents a summary of the identified metabolites and their quantification.

Table 6.132 Summary of Identified Urine and Faecal Metabolites following Repeated Oral Dosing of 10 mg/kg bw [14C]-M-01.

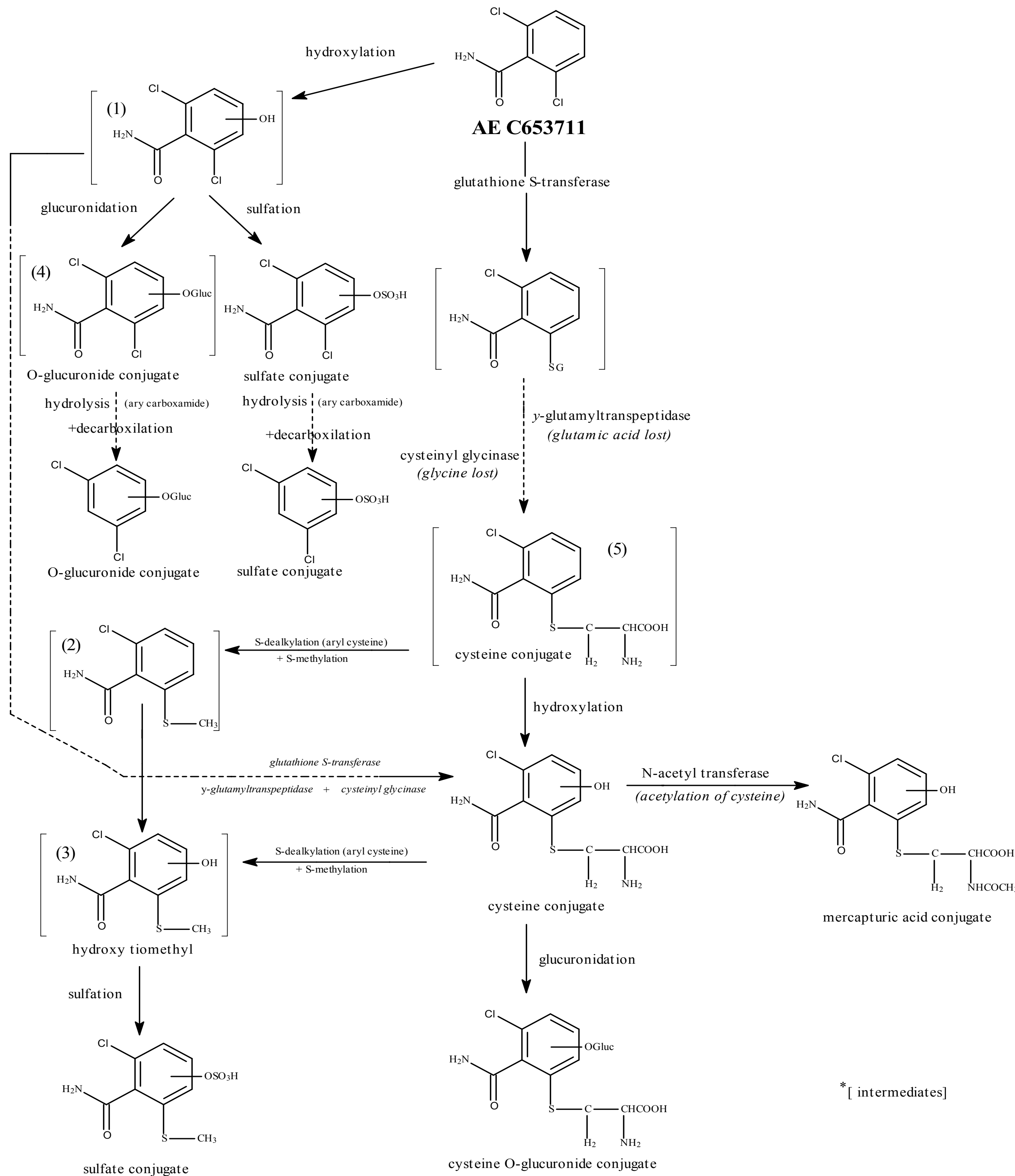
Radioactive peaks	Structural Identification (Key funtional groups)	% Dose	
		♂	♀
URLD/3	cysteine and O-glucuronide conjugate of chlorobenzamide	3.36	5.19
URLD/6	cysteine conjugate of hydroxy-chlorobenzamide	4.20	12.36
URLD/9	mercapturic acid conjugate of hydroxy-chlorobenzamide	15.48	16.00
URLD/10	O-sulfate conjugate of dichlorobenzamide O-sulfate conjugate of thiomethyl-chlorobenzamide	5.47	5.44
URLD/13	mercapturic acid conjugate of hydroxy-chlorobenzamide	0.77	1.04
URLD/15	O-glucuronide conjugate of dichlorophenyl	0.17	0.32
URLD/16	mercapturic acid conjugate of hydroxy-chlorobenzamide	0.20	0.61
URLD/18	2,6-dichlorobenzamide (M-01)	9.49	11.72
FRLD/11		10.40	7.74
URLD/19	O-sulfate conjugate of dichlorophenyl	0.71	0.81

Overall, the multiple dosing did not have any significant impact in the absorption, distribution, metabolism and elimination compared to results after single oral dosing. Thus, the results in this study showed that the routes and the rates of excretion were maintained despite the multiple dosing, which meant that most of the radioactivity was eliminated via the urinary route within the 72 hours post multiple dosing. The distribution pattern in the tissues was similar between the males and females albeit with higher levels being observed for the males. In terms of concentration the males were found to possess tissue levels that were a mean of 6.5 times (±2.5 times) higher than those observed following a single oral low dose of 10 mg/kg (section 6.8.1.1.b), whilst the females displayed levels that were a mean of 3.1 times (±1.9) those observed following a single oral low dose (section 6.8.1.1.b). As these increases were less than half the increase in the amounts of administered product, it would appear that M-01 was not subject to bioaccumulation. Moreover, in terms of %dose, the proportion of radioactivity remaining in the tissues 144 hours post the last of 14 daily administrations of [14C]-M-01 was lower than that observed 144 hours following a single oral low dose. Thus, the mean values in tissues after single oral dose were 2.21 % for males and 1.71 % for females; whilst the mean values in tissues after repeat dose were 1.07 % for males and 0.69 % for females. The metabolism of M-01 after multiple dosing was the same compared to single oral dosing, where the biotransformation leading to the mercapturic acid conjugate was the principal metabolic pathway for

elimination of the test product. The metabolism of M-01 was completed with the excretion of several O-sulphate and O-glucuronide conjugates that were mainly eliminated in urine. The unchanged pared compound was seen in urine and faecal samples.

The proposed metabolic pathway is shown on the following page in Figure 6.8.

Figure 6.8 Proposed Metabolic Pathway following Repeated Oral Dosing with 10 mg/kg bw [14C]-M-01



intermediates (1), (2), (3) & (4) were confirmed in the High Dose Study Ref. 4

d) Acute oral toxicity of M-01

Study	M-01 : Acute toxicity in the rat after oral administration
Reference	Schüngel, M., 12/12/2003
Date performed	15/10/2003 – 26/11/2003
Test facility	[redacted] Wuppertal, Germany
Report reference	Laboratory reference no.: AT00875 Notifier reference no.: Dossier ref: C038678
Guideline(s)	OECD 423 (2001)/ EU (=EEC) 67/548/EEC (1967)/ USEPA : OPPTS 870.1100 (1998)
Deviations from the guideline	No significant deviations.
GLP	Yes and quality assured
Test material	Batch no. 8808018, purity 97.0%)
Study acceptable	Yes

In an acute oral toxicity study (2001) by the acute toxic class method, groups of 3 male and 3 female fasted Wistar rats were each administered by gavage a single oral dose of 2000 mg/kg bw M-01 (batch 8808018, purity 97.0%) in demineralised water with the aid of 2% Cremophor EL followed by a 300 mg/kg bw dose (10 ml/kg bw dose volume). Mortality and signs of reactions to treatment were recorded during a subsequent 14-day observation period. Decedents and animals killed on day 15 were necropsied.

The study was certified to be GLP compliant and satisfied the essential requirements of OECD guideline # 423 (2001) for acute oral toxicity by the Acute Toxic Class Method. Stability and homogeneity of the test substance was confirmed analytically.

Mortalities following a dose of 2000 mg/kg bw were 2/3 males and 3/3 females. Deaths occurred from 6 hours to 6 days after the administration. Clear clinical signs such as abdominal/lateral position, decreased motility, poor reflexes, reduced reactivity, spasmodic state, uncoordinated gait, laboured breathing, tachypnoea, chromodacryorrhoea, increased lacrimation, closed eyelids, narrowed palpebral fissure and piloerection were recorded.

At 300 mg/kg bw, no mortality was observed. Clinical signs observed at 300 mg/kg bw were decreased motility, uncoordinated gait and narrowed palpebral fissure were the main clinical signs. There was a vigorous depression in body weight gain (-57% when compared to 300 mg/kg bw males), in the surviving males treated with 2000 mg/kg bw. At 300 mg/kg bw, there were no significant effects on body weights in either males or females.

In animals that died or were killed in a moribund state during the observation period the macroscopic examination revealed changes including discolouration and paleness of the liver and the spleen. There were no other treatment-related findings at necropsy.

The acute oral LD50 of M-01 was 2000 mg/kg bw in males and 500 mg/kg bw in females. According to the OECD 423 guideline, M-01 should be classified as category 4 (harmful).

(Schüngel, 2003d)

e) Acute oral toxicity of M-01

Study	Preliminary Toxicity studies with 2,6-dichlorobenzamide. A. Acute oral toxicity to rats.
Reference	Kemp, A. et al (1967a)
Date performed	15/10/2003 – 26/11/2003
Test facility	[redacted] The Netherlands.
Report reference	Laboratory reference no.: 56645/01/1967; Notifier reference no.: *Tox 99324*. Dossier ref: C040448
Guideline(s)	OECD 423 (2001)/ EU (=EEC) 67/548/EEC (1967)/ USEPA: OPPTS 870.1100 (1998)
Deviations from the guideline	No significant deviations.
GLP	Not specified
Test material	Not specified
Study acceptable	Yes, as additional source of information

In a study, groups of 5 male and 5 female fasted SPF Wistar rats were administered by gavage 2,6-dichlorobenzamide suspended in 1% w/v aqueous tragacanth (dose volume, 10 ml/kg) at dose levels of 1000, 2150, 4640 and 10000 mg/kg bw. The animals were examined on every day for 14 consecutive days for any abnormal symptoms. The medium lethal dose was subsequently estimated from the tables of Horn.

The study was not certified to be GLP compliant however, the methodology is considered to be consistent with that of the former OECD guideline # 401. It is considered to provide limited but corroborative information on acute toxicity. The mean bodyweight of animals used in the study was 190 g for males and 150 g in females.

Mortalities occurred at all dose levels tested and was 1/5, 4/5, 5/5 and 5/5 in males and 0/5, 3/5, 4/5 and 5/5 in females at dose levels of 1000, 2150, 4640 and 10000 mg/kg bw respectively (Table 6.89). The signs of toxicity in animals at dose levels of $\geq$ 2150 mg/kg bw including one animal at 1000 mg/kg bw which died was progressive narcosis to the plane of surgical anaesthesia followed by exitus probably due to medullary depression. Signs of toxicity in survivors included prostration, relaxed limbs, absent righting reflex but corneal reflex present; miosis and rapid but shallow respiration

Table 6.133 Summary of the mortality and clinical signs observed in the acute oral toxicity in rats administered 2,6-dichlorobenzamide.

BAM (mg/kg bw)	Males	Females	Symptoms
1000	1/5	0/5	Prostrate, limbs relaxed, righting reflex absent but corneal reflex present; miosis and rapid but shallow respiration (refers to surviving animals only).
2150	4/5	3/5	Progressive narcosis to the plane of surgical anaesthesia. Exitus probably due to medullary depression (refers also to death at 1000 mg/kg).
4640	5/5	4/5	
10000	5/5	5/5	

The symptoms began to appear 10 minutes after treatment. Complete regression of symptoms in surviving animals occurred 24-28 hours later. All deaths occurred between 3 and 72 hours after treatment.

The LD50 values with 95% confidence levels were calculated after a 14-day observation period and were found to be 1470 (951–2270) and 2330 (1430–3780) mg/kg bw for male and female rats, respectively.

(Kemp et al, 1967a)

f) Ames test on M-01

Study	Evaluation of the possible mutagenic activity of 2, 6-dichlorobenzamide in the Ames salmonella/microsome test.
Reference	Koom, J. 1992a
Date performed	5/11/1992 – 20/11/1992
Test facility	Solvay Duphar B.V, The Netherlands
Report reference	Laboratory ref 56645/69/1992/ Notifier reference no. Dossier ref: C0040455
Guideline(s)	OECD 471 (1984); USEPA TSCA 798.5265 (1985); JMAFF, 4200 (1985); EEC 84/449/ECC (1984)
Deviations from the guideline	No significant deviation
GLP	*Yes and QA*
Test material	Batch no., Not provided.
Study acceptable	Yes

In a study (1992), the genotoxic potential of 2, 6-dichlorobenzamide was investigated in *Salmonella typhimurium* TA 1535, TA 1537, TA 1538, TA 98 and TA 100 strains. Four concentrations of the test substance were tested in triplicate in each strain. The test substance was tested in the presence and absence of S9-mix in each strain, in two independent experiments. S9-mix, cofactors mix and the test substance solutions were checked for sterility. The viable count of each culture was made by plating appropriate dilutions of the cultures on agar plates. Each culture was also examined for the number of spontaneous revertants. Revertant colonies were counted automatically with an Artek 880 colony counter. Reduced background growth or a decreased number of colonies indicates that a test compound is toxic for the bacterial strain used. Selection of an adequate range of doses was based on a preliminary toxicity test with strain TA 100, both in the presence and the absence of S9-mix. Six concentrations

were tested in duplicate for toxicity. The highest concentration of test substance used in the mutation assay was that which gave a reduced survival on selective agar plates. If no toxicity was observed, the highest concentration used in the mutation assay was the highest soluble concentration in the top agar. If solubility was good a limit concentration of 5 mg/plate was used as the highest concentration in the test.

A positive response in the assay system is considered to be a two-fold or greater increase in the mean number of revertant colonies appearing in the test plates over and above the background spontaneous reversion rate observed with the solvent control, together with evidence of a dose-response relationship. This response has to be greater than the laboratory's historical data of the solvent control. The positive response has to be reproducible in an independent experiment.

The study was certified to be GLP compliant and satisfied the essential criteria of OECD guideline # 471 (1984).

Strain TA 1538 showed an increased spontaneous mutant frequency (92-253) in the first mutation assay. Therefore the results of strain TA-1538 were not reported and the strain was not used in the second mutation assay. The use of four strains was considered to be still in accordance with the guidelines.

Toxicity and/or precipitation of the test substance was not observed at any of the concentrations tested in the preliminary study. It was therefore concluded that the highest concentration in the mutation assay with BAM should be 5000 µg/plate both in the presence and in the absence of the S9-mix, in accordance with the guidelines.

The following concentrations were used in the main study: 625; 1,250; 2,500 and 5,000 µg/plate, both in the presence and in the absence of the S9-mix. In the first as well as in the second mutation assay no increase of revertant colony counts was observed at any of the four tester strains, both in the presence and in the absence of the S9-mix.

The test substance 2,6-dichlorobenzamide (BAM) showed no evidence of mutagenic potential, either in the presence or in the absence of the S9-mix in this in vitro gene mutation assay ("Ames test") at the dose levels tested.

(Koom, 1992a)

g) Bacterial gene mutation assay with M-01

Study	M-01: Salmonella/microsome test – plate incorporation and preincubation method
Reference	Herbold, B., 2003j
Date performed	21/11/2003 – 4/12/2003
Test facility	Bayer HealthCare, molecular and genetic toxicology, Wuppertal, Germany.
Report reference	Laboratory ref AT00853/ Notifier reference no. Dossier ref: C038670
Guideline(s)	OECD 471 (1997); USEPA OPPTS 870.5100 (1998); EEC 2000/32/EC (2000)
Deviations from the guideline	No significant deviation
GLP	Yes and QA
Test material	Batch no., 08018ET, purity 96.2%.
Study acceptable	Yes

In a study (2003), the potential of M-01 to induce reverse gene mutation in bacteria. Histidine dependent auxotrophic mutants of Salmonella typhimurium (strains TA1535, TA1537, TA98, TA102 and TA100) were exposed to M-01 (batch N° 08018ET, purity 96.2%) diluted in dimethyl sulphoxide, which was also used as a negative control.

Two independent mutation tests were performed in the presence and absence of liver preparations from Aroclor 1254-induced rats (S9 mix). The first was a standard plate incorporation assay, the second involved a pre-incubation stage. Dose levels of up to 5000 µg/plate were tested in the mutation tests.

The study was certified to be GLP complaints and satisfied the essential requirements of OECD guideline # 471.

There was no indication of a bacteriotoxic effect of M-01 at doses of up to and including 5000 µg/plate. The total bacteria counts consistently produced results comparable to the negative controls, or differed only insignificantly. No inhibition of growth was noted. Higher doses had only in the plate incorporation trial a weak, strain-specific bacteriotoxic effect. Therefore they could nevertheless be used for assessment purposes. No bacteriotoxic effects were observed under preincubation conditions.

None of the five strains concerned showed in the plate incorporation test a dose-related and biologically relevant increase in mutant counts over those of the negative controls (tables 6.90 and 6.91). This applied both to the tests with and without S9 mix and was confirmed by the results of the preincubation trials. The positive controls increased mutant counts to well over those of the negative controls, and thus demonstrated the system's sensitivity and the activity of the S9 mix.

Table 6.134 Revertant colony counts obtained per plate using *S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA102 – (plate incorporation assay).

Treatment	Concentration (µg/plate)	Metabolic activation +/- S9	Mean revertant colony counts in strains				
			TA 98	TA 100	TA 1535	TA 1537	TA 102
M-01	5000	-	13	138	21	6	196
	1581	-	14	158	18	7	186
	500	-	14	144	14	6	215
	158	-	19	145	17	6	215
	50	-	14	130	17	6	237
	16	-	15	129	17	7	226
Solvent control	0	-	14	131	17	8	226
M-01	5000	+	31	165	9	11	277
	1581	+	28	163	9	10	268
	500	+	28	153	9	10	261
	158	+	31	149	8	8	277
	50	+	26	153	9	10	241
	16	+	31	153	11	9	262
Solvent control	0	+	24	136	10	9	267
Sodium azide	10	-	NA	NA	645	NA	NA
4-NPDA	0.5-10*	-	143	NA	NA	85	NA
2-nitrofluorene	0.2	-	NA	331	NA	NA	NA
MMC	0.2	-	NA	NA	NA	NA	531
2-aminoanthracene	3	+	1080	1385	210	379	509

NA : not applicable
MMC : Mitomycin C
* 4-NPDA : 4-Nitro-1,2-phenylene diamine - 0.5 µg/plate for TA 98 and 10 µg/plate for TA 1537

Table 6.135 Revertant colony counts obtained per plate using S. typhimurium strains TA 98, TA 100, TA 1535 and TA 1537 and TA 102–preincubation assay

Treatment	Concentration (µg/tube)	Metabolic activation +/- S9	Mean revertant colony counts in strains				
			TA 98	TA 100	TA 1535	TA 1537	TA 102
M-01	5000	-	19	130	14	5	214
	1581	-	24	147	11	9	239
	500	-	25	147	12	4	248
	158	-	22	144	14	9	245
	50	-	21	154	12	6	263
	16	-	26	153	13	6	256
Solvent control	0	-	18	161	11	8	253
M-01	5000	+	42	195	9	13	259
	1581	+	39	210	9	10	278
	500	+	32	197	9	10	299
	158	+	35	179	8	10	275
	50	+	37	184	7	10	291
	16	+	30	182	9	8	248
Solvent control	0	+	34	187	9	12	278
Sodium azide	10	-	NA	NA	674	NA	NA
4-NPDA	0.5-10*	-	147	NA	NA	126	NA
Nitrofurantoin	0.2	-	NA	514	NA	NA	NA
Cumene hydroperoxyde	50	-	NA	NA	NA	NA	526
2-aminoanthracene	3	+	1164	1421	165	291	425

P : precipitation
NA : not applicable
4-NPDA : 4-Nitro-1,2-phenylene diamine - 0.5 µg/plate for TA 98 and 10 µg/plate for TA 1537

M-01 was not mutagenic without and with S9 mix in the plate incorporation as well as in the preincubation modification of the Salmonella/microsome test.

(Herbold,2003j)

h) V79/HPRT test on M-01

Study	M-01 : V79/HPRT-test in vitro for the detection of induced forward mutations
Reference	Herbold, B. ; 2003d
Date performed	9/5/2003 – 16/7/2003
Test facility	Bayer HealthCare, molecular and genetic toxicology, Wuppertal, Germany.
Report reference	Laboratory ref: AT00610/ Notifier reference no. Dossier ref: C035434
Guideline(s)	OECD 476 (1997); USEPA OPPTS 870.5300 (1998); EEC (EU) 2000/32/EC (2000)
Deviations from the guideline	No significant deviation
GLP	Yes and QA
Test material	Batch no., 08018ET, purity 96.2%
Study acceptable	Yes

In a study (2003), the potential for M-01 (batch N° 08018ET, purity 96.2%) to induce point mutations at the hypoxanthine-guanine phosphoribosyl transferase locus (forward mutation assay) in V79 cell cultures after treatment with concentrations of up to and including 5000 µg/ml, both with and without S9 mix was investigated in two independent experiments.

The study was certified to be GLP compliant and satisfied the essential criteria of OECD guideline # 476.

M-01 was tested up to its limit of solubility under culture conditions. Precipitation of M-01 in the culture medium was observed at 3000 µg/ml and above. M-01 did not induce decreases in survival to treatment nor decreases in relative population growth in tests with and without S9 mix. Without and with S9 mix there was no biologically relevant increase in mutant frequency above that of the vehicle controls (Tables 6.92 and 6.93). Positive controls induced clear mutagenic effects and demonstrated the sensitivity of the test system and the activity of the S9 mix.

Tables 6.136 Relative survival and mean mutation frequency (mutant colonies per 1 millions cells) – Experiment 1-without S9 mix

Treatment	Concentration (µg/ml)	Relative survival (%)	Mutation frequency
M-01	5000	78.3p	2.45
	3000	81.2p	0.65
	1000	101.3	1.75
	500	72.4	0.70
	250	109.6	0.85
	125	94.1	1.95
Negative control	0	87.4	1.70
Solvent control	0	100.0	0.50
EMS	900	17.9	508.95
M-01	5000	74.6p	3.05
	3000	93.2p	1.85
	1000	77.6	2.65
	500	75.6	3.65
	250	100.9	0.75
	125	84.45	0.25
Negative control	0	95.3	2.85
Solvent control	0	100.0	1.70
EMS	900	10.5	412.9

P : precipitation
EMS : ethyl methanesulfonate

Tables 6.137 Relative survival and mean mutation frequency (mutant colonies per 1 millions cells) –Experiment 2-with S9 mix

Treatment	Concentration (µg/ml)	Relative survival (%)	Mutation frequency
M-01	5000	115.7p	0.55
	3000	110.1p	0.35
	1000	92.1	0.75
	500	115.6	1.10
	250	133.1	0.25
	125	96.6	0.50
Negative control	0	120.5	0.80
Solvent control	0	100.0	0.80
DMBA	20	30.9	32.65
M-01	5000	78.8p	9.30
	3000	77.5p	2.10
	1000	83.7	1.45
	500	110.5	4.90
	250	116.5	4.80
	125	107.7	0.00
Negative control	0	132.8	1.10
Solvent control	0	100.0	2.85
DMBA	20	93.3	65.35

P : precipitation
DMBA : dimethylbenzanthracene

M-01 was not mutagenic in the V79/HPRT Forward Mutation Assay both with and without metabolic activation under the conditions of the assay.

(Herbold, 2003d)

i) DNA repair test on M-01

Study	Evaluation of DNA repair inducing ability of 2,6-dichlorobenzamide (BAM) in a primary culture of rat hepatocytes (with independent repeat)
Reference	Waart E.J. van de; 1993b
Date performed	19/10/2002 – 11/3/2003
Test facility	Notox B.V., The Netherlands.
Report reference	Laboratory ref: 56345/23/93/ Notifier reference no. Dossier ref: C034068
Guideline(s)	OECD 482 (1986); USEPA : 798.5550 (1989); EEC (EU) 2000/32/EC (2000)
Deviations from the guideline	No significant deviation
GLP	Yes and QA
Test material	Batch no., FUX001000/FUN81G02C, purity 100%
Study acceptable	Yes

In a study (2003), the potential of 2,6-dichlorobenzamide (BAM) to induce DNA repair or unscheduled DNA synthesis (UDS) in a primary culture of rat hepatocytes was investigated. A freshly isolated primary culture of rat hepatocytes is exposed to a given test substance concentration for 16 h in the presence of tritiated thymidine (3HTdR). Most of the damaged DNA is repaired during that time by excision repair, thereby incorporating 3HTdR (UDS). Incorporation of 3HTdR is measured by an autoradiographic method. UDS is determined by counting the number of silver grains resulting from 3HTdR incorporation in the hepatocyte nucleus. Selection of adequate concentrations for the UDS-assay was based on a preliminary cytotoxicity test, with a (generally 4-log) range of test substance concentrations in half-log steps and treated with trypan blue. The concentration which produced a 90 % decrease in viability as compared to the control (EC10) was determined. If possible the highest dose level used in the UDS-assay was the EC10. The other four dose levels were evenly spaced in between the approximate EC10 and a dose level which showed viability comparable with the control. Negative and positive controls were included in the assay in presence and absence of metabolic activation (S9 mix).

BAM (batch FUX001000/FUN81G02C, purity 100 %) was added together with 3HTdR (10 uCi/ml; specific activity 18-30 Ci/mmol). Every dose level including positive and solvent controls was tested in triplicate. The cells were exposed overnight (18 h). The whole procedure was repeated once so that two independent experiments were carried out. After fixation of the cells the coverslips were mounted on microscopic slides and revealed by autoradiography.

The study was certified to be GLP compliant and was conducted in accordance with OECD guideline # 482 (1986)

The range finding experiment was carried out with test substance concentrations from 0.1 to 1000 µg/ml and doses selected for scoring of UDS were 0, 3, 10, 33, 100, 333 and 1000 µg/ml. No increase in the number of grains per nucleus or cytoplasm was detectable while positive controls produced significant increases in the number of grains per nucleus (Tables 6.94 and 6.95).

Tables 6.138 Induction of UDS in rat hepatocytes - Experiment 1

Test substance	Concentration (µg/ml)	Mean percentage of viable cells	Mean nuclear grain count corrected for cytoplasm
BAM	0	100	2
	3	89	1
	10	78	2
	33	88	1
	100	91	1
	333	57	1
	1000	61	0 P
4-NQO	10	75	44
DMBA	50	50	47

P : precipitation
4-NQO : 4-Nitroquinoline-N-oxide = positive control without S9 mix
DMBA : 7,12-Dimethylbenzanthracene = positive control with S9 mix

Table 6.139 Induction of UDS in rat hepatocytes - Experiment 2

Test substance	Concentration (µg/ml)	Mean percentage of viable cells	Mean nuclear grain count corrected for cytoplasm
BAM	0	100	-5
	3	83	-5
	10	93	-7
	33	82	-5
	100	98	-6
	333	87	-6
	1000	80	-6 P
4-NQO	10	97	143
DMBA	50	93	146

P : precipitation
4-NQO : 4-Nitroquinoline-N-oxide = positive control without S9 mix
DMBA : 7,12-Dimethylbenzanthracene = positive control with S9 mix

2,6-dichlorobenzamide (BAM) was negative in the DNA-repair assay using primary cell cultures of rat hepatocytes.

(Van de Waart, 1993b)

j) Micronucleus test on M-01

Study	Micronucleus test in bone marrow cells of the mouse with 2,6-dichlorobenzamide (BAM).
Reference	Waart E.J. van de; 1993a
Date performed	28/9/1992 – 30/11/1992
Test facility	RCC NOTOX., The Netherlands.
Report reference	Laboratory ref: 56345/13/93/ Notifier reference no. Dossier ref: C034071
Guideline(s)	OECD 474 (1983); USEPA : 798.5395 (1989); EEC (EU) 84/449/EEC (1984)
Deviations from the guideline	No significant deviation
GLP	Yes and QA
Test material	Batch no., FUX001000/FUN81G02C, purity 100%
Study acceptable	Yes

In a study (1992), the genotoxic potential of 2,6-dichlorobenzamide (BAM) was investigated in the in vivo micronucleus assay in mice. Three groups each comprising 5 males and 5 females, received a single oral dose of 250 mg/kg bw BAM (batch FUX001000/FUN81G02C, purity 100%). The dose selected was based on the findings of a range-finding pilot study and was considered to be the maximum tolerated acute dose). Bone marrow was sampled at 24, 48 and 72 hours after dosing. The number of micronuclei per 1000 polychromatic erythrocytes in mouse bone marrow was recorded. Corresponding vehicle (corn oil) treated groups served as negative controls. Bone marrow from a positive control group, treated with a single oral dose of cyclophosphamide (CPA) at 50 mg/kg, was harvested at 48 hours after dosing only.

The study was certified to be GLP compliant and satisfied the essential requirements of OECD guideline # 474. In a preliminary study animals (3 males and 3 females/dose group) were dosed orally with 4000, 2000, 1000 (3 males only), 500, 250 and 100 mg/kg bw. (groups 1-6 respectively). Higher concentrations could not be dosed because of aggregation of the test substance in suspension. Animals of group 1 and 2 died just after dosing. Male animals of group 3 died one day after dosing. All animals of group 4 were comatose after dosing and all female animals died or stayed comatose. Animals of group 5 showed ataxia (female) and lethargy (male and female) on the day of dosing, but recovered the next day. Animals of group 6 showed lethargy after dosing but all animals recovered. The Rappporteur does not consider these observations as indicative of neurotoxicity requiring further investigation in this area and further, there are clear dose levels at which no clinical signs are seen in short term and long term studies. Based on the results of this pilot study 250 mg/kg body weight was selected as an appropriate dose for the Micronucleus Test.

In the main study, the mean number of micronuclei scored in the test substance treated groups was compared with the corresponding control groups. No decrease in the ratio of polychromatic/normochromatic erythrocytes was observed (no toxic effect on the erythropoiesis) at a dose level of 250 mg/kg (Table 6.96). No increase in the frequency of micronuclei was observed in the polychromatic erythrocytes of the bone marrow of test substance treated animals.

Table 6.140 Mean number of micronuclei polychromatic erythrocytes (per 1000 cells) and mean ratio of polychromatic erythrocytes to total erythrocytes of male and female mice

Treatment	Sampling time (hours)	Sexes	Ratio PCE/NCE	MNPCE/1000 PCE
BAM	24	Males	1.01	1.0
	48		0.89	0.2
	72		0.97	0.0
Vehicle	24		1.01	0.4
	48		0.96	0.8
	72		0.97	1.0
CP	48		0.32	16.8*
BAM	24	Females	1.00	0.6
	48		1.04	0.4
	72		0.96	0.2
Vehicle	24		1.01	0.2
	48		1.03	0.4
	72		1.05	0.2
CPA	48		0.60	8.8*

CPA : cyclophosphamide
* $p < 0.05$ using non parametric Wilcoxon ranking test

The incidence of micronuclei in the control animals was found to be in the range of historical data while animals treated with cyclophosphamide showed a decrease in the ratio of polychromatic to normochromatic erythrocytes, which reflects a toxic effect of this compound on the erythropoiesis. The positive control substance induced in both sexes a statistically significant increase in the number of micronuclei confirming the sensitivity of the assay.

2,6-dichlorobenzamide was not clastogenic in the micronucleus test under the experimental conditions of the assay.

(Van de Waart, 1993a)

k) 13-week dietary study in rats with M-01

Study	Dietary administration of 2,6-dichlorobenzamide to male and female rats for 13 weeks
Reference	Boschman T., Kemp A., Linde H.M. van der; 1967a
Date performed	Not provided
Test facility	[redacted] The Netherlands
Report reference	Laboratory ref: 56645/2/67/ Notifier reference no. Dossier ref: C0040455
Guideline(s)	Not stated.
Deviations from the guideline	No significant deviation
GLP	*No*
Test material	Batch no.: 133/2/4/104, purity unknown.
Study acceptable	Yes

In a study (1967), groups of 10 male and 10 female Wistar rats were administered in the diet 2,6-dichlorobenzamide (batch N° 133/2/4/104, purity unknown) at concentrations of 0, 50, 180, 600 and 2300 ppm (corresponding to to 0, 4, 14, 49 and 172 mg/kg/day in combined sexes) for 13 weeks. The animals were regularly observed for signs of toxicity; bodyweights and food intake were determined weekly. The effect of the 2,6-dichlorobenzamide on skeletal muscle tone was measured at 4 intervals during the experiment. Clinical laboratory analyses of blood and urine samples were performed throughout the study. At the end of the treatment period, the clearance of bromosulphthalein by the liver and the blood clotting time were evaluated. Organ weights were determined and tissues were subjected to gross and histopathological investigations. Liver content glycogen was also recorded.

The study was certified to be GLP compliant and was not conducted in accordance with a specified guideline.

There was no treatment-related mortality during the treatment period. Clinical signs were confined to females treated at 600 and 2300 ppm which presented with hair loss during the latter treatment period. Food consumption and body weight gain were reduced in females at dose levels of ≥ 600 ppm and in males at 2300 ppm. At 2300 ppm, mean body weights were reduced from week 2 up to termination. Body weight gains were significantly reduced throughout the study in all animals at 2300 ppm and in females at 600 ppm (Table 6.97). No treatment-related body weight and food consumption changes were observed in animals at 50 and 180 ppm and in males at 600 ppm.

Table 6.141 Mean body weight and food consumption in male and female rats at termination.

Weeks 2 - 11	Dose level (ppm)									
	Males					Females				
	0	50	180	600	2300	0	50	180	600	2300
Mean body weights gain (g) (% control)	159	165	152	146	111** (70)	66	69	67	54** (82)	46** (70)
Mean food consumption (g) (% control)	1311	1344	1297	1255	1103** (84)	980	1010	1010	907** (93)	814** (83)

** $p < 0.01$; significantly different from control using the Wilcoxon test

A significant reduction in skeletal muscle tone in both sexes was observed at 600 and 2300 ppm in both males and females (Table 6.98).

Table 6.142 Mean muscle relaxation scores throughout the treatment period

ays	Dose level (ppm)									
	Males					Females				
	0	50	180	600	2300	0	50	180	600	2300
Day 4	4	7	6	17*	27*	10	7	19	17	30*
Day 21	5	6	13	16	34*	10	7	8	13	13*
Day 91	6	11	11	10	34*	7	2	9	21*	25*
Day 92	7	11	11	16	36*	6	6	10	23*	27*

** $p < 0.05$; significantly different to controls using the Wilcoxon test

No treatment-related haematological changes were observed at any dose levels. Blood chemistry parameters at 2300 ppm showed increased total protein and cholesterol concentrations in all animals at the end of the treatment period compared with controls (Table 6.99). Urea levels were increased in males at 600 and in all animals at 2300 ppm. No treatment-related clinical chemistry changes were observed at 50 and 180 ppm.

Table 6.143 Mean blood chemistry parameters at termination

Blood parameters	Dose level (ppm)									
	Males					Females				
	0	50	180	600	2300	0	50	180	600	2300
Total protein (g/100 ml)	6.1	6.1	6.0	6.2	6.8**	6.2	6.2	6.1	6.2	6.6
Cholesterol (mg/100 ml)	97	87	108	116	131**	87	100	92	95	122**
Urea (mg/100 ml)	29	30	32	37	45**	36	37	40	39	47**

**$p < 0.01$; significantly different from control using the Wilcoxon test

Urinalysis showed no treatment-related changes at any dose levels. Blood coagulation times showed a significant decrease of the thrombotest-time in both sexes at 2300 ppm compared with controls (Table 6.100).

Table 6.144 Mean blood coagulation times at termination

	Dose level (ppm)									
	Males					Females				
	0	50	180	600	2300	0	50	180	600	2300
Thrombotest-time (seconds)	66	-	-	-	52**	56	-	-	-	49*

* $p < 0.05$; ** $p < 0.01$ - significantly different from control using the Wilcoxon test

There were no clear treatment-related changes in relative organ weights at termination. Gross examination at necropsy and microscopic observation of organs and tissues did not reveal any treatment-related changes.

The continuous dietary administration of 2.6-dichlorobenzamide to male and female rats for 13 weeks produced reduced body weight gains and food consumption as well as reduced skeletal muscle tone. No toxicologically significant changes were seen in the haematology, blood chemistry or urinalyses investigations. The changes described were not associated with any histopathological findings. No effects on the liver or kidney function were observed.

The NOAEL for 2.6-dichlorobenzamide in the 90-day dietary study was 180 ppm (equivalent to 14 mg/kg bw/day) based on reduction in food consumption and bodyweight gain, reduced skeletal muscle tone at dose level of ≥ 600 ppm (49 mg/kg bw/day).

(Boschman, Kemp, Van Der Linde, 1967a)

k) 2-year dietary study in rats with M-01

Study	Effects of BAM in dietary administration to rats for two years
Reference	Wheldon G.H., 1971a; Johnson S.F., 1996
Date performed	11/1968 – 11/1970
Test facility	[redacted] England
Report reference	Laboratory ref: 56645/2/67/ Report no. PDR/49 3980/71/138 Dossier ref.: C034294/ Addendum C034296
Guideline(s)	US EPA - FIFRA 83-1 and 83-2
Deviations from the guideline	No significant deviation
GLP	Yes
Test material	Batch no.: 195, purity unknown.
Study acceptable	Yes

Groups of 35 male and 35 female Sprague Dawley CD rats were administered in the diet 2,6-dichlorobenzamide (BAM) at nominal concentrations of 0 (control), 60, 100, 180 or 500 ppm (corresponding to 0, 2.0, 3.5, 5.7 and 17.6 in males and 0, 2.7, 4.1, 8.6 and 21.3 mg/kg bw in females as determined on week 106) for 2 years. During the study, clinical condition, bodyweight, food and water consumption, ophthalmoscopic

examination, haematology, blood chemistry, urinalysis, organ weight, macroscopic and microscopic pathology investigations were undertaken.

The study was certified to comply with GLP. It is considered informative.

No treatment-related death occurred during the course of the study at any dose levels. At 500 ppm, statistically significant reduced bodyweight gains were observed in both males (-16% compared to controls on week 106) and females (-26% compared to controls on week 106). No treatment-related changes in body weight gain were observed at the lower dose levels. Ophthalmoscopic examination between controls and high dose animals did not show any treatment-related changes. Food consumption in females at 500 ppm was slightly reduced (by 8% when compared to controls).

Haematology tests showed minor depression of red cell parameters (haemoglobin concentration, erythrocyte counts and haematocrit) occasionally mainly in males and to a lesser extend in females at 500 ppm although statistical significance was not attained on every occasions (Table 6.101). At 180 ppm, no treatment-related changes of toxicological significance were observed in males and females.

Table 6.145 Group mean haematological changes on week 106

Week	Dose level (ppm)									
	Males					Females				
	0	**60**	**100**	**180**	**500**	**0**	**60**	**100**	**180**	**500**
Hematocrit (%)	46	-	-	45	**41***	42	-	-	43	43
Haemoglobin (g%)	14.9	-	-	14.8	**13.5***	14.2	-	-	13.9	**13.5**
RBC ($x10^6$/cmm)	7.80	-	-	7.69	**7.34**	7.12	-	-	6.78	7.29

* $p< 0.05$; significantly different to controls using Student's t test

Blood chemistry and urinalysis did not show any treatment-related changes at the high dose level.

No treatment-related relative or absolute organ weight changes were observed at any dose levels. Gross examination at necropsy did not reveal any treatment-related abnormalities.

The only treatment-related histopathological findings were confined to the liver and were only observed in females at 500 ppm (Table 102). These liver changes were characterized by hepatocyte vacuolation and degeneration as well as fat deposition. In addition, higher incidence (non statistically significant) of hepatoma was found in 4/20 females subjected to liver histology. Other histopathological findings were observed in some tissues. As these findings were observed among all groups including controls and frequently recorded in rats of this age and strain, they were not considered to be related to treatment.

Table: 6.146 Liver histopathology in males and females sacrificed on week 107

	Dose level (ppm)									
	Males					Females				
	0	60	100	180	500	0	60	100	180	500
N° of rats subjected to liver histology	7	10	10	10	15	8	10	10	10	20
Vacuolation, fat deposition, hepatocyte degeneration	0	1	1	0	1	0	3	0	2	9
Hepatoma	1	0	1	0	1	0	0	0	0	4

The dietary administration of 2,6-dichlorobenzamide (BAM) for 2 years in male and female rats produced histopathological liver changes in females at 500 ppm. No carcinogenic activity of BAM was detected in this study up to and including 500 ppm. The NOAEL in the 2-year dietary study 2,6-dichlorobenzamide (BAM) was 180 ppm (equivalent to 5.7 and 8.6 mg/kg bw/day in males and females, respectively) based on reduction in body weight gain in males and females and histopathological liver changes in females at 500 ppm (17.6 and 21.3 mg/kg bw in males and females respectively).

(Wheldon, 1971a; Johnson S.F., 1996)

l) Re-examination of histopathology from 2-year dietary study in rats with M-01

Study	Re-assessment of liver lesions/tumours from study PDR/49 ; BAM : dietary administration to rats for 2 years
Reference	Connick, H., Crome, S.J. and Gopinath, C., 1996a
Date performed	November 20, 1995 to May 1996
Test facility	[redacted] England
Report reference	Laboratory ref: / Report no. URL97/961569 Dossier ref.: C034295
Guideline(s)	US EPA - FIFRA 83-2
Deviations from the guideline	No significant deviation
GLP	Yes
Test material	Batch no.: 195, purity unknown.
Study acceptable	Yes

In a study (1996), a re-evaluation of the pathological findings in slides produced from liver sections taken in a rat study of 2 years duration (Huntingdon Research Centre report number PDR/49 3980171/138) conducted at Huntingdon Life Sciences between November 1968 and November 1970 and reported in October 1971 (see above C034294) was performed. Groups of 35 male and 35 female Sprague Dawley CD rats were administered in the diet 2,6-dichlorobenzamide (BAM) at nominal concentrations of 0 (control), 60, 100, 180 or 500 ppm (corresponding to 0, 2.0, 3.5, 5.7 and 17.6 in males and 0, 2.7, 4.1, 8.6 and 21.3 mg/kg bw in females as determined on week 106) for 2 years. During the study, clinical condition, bodyweight, food and water

consumption, ophthalmoscopic examination, haematology, blood chemistry, urinalysis, organ weight, macroscopic and microscopic pathology investigations were undertaken. All rats killed in extremis, found dead during the study or sacrificed at termination were subjected to detailed macroscopic examination and, where practicable, a full spectrum of tissue samples were preserved in buffered 4 % formaldehyde saline. Microscopic examination was initially confined to all rats that died during the study, all rats from Group 5 and 7 males and 8 females from the control group killed at termination. In addition, liver sections from 10 males and 10 females and mammary tissue from all rats in Groups 2, 3 and 4 were examined due to changes observed at 500 ppm.

The study was certified to comply with GLP requirements and was in accordance with USEPA FIFRA 83-2.

Histopathological examination of the liver sections available revealed a slightly increased incidence of neoplastic hepatocellular adenomas in female rats only at 500 ppm in comparison with concurrent controls (5/35, p = 0.049). No hepatocellular carcinomas were observed in females at any dose. No increase in hepatocellular tumours were seen in treated male rats (Table 6.103).

Table 6.147 Liver neoplastic findings

	Dose level (ppm)									
	Males					Females				
	0	**60**	**100**	**180**	**500**	**0**	**60**	**100**	**180**	**500**
N° livers examined	26	28	32	25	34	24	28	27	32	35
Hepatocellular adenoma	1	0	1	0	1	0	1	0	0	**5**
Hepatocellular carcinoma	2	1	2	1	0	0	0	0	0	0

Non neoplastic findings of increased incidences of focal and areas of eosinophilic and basophilic hepatocytes were detected in rats of both sexes receiving 100, 180 or 500 ppm; these findings were generally more pronounced amongst females (Table 6.148). In addition, an increased incidence of vacuolation of centrilobular hepatocytes was evident in both sexes receiving 500 ppm. This change was associated with/masked by the areas/foci of eosinophilic hepatocytes in some animals receiving 500 ppm.

Table 6.148 Liver non neoplastic findings

Parameter	Dose level (ppm)									
	Males					Females				
	0	**60**	**100**	**180**	**500**	**0**	**60**	**100**	**180**	**500**
N° livers examined	26	28	32	25	34	25	28	28	32	35
Eosinophilic hepatocytes-focal	6	12	**17****	11	**21****	5	4	7	16*	**23****
Eosinophilic hepatocytes-area	1	3	0	2	4	2	2	1	5	**18****
Basophilic hepatocytes-focal	7	11	5	6	9	9	10	6	14	**23***

*$p < 0.05$; ** $p < 0.01$ –significantly different from controls using Fisher's Exact test

The dietary administration of 2,6-dichlorobenzamide for 2 years in male and female rats produced liver changes in animals at 500 ppm. Neoplastic changes were characterized by a slight increased incidence of benign hepatocellular adenoma in females at 500 ppm of non statistical significance.

Summary

Following single oral administration of [14C]-M-01 to the male and female rat at the rates of 10 and 150 mg/kg most of the administered radioactivity was eliminated in the urine (ca 82 %dose) although the rate of elimination was relatively slow. Lower levels (ca 13 %dose) were eliminated via the faeces. The highest concentrations in tissues were seen in the kidney (ca 0.57 µg equiv./g) and liver (ca 0.44 µg equiv./g) for the 10mg/kg dose group and in the skin & fur (3.8 to 5.0 µg equiv./g), kidneys (2.8 to 3.0 µg equiv./g) and liver (2.1 to 2.3 µg equiv./g) for the 150 mg/kg dose group. Tissue concentrations therefore increased by approximately five-fold for a fifteen-fold increase in dose rate. Overall, multiple dosing (14 daily doses at 10 mg/kg) did not have any significant impact in the absorption, distribution, metabolism and elimination compared to results after single oral dosing. Thus, the results in this study showed that the routes and the rates of excretion were maintained despite the multiple dosing, which meant that most of the radioactivity was eliminated via the urinary route. The distribution pattern in the tissues was also similar between single and multiple dosing with the highest mean concentrations observed in the skin & fur (3.0 µg equiv./g), kidney (1.9 µg equiv./g) and liver (1.3 µg equiv./g). Bioretention or accumulation was therefore not indicated. The routes of biotransformation was similar between dose levels and sexes with hydrolysis of the amide group to form AE C416656, hydroxylation to form hydroxy-BAM (possibly M-04) and subsequent conjugation with either glucuronic acid or sulphate, and the loss of a chlorine atom following glutathione conjugation. Further metabolism of the glutathione group to the mercapturic acid or S-methyl metabolites was observed.

M-01 was shown to be of relatively low acute oral toxicity with an LD50 of 500 mg/kg in female rats and 2000 mg/kg bw determined by the acute toxic class method. Hi=owever, an LD50 of 1470 (951–2270) and 2330 (1430–3780) mg/kg bw for male and female rats was determined in an older pre-GLP study in which toxicity to females was less than in males. M-01 thus qualifies for an Xn classification according to the current European directive. It is notable that this metabolite is of greater acute oral toxicity than the parent, fluopicolide (LD50 > 5000 mg/kg bw), However, this difference in toxicity between fluopicolide and BAM for acute toxicity is not considered relevant under the groundwater metabolite assessment guidance because BAM is not classified as TOXIC nor is Fluopicolide.

The genotoxicity profile of M-01 was assessed in three in vitro and one in vivo assays and no evidence of genotoxicity was observed in any assays. The *in vitro* studies were the bacterial gene mutation assay in bacterial cells, V79/HPRT gene locus assay, and unscheduled DNA synthesis assay and the mouse micronucleus assay *in vivo*.

In a 13-week toxicity study performed in CD rats with M-01 at doses up to 2300 ppm reduced body weight gains and food consumption was observed at dose levels of ≥ 600 ppm but no target organ toxicity was observed. The NOAEL of M-01 was 180 ppm (equivalent to 14 mg/kg bw/day) in both males and females.

In a carcinogenicity study performed in CD rats with M-01 at doses up to 500 ppm, the liver as the principal target organ with a slightly increased incidence (of non statistical significance) of hepatocellular adenoma in females at 500 ppm. No carcinogenic effect was seen after a 2-year treatment with M-01. The NOAEL was 180 ppm, 5.7 mg/kg bw/day in males and 8.6 mg/kg bw/day in females based on reduction in bodyweight gain in both sexes and histopathological changes in the liver in females only.

In conclusion, these data showed that the toxicological profile of the metabolite M-01 is similar to that of fluopicolide based on the principles of the guidance document for the assessment of groundwater metabolites (EU Guidance Document - SANCO/221/200-rev 10, 25 February 2003).

APPENDIX 3

Position Paper

Subject:

The non-relevance of the fluopicolide metabolite M01 (AE C653711): 2,6-dichlorobenzamide (also known as BAM)

Authors:
C. Leake, E. Mackenzie, C. Kley, B. Mallyon, D. Renault, S. Pross, M. Theurig

Date:

11th April 2008

Bayer CropScience AG
Alfred Nobel Straße 50
D-40789 Monheim/Rhein
Germany

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This report is confidential. No part of this report or any information contained there in may be disclosed to any third party without the prior written authorisation of Bayer CropScience.

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1. Summary

Fluopicolide is a novel fungicide discovered and developed by Bayer CropScience, active against *Oomycetes* fungi which are responsible for late blight diseases on a wide range of crops including potatoes and vines. In 2007 it was rated by independent potato experts in Europe as the best potato late blight product (showed the best biological performance in independent trials as well as the best spectrum in controlling leaf, stem and tuber blight).

During the toxicology expert meeting organised by EFSA (European Food Safety Authority) it was requested to demonstrate the non relevance of one of the metabolites, namely M01 (AE C657311) also known as 2,6-dichlorobenzamide or BAM. This document has been prepared to summarise all the available information on this metabolite, to provide the risk assessment and to demonstrate that all the criteria for non-relevance have been met.

➢ **Exposure Assessment**

A maximum PEC_{gw} value for M01 (AE C653711) was estimated to be 6.3 µg/L in vines, and 3.2 µg/L and 2.1 µg/L in potatoes for the representative scenarios of application one year in two or one year in three, respectively.

➢ **Pesticidal / biological assessment**

M01 (AE C653711) showed no evidence of biological activity in fungicide assessment studies whereas the parent fluopicolide showed >90% control. It has also been assessed as part of the dichlobenil dossier and shown no herbicidal activity.

➢ **Toxicological assessment**

M01 has been shown:

- not to be genotoxic in an Ames, HPRT and UDS tests *in-vitro*, and in a micronucleus test *in-vivo*
- the majority is excreted via urine, both unchanged and following biotransformation, small quantities were excreted via the faeces and very low quantities were retained, showing that it is not subject to bioaccumulation
- to have a LD_{50} in the range between 500 and 2330 mg/kg and is therefore not toxic (T) or very toxic (T+)
- not to be carcinogenic
- not to be a reproductive toxicant

and is therefore not toxicologically relevant.

- **Refined Risk Assessment**.

    - A worst-case exposure assessment as recommended by the Rapporteur Member State, UK (PSD), was conducted based on the intake of M01 (AE C653711) by an infant weighing 8.7 kg and drinking 2 litres of water (*e.g.* in infant formula) containing 10 µg M01 (AE C653711)/litre water (in excess of the maximum predicted concentration) and also taking into account exposure via food intake from fluopicolide residues. The Theoretical Maximum Daily Intake on this basis was 0.00274 mg/kg/day.

    - It has been shown that when all sources of the diet are included; primary crops, rotational crops and water, M01 will contribute, as a worst-case, no more than 6% of the acceptable daily intake in total. The worst-case contribution from water is only 5% of the ADI.

- **Calculation of Margin of Safety**

    - In comparison with the lowest NOEL for M01 (AE C653711) from the dog 2-year study (*i.e.* 4.5 mg/kg/day), on which basis an ADI of 0.045 mg/kg/day is proposed, it can be seen that the Margin of Safety (MOS) is **1642** (*i.e.* 4.5 mg/kg/day divided by 0.00274 mg/kg/day).

    - In comparison with the ADI for the parent fluopicolide of 0.08 mg/kg/day it can be seen that the MOS from the NOEL for the parent fluopicolide is **2920**.

    - In either case it is clear that there is no significant risk to consumers from exposure of M01 (AE C653711) based on the proposed uses of fluopicolide.

- **Ecotoxicological assessment**

    - M01 (AE C653711) has shown to be not toxic to any of the tested aquatic organisms. Therefore it can be considered as not ecotoxicologically relevant in aquatic systems.

- **Therefore,** it is concluded that M01 (AE C653711) is not relevant as it fully meets the criteria for non-relevance according to the EU guidance document 91/414/EEC – Sanco/221/2000-rev 10, 25 February 2003.

M01 (AE C653711) has clearly and comprehensively been shown to be non-relevant.

2. Introduction

Fluopicolide is a new fungicide with a novel mode of action. Fluopicolide belongs to a new chemical class of fungicides and is highly effective on a broad spectrum of *Oomycetes* such as *Phytophthora infestans*, *Plasmopora viticola* and various *Pythium* species. These very destructive diseases are also known as “blight”, *e.g.* potato blight. Fluopicolide’s unique and novel mode of action is a valuable tool for anti-resistance management, controlling all already known resistant strains to other fungicides. In the Europe Blight Workshop in Bologna in 2007, the fluopicolide based combination with propamocarb-HCl was rated by independent potato experts in Europe as the best potato late blight product (showed the best biological performance in independent trials as well as the best spectrum in controlling leaf, stem and tuber blight). (Bradshaw, N. J., 2007)

	Effectiveness				Action mode			
Product	**Leaf blight**	**New growing point**	**Stem blight**	**Tuber blight**	**Protect-ant**	**Curative**	**Anti-sporulant**	**Rainfast-ness**
fluopicolide + propamocarb	**+++**	**++**	**++**	**+++**	**+++**	**++**	**++(+)**	**++(+)**
benthiavalicarb + mancozeb	+++	?	+(+)	+(+)	+++	+(+)	+	++(+)
cymoxanil + mancozeb	++(+)	?	+(+)	0	++	++	+	++
dimethomorph + mancozeb	++(+)	?	+(+)	++	++(+)	+	++	++(+)
cyazofamid	+++	?	+	+++	+++	0	0	+++
fluazinam	+++	?	+	++(+)	+++	0	0	++(+)
zoxamide + mancozeb	+++	?	+	++	+++	0	0	++(+)
The scores of individual active ingredients are based on the label recommendation								
Key to ratings : 0 = no effect ; + = reasonable effect ; ++ = good effect ; +++ very good effect ; ? = insufficient experience								

As part of a comprehensive development program of scientific studies, the environmental fate of fluopicolide has been determined in laboratory and field studies. In conducting these studies the metabolite M01 (AE C653711) has been found to be potentially mobile in soil.

This position paper summarises the results of investigations with the metabolite M01 (AE C653711) in a stepwise approach according to the Guidance Document on the assessment of relevance of metabolites under the EU Council Directive 91/414 EEC, dated 25th February 2003, Sanco/221/2000 – rev. 10, with special focus on its toxicological properties and the dietary risk assessment.

3. Environmental behaviour of M01 (AE C653711)

3.1. Degradation of Fluopicolide in soil resulting in the formation of M01 (AE C653711)

The metabolic pathway of fluopicolide has been determined as proceeding via hydroxylation to form AE 0608000 which is rather rapidly degraded by cleavage of the straight chain bridge to form the carboxylic acid M02 (AE C657188) and the amide M01 (AE C653711). Both metabolites are further degraded resulting in the formation of carbon dioxide and unextractable residues.

AE C638206

M03
(AE 0608000)
major transient metabolite 11 %

M02
(AE C657188)
minor metabolite 7%

unextracted residues
and carbon dioxide

M01
(AE C653711)
major metabolite maximum 25% in EU studies and 40% in US studies

M02 (AE C657188) is only a minor metabolite in soil and degrades very quickly.
In a comprehensive range of laboratory, outdoor and field studies the fate and mobility of the metabolite M01 (AE C653711) in soil was investigated.

In six field dissipation studies (three in Germany, two in France and one in Spain), due to the known mobility of M01 (AE C653711), soil samples were generally taken to depths of between 50 and 90 cm over the two year period of the studies. Soil residues of M01 (AE C653711) were found to remain in the upper soil layers and were generally not detected above the LOQ (0.005 mg/kg)

below a depth of 30 cm. However since there was a possibility of M01 (AE C653711) leaching at low levels below this LOQ a field leaching study was conducted to quantify the amount present in soil water at lower depths.

3.2 Field leaching study

The field leaching study was conducted with fluopicolide by Pollman, B., 2003, on a sandy soil in Germany to quantify the leaching potential of fluopicolide and its primary metabolite M01 (AE C653711) under realistic worst case conditions. The top soil was a low organic carbon silty sand, overlying sand subsoil. The soil properties were significantly more stringent than the lysimeter guideline requirements.

Fluopicolide, formulated as a suspo-emulsion was applied to lettuce at growth stage BBCH 14 to 19 between May and October 2000, at the rate required to achieve a total application of 400 g a.i./ha. A total of 45 suction samplers were employed to collect soil water at 5 different depths throughout the soil profile down to 150 cm. Samples of soil water were collected at intervals over a three year period and analysed by a LC/MS/MS method.

Soil leaching conditions were prevalent between May and July 2000 and from September 2000 to May 2001 in the first year of the study. Unusually wet conditions caused immediate downward movement of the bromide tracer and of the parent compound and metabolites. In the second year the leaching period was from September 2001 to June 2002 and in the third year from October 2002 to March 2003.

Annual average concentrations of fluopicolide throughout the study in the deeper soil layers were below < 0.1 µg/L. The metabolites M03 (AE 0608000) and M02 (AE C657188) were rapidly degraded, were not mobile and would not be expected to reach groundwater at concentrations exceeding 0.1 µg/L.

The metabolite M01 (AE C653711) was found to be mobile and moderately degraded in soil and consequently residues were detected in soil water at all soil depths. Maximum annual average concentrations for each of the three years of the study in the deeper soil layers were 4.4 µg/L at 85 cm (second year), 2.9 µg/L at 120 cm (third year) and 2.4 µg/L at 150 cm depth (third year).

Annual Average Concentrations in Soil Water at different depths

Time Period	Arithmetic mean annual concentration (µg/L) of M01 AE C653711	Depth
Year 1	5.320	30 cm
Year 2	6.691	
Year 3	2.930	
Year 1	3.257	50 cm
Year 2	5.764	
Year 3	3.375	
Year 1	0.845	85 cm
Year 2	4.361	
Year 3	3.346	
Year 1	0.282	120 cm
Year 2	2.548	
Year 3	2.928	
Year 1	0.085	150 cm
Year 2	1.302	
Year 3	2.415	

LOQ = Limit of quantification (0.075 µg/L)

In conclusion the residues of the metabolite M01 (AE C653711) below 120 cm will not exceed an annual average concentration of 5 µg/L and concentrations in groundwater would be expected to be lower based on the observed decline in concentrations at soil depths of 120 to 150 cm.

3.3 Predicted Environmental Concentrations in groundwater – FOCUS calculations

The leaching behaviour of fluopicolide and its metabolite M01 (AE C653711) was investigated for the use in vines and potatoes according to the European GAP by Kley, C. and Ellerich, C., 2007a and 2007b. Model calculations with the FOCUS PELMO and FOCUS PEARL models were carried out according to the FOCUS groundwater requirements.

In vines, a scenario of 3 applications of 133 g fluopicolide per hectare at 10 day intervals each year was chosen as a worst-case. In potatoes 4 applications of 100 g fluopicolide per hectare at 5 day intervals every one in two years and one in three years was assessed in accordance to agricultural practice. Due to nematode limitations potatoes are normally planted only every 3rd year in the same field. Sorption parameters for the metabolite M01 (AE C653711) were taken from laboratory batch/equilibrium experiments.

Compound	FOCUS scenario	DT_{50} (days)	K_{oc} (L/kg)	Freundlich exponent 1/n
M01 (AE C653711)	All	137.7	40.9	0.9158

Predicted 80th percentile average groundwater concentrations of M01 (AE C653711, BAM) **in vines** at 1 m depth (3 x 133 g/ha, 60 + 2 · 70% int., 10-d interval, every year; FOCUS PEARL, incl. sorption kinetic and FOCUS PELMO, no sorption kinetic)

Scenario	Annual PEC_{gw} in µg/L of M01 (AE C653711 (BAM)) in vines	
	PEARL	PELMO
Châteaudun	4.887	5.003
Hamburg	5.879	6.265
Kremsmünster	4.389	4.862
Piacenza	4.515	4.891
Porto	1.553	1.981
Sevilla	3.630	4.118
Thiva	3.875	4.645

Predicted 80th percentile average groundwater concentrations of M01 (AE C653711, BAM) in **potatoes** at 1 m depth (4 x 100 g/ha, 2 · 50 + 2 · 80 % int., 5 d interval, application **every 2 and 3 years**; FOCUS PEARL, incl. sorption kinetic and FOCUS PELMO, no sorption kinetic)

Scenario	Annual PEC_{gw} in µg/L of M01 (AE C653711 (BAM)) in potatoes			
	every 2 years		every 3 years	
	PEARL	PELMO	PEARL	PELMO
Châteaudun	2.428	1.913	1.602	1.223
Hamburg	3.153	3.152	2.100	2.003
Jokioinen	2.609	2.073	1.597	1.331
Kremsmünster	2.281	1.986	1.553	1.224
Okehampton	2.554	2.542	1.701	1.627
Piacenza	2.274	2.357	1.582	1.526
Porto	0.700	0.471	0.436	0.303
Sevilla	1.546	0.056	0.910	0.034
Thiva	1.950	0.830	1.292	0.559

In conclusion a maximum PEC_{gw} value for M01 (AE C653711) was estimated to be 6.3 µg/L in vines and 3.2 µg/L and 2.1 µg/L in potatoes for the representative scenarios of application one year in two or one year in three, respectively.

4. Assessment of the relevance of the metabolite M01 (AE C653711)

As given in the EU Guidance Document on the assessment of the relevance of a metabolite in groundwater of substances regulated under Council Directive 91/414/EEC – Sanco/221/2000-rev 10, 25 February 2003, a program of relevance testing has been completed.

The program followed the step-wise approaches as outlined in the Guidance Document:

Step 1: Exclusion of degradation products of no concern

The metabolite M01 (AE C653711) contains a phenyl ring and is similar to natural substances from soil organic matter. However, since it contains more than four carbon atoms and it is therefore not automatically of no concern.

Step 2: Quantification of potential groundwater contamination

A comprehensive range of studies have been conducted under laboratory, outdoor and field conditions to quantify the potential concentrations in groundwater and these have shown highest concentrations in the range of 2 µg/L to 6.3 µg/L.

Step 3: Hazard assessment – identification of relevant metabolites

Progressing to step 3 requires the assessment to be conducted in three stages:

Stage 1: screening for biological activity
Stage 2: screening for genotoxicity
Stage 3: screening for toxicity

A comprehensive series of hazard assessment studies have been conducted according to Step 3 and M01 (AE C653711) has been shown to have no fungicidal activity, no herbicidal activity and not to be genotoxic or classified for toxicity. These results are summarised on the next pages.

Step 4: Exposure assessment – threshold of concern approach

The exposure assessment shows that M01 (AE C653711) is above threshold of concern which is given in the Guidance Document as 0.75 µg/L and therefore has been subject to a refined risk assessment.

Step 5: Refined risk assessments for the non relevant metabolites

The refined risk assessment using a worst-case value of 10µg/L (trigger value described in the above-mentioned guidance document) has shown there is no concern when taking all contributions to the diet into consideration (overall intake being less than 6% of the ADI).

4.1 Step 3 Hazard assessment- screening for biological relevance

One of the key stages in the assessment of potential relevance of a metabolite is the determination of biological activity by comparing the activity against the biological target of the parent. Included in this assessment is the structure-activity relationship and the necessary functional groups to give the fungicidal activity that is present in the parent fluopicolide molecule.

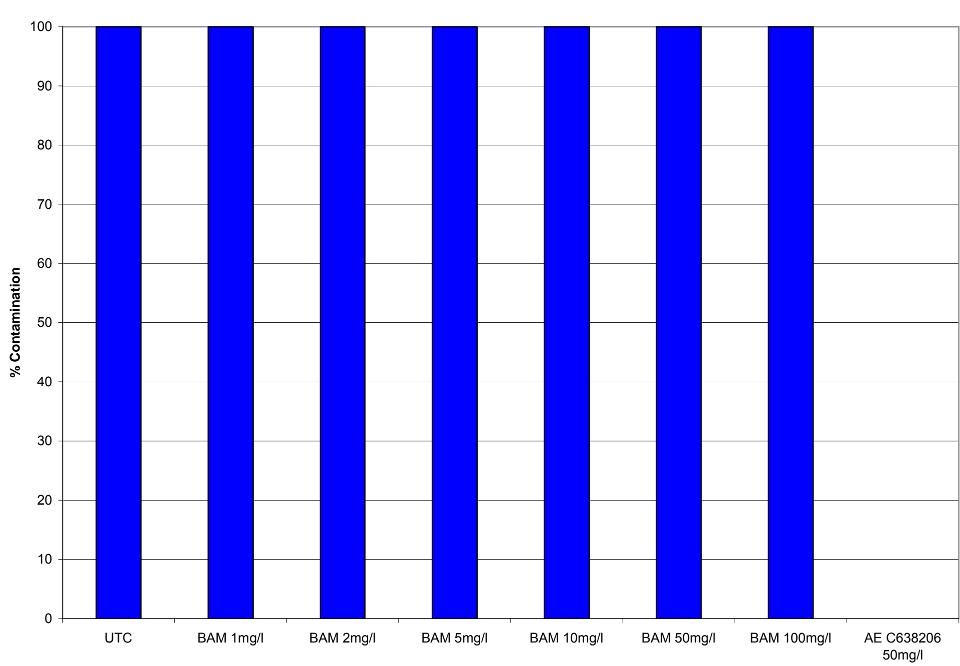
It is known from the biological screens that both the pyridine and phenyl ring parts of the molecule are required for fungicidal activity. Therefore metabolites without both these rings would be predicted to have no fungicidal activity.

M01 (AE C653711) was tested for fungicidal activity in comparison with the parent fluopicolide (Latorse, M.P., and Flahout, J. 2004).

Code	Other identifiers	structure	formula
Fluopicolide	**Parent**		**2,6-dichloro-N-{[3-chloro-5-(trifluoro-methyl)-2-pyridyl]methyl}benzamide** **$C_{14}H_8Cl_3F_3N_2O$** **MW = 383.59**
M01 (AE C653711)	**BAM**		**2,6-dichlorobenzamide** **$C_7H_5Cl_2NO$** **MW = 190.0**

M01 (AE C653711) was formulated as an SC formulation and tested at 100 mg/L irrespective that the molecular weight is approximately 50 % of the parent fluopicolide resulting in an over treatment. In addition M01 (AE C653711) was tested over a range of concentrations (0.1, 1, 3, 10, 30, 50 and 100 mg/L).

Activity of M01 (AE C653711 BAM) against late blight on potatoes



Overall the results showed no effect of M01 (AE C653711) at a range of concentrations from 1mg/L to 100 mg/L, whereas in comparison there was complete control from the parent fluopicolide at 50 mg/L.

After one treatment of 100 g a.i./ha of M01 (AE C653711) to vines no phytotoxicity was observed.

The results of fluopicolide and M01 (AE C653711) against downy mildew on grape vines in a detached leaf test showed that the metabolite M01 (AE C653711) had no biological activity, whereas at the same time the parent compound fluopicolide showed > 90 % control.

The results of fluopicolide and M01 (AE C653711) against late blight on potatoes in a detached leaf test also showed that the metabolite had no biological activity, whereas at the same time the parent compound fluopicolide showed > 90 % control in all the treatments.

Additional confirmation of M01's (AE C653711) lack of fungicidal activity was shown in a series of studies by Lechelt-Kunze, C. 2003a-d, with soil fungi *Mucor circinelloides*, *Phytophtora nicotianae, Cladorrhinum foesundissimum, Penicillium janthinellum*, and *Suillus granulatus*, where the metabolite M01 (AE C653711) showed no inhibition of growth over a range of days.

Since M01 (AE C653711) is also a metabolite of the herbicide dichlobenil it has been tested for herbicidal activity by Chemtura as part of relevance testing in the dichlobenil dossier and was found to be not herbicidally active.

In conclusion M01 (AE C653711) showed no evidence of biological activity in fungicide assessment studies, whereas the parent fluopicolide showed >90% control. As part of the dichlobenil dossier it was also shown that M01 was not active as a herbicide, compared to dichlobenil.

4.2 Step 3 Hazard assessment- screening for genotoxicity

In the guidance document there is a requirement that metabolites that have shown some potential to be mobile and are not biologically active should be screened for their genotoxic activity in a series of three *in vitro* genotoxicity studies. These three study types are the Ames test, gene mutation test with mammalian cells and the chromosome aberration test. The guidance document also states that any equivocal results from *in vitro* studies should be substantiated by *in vivo* experiments.

The genotoxicity of M01 (AE C653711) was actually assessed in four *in vitro* assays and one *in vivo* assay and no evidence of genotoxicity was observed in any of these assays.

In two *in vitro* Ames tests run in a bacterial system (Koorn, J., 1992 and Herbold, B., 2003) M01 (AE C653711) was considered to be non-mutagenic with and without metabolic activation in the several bacteria strains assayed.

In an *in vitro* V79/HPRT-test for the detection of induced forward mutations (Herbold, B., 2003a,), M01 (AE C653711) was shown to be non-mutagenic both with and without metabolic activation.

M01 (AE C653711) was also negative in a DNA-repair assay using primary cell cultures of rat hepatocytes (Waart E.J. van der, 1993a).

In addition, an *in vivo* assay was conducted in mouse bone marrow cells (Waart E.J. van de 1993b), clearly showing that M01 (AE C653711) was not clastogenic.

Summary of genotoxicity testing with M01 (AE C653711)

Test system		Results	Reference
In vitro	**Ames test**	**Negative**	**Koorn J., 1992** **Herbold B., 2003**
	HPRT test V79	**Negative**	**Herbold B., 2003a**
	UDS in rat hepatocyte	**Negative**	**Waart E.J. van der, 1993a**
In vivo	**Micronucleus test in mouse bone marrow**	**Negative**	**Waart E.J. van der, 1993b**

In conclusion, M01 (AE C653711) is not genotoxic.

4.3 Step 3 Hazard assessment- screening for toxicity

In addition to genotoxicity testing, toxicity testing has been conducted to determine whether the metabolite has certain toxicological properties which from a regulatory perspective would qualify it to be classified as relevant.

The EU Guidance Document states that if the parent compound is:

- acutely or chronically toxic or very toxic (T followed by R25, R24, R23 or R48, or T+ followed by R28, R27, R26 or R39), the metabolite must be tested for acute or chronic toxicity.
- toxic to reproduction (any category with R60, R61, R62 or R63), the metabolite must be shown not to qualify for the same classification,
- a carcinogen (category 1 or 2 followed by R45) all metabolites are considered to be relevant. For parent active substances classified as carcinogen category 3 (followed by R40) convincing evidence must be provided that the metabolite will not lead to any risk of carcinogenicity.

Taking into account that the parent compound fluopicolide was not classified for any endpoint, there is no strict requirement to undertake further testing with M01 (AE C653711) to evaluate its potential for acute, chronic, reproductive or carcinogenic potential.

However, there are several studies available on M01 (AE C653711) which were conducted a long while ago for historic reasons, and in addition there are some newer studies which were conducted to assess the relevance of M01 (AE C653711) and to better characterise the toxicity of this metabolite.

None of these studies provide evidence that M01 (AE C653711) should be classified for acute, chronic, reproduction or carcinogenic effects. Furthermore, the expert meeting (PRAPeR 39) concluded that M01 (AE C653711) should not be classified R40 for carcinogenicity.

4.3.1 Overview of metabolism studies in rats

The metabolite M01 (AE C653711) has been observed in the rat, where it was found in both male and female rat liver at 8 hours post-dosing of fluopicolide. (Fisher P.J., 2003). Hence M01 (AE C653711) is termed a “common metabolite”.

The metabolism of [^{14}C-phenyl] M01 has been investigated following a single oral high dose by Gutierrez, L. 2003 and also by the same author at a single oral low dose Gutierrez, L. 2003a. In addition a study has been conducted in which M01 was repeatedly dosed to rats at a low dose rate, Gutierrez, L 2003b.

The results of the single oral high dose showed that most of the administered radioactivity was eliminated in the urine (69-78 %) whilst only low levels were eliminated via the faeces (Gutierrez, L. 2003). Overall the quantification of radioactivity in the tissues showed low residual levels with sum totals of 1.17 % and 1.21 % for male and female rats, respectively (mean values). The highest concentration in tissues were seen in skin and fur, liver and kidney where the mean values ranged between 2.10 and 5.10 µg equivalents/g. Unchanged M01 (AE C653711) was seen in urine and

faecal samples from male and female rats. The biotransformation of M01 proceeded via diethyl-s-cysteine, o-glucuronidase, o-sulphatase enzymes and N-glucuronidase enzymes.

A similar result was obtained from the single oral low dose (Gutierrez, L., 2003a), with 66-70 % excreted via urine and 12-13 % via the faeces. Again levels in the tissues were relatively low.

In the repeat low dose oral A.D.M.E. study, Gutierrez, L., 2003b, it was shown that multiple dosing had no significant impact in the absorption, distribution, metabolism and elimination compared to the results after single oral dosing. The majority of the radioactivity was eliminated via the urinary route within 72 hour post multiple dosing. In terms of concentration the males were found to possess tissue levels that were a mean of 6.5 times (± 2.5 times) higher than those observed following a single oral low dose of 10 mg/kg, whilst the females displayed levels that were a mean of 3.1 times (± 1.9) those observed following a single oral low dose. As these increases where less than half the increase in the amounts of administered M01 it can be concluded that M01 is not subject to bio-retention.

In conclusion, the majority of the M01 (AE C653711) is excreted via urine, both unchanged and following biotransformation, small quantities were excreted via the faeces and very low quantities were retained showing that it was not subject to bioaccumulation.

4.3.2 Overview of acute oral toxicity studies

M01 (AE C653711) was shown to be of low acute oral toxicity.

In a first non-GLP study the LD_{50} values (with 95% confidence intervals) for males and females were found to be 1470 (951-2270) mg/kg and 2330 (1430-3780) mg/kg, respectively (Kemp, A. van der Linde, H.M. 1967).

A more recent GLP-study was conducted according to the current OECD 423 guideline (Schuengel, M., 2003). The highest starting dose level, which was selected to be 2000 mg/kg, induced mortality in 2 out of 3 males and 3 out of 3 females. Following the recommendations of the stepwise procedure with fixed dose levels, a second experiment was carried out at 2000 mg/kg in males and at 300 mg/kg in females. No mortality occurred in either sex. On the basis of the testing scheme presented on page 13 of the guideline, the LD_{50} cut-off was established at 2000 mg/kg in males and at 500 mg/kg in females. The value observed in females in this study is slightly lower than the one reported in the first non-GLP study. This minor difference likely results from the experimental procedure set by the OECD 423 guideline, which requires to test 2000 mg/kg as a starting point and then 300 mg/kg as a second step without any possibility to test intermediate dose levels in between in order to establish a precise experimental LD_{50}.

In conclusion, the results of both studies consistently showed that M01 (AE C653711) was not toxic (T) or very toxic (T+).

4.3.3 Overview of the key studies to evaluate the acute/chronic/long-term toxicity of M01 (AE C653711)

A number of studies have been conducted with M01 (AE C653711) to investigate its potential acute, short-term, long-term and reproductive toxicity. This study list was prepared from the US EPA Reregistration Eligibility Decision (RED) of dichlobenil (1998) and the US EPA Human Health Risk Assessment for fluopicolide (November 2007) see references.

Study Type	Year/ Study design	Results	Reference
Acute oral rat (gavage) *	**1967**	**LD_{50} = 1470 mg/kg [951-2270] (M) and 2330 mg/kg [1430-3780](F)**	**Kemp, A.**
Acute oral rat (gavage) *	**2003**	**$LD_{50} \geq 2000$ mg/kg (M) and $LD_{50} \gtrsim 500$ mg/kg (F)**	**Schuengel, M.**
90-day oral rat (dietary) *	**1967** **0, 50, 180, 600, or 2300 ppm (equal to 0, 4, 14, 49, or 172 mg/kg/day)**	**NOAEL = 180 ppm = 14 mg/kg/day** **LOAEL = 49 mg/kg/day based on decreased body weight gain (M), food intake and clinical signs (M&F)**	**Boschman, T.**
90-day oral dog (dietary) *	**1967** **0, 100, 300, or 2000 ppm (equal to 0, 7.5, 22.5, or 150 mg/kg/day)**	**NOAEL = 300 ppm = 22.5 mg/kg/day** **LOAEL = 2000 ppm = 150 mg/kg/day based on clinical signs (thin appearance, dull coat, hair loss) and increased liver weight and serum alkaline phosphatase concentrations (F) and clinical signs (thin appearance, dull coat, hair loss) (M)**	**Walker, A.I.T.**
2-year oral rat (dietary) * ***	**1967** **0, 60, 100, 180, or 500 ppm [equal to 0/0, 2.2/2.8, 3.6/4.7, 6.5/8.5 or 19/25 mg/kg/day (M/F)]**	**NOAEL = 180 ppm = 6.5 mg/kg/day (M) and 100 ppm = 4.7 mg/kg/day (F)** **LOAEL = 500 ppm = 19 mg/kg/day (M) and 180 ppm = 8.5 mg/kg/day (F) based on decreased body weights and histological liver changes in females**	**Wheldon, G.H.**
	1996	**Re-assessment of Liver lesions/tumours**	**Connick, H.,Crome, S.J. and Gopinath, C.**
	1996	**Homogeneity/Stability data addendum to report**	**Johnson, S.F.**
	2006	**Re-Assessment of liver lesions/tumours – complimentary statistical analysis**	**Pallen, C.**
	2007	**Expert opinion on the carcinogenic potential of BAM (2,6-dichlorobenzamide)**	**Gopinath, C.**

	2008	Letter from the conducting laboratory on the carcinogenic potential of BAM (2,6-dichlorobenzamide	Pilling, A.
2-year oral dog (dietary) ** ***	1971 0, 60, 100, 180, or 500 ppm (equal to 0, 1.5, 2.5, 4.5, or 12.5 mg/kg/day)	NOAEL = 4.5 mg/kg/day LOAEL = 12.5 mg/kg/day based on decreased body weight and body weight gain	Wilson, A.B. and Thorpe, E.
3-generation reproduction rat study (dietary) ** ***	1971 0, 60, 100, or 180 ppm (equivalent 0, 4.5, 7.5, or 13.5 mg/kg/day)	Parental NOAEL = 180 ppm = 13.5 mg/kg/day Parental LOAEL was not observed. Reproductive NOAEL = 180 ppm = 13.5 mg/kg/day Reproductive LOAEL was not observed. Offspring NOAEL = 180 ppm = 13.5 mg/kg/day Offspring LOAEL was not observed.	Hine, C.H., Eisenlord, G. and Loquvam, G.S.
Developmental toxicity oral rabbit (gavage) ** ***	1986 0, 10, 30, or 90 mg/kg/day	Maternal NOAEL = 30 mg/kg/day Maternal LOAEL= 90 mg/kg/day based on increased incidences of clinical signs and decreased body weight gain and food consumption during dosing Developmental NOAEL = 30 mg/kg/day Developmental LOAEL = 90 mg/kg/day	McIntyre, M.

* = Studies submitted as part of the flupicolide dossier
** = Studies not submitted as part of the fluopicolide EU dossier
*** See appendix III for a summary of this study

Summary of US EPA review program and fluopicolide registration.

Studies that were included in the fluopicolide dossier submission in the EU are included in this list and marked with a single asterisk “*”. Some studies are included in this list that were not submitted as part of the European fluopicolide dossier, since they were not required but were included in the negative reference list of the dichlobenil dossier for completeness. These studies are shown with a double asterisk “**”. This includes a 2-year dog study, a rat multi-generation study and a rabbit developmental study. These studies did not raise any concerns. The 2-year dog study was chosen by US EPA for endpoint setting for risk assessment purposes.

During the fluopicolide EU Expert Meeting on Toxicology (PRAPeR 39 expert meeting) it was indicated that in the US EPA RED of dichlobenil (1998) EPA raised concerns about the quality of

some M01 (AE C653711) studies. EPA requested data on the stability/homogeneity of the test compound and a peer review assessment of histopathology of the 2-year rat study. BCS was informed by Chemtura that the peer review for liver re-assessment requested by EPA in 1995 to upgrade the 2-year rat study was submitted to EPA in 1996. Although the RED was issued in 1998 it was essentially completed a year earlier. The Connick *et. al.* (peer review for liver re-assessment, 1996) and Johnson studies (stability/homogeneity of test compound, 1996) were not reviewed by EPA in time to be part of the RED, even though the Connick *et. al.* (1996) study was listed in the bibliography. BCS and Chemtura also understand that M01 (AE C653711) was never sent to the Cancer Peer Review Committee (CPRC) following submission of the Connick *et.al.* (1996) peer review liver reassessment.

The Connick *et. al.* (1996) study was again submitted and evaluated during the fluopicolide registration process in Europe in 2004 and the USA in 2005. As a consequence US EPA again concluded in November 2007 that M01 (AE C653711) has shown no evidence of carcinogenicity in the chronic rat study.

None of the studies indicate that M01 (AE C653711) should be classified for acute, chronic, reproductive or carcinogenic effects. In all the repeat dose studies clear no effect levels were established and there were no major findings of concern. Both the US EPA and the PRAPeR 39 expert meetings concluded that M01 (AE C653711) is not carcinogenic.

The US EPA conducted a combined risk assessment of M01 (AE C653711) derived from both uses of fluopicolide and dichlobenil and following this review **granted registration of fluopicolide on a wide range of crops and ornamentals in early 2008,** (Environmental Protection Agency 2007). Having reviewed all the available data US EPA did not formally classify M01 (AE C653711) for carcinogenicity.

4.3.4 Determination of the Acceptable Daily Intake of M01 (AE C653711)

The Acceptable Daily Intake (ADI) is defined as an estimate of the amount of a substance, expressed on a body weight basis that can be ingested daily over a life-time period without appreciable health risk. The ADI is traditionally derived from the No Observed (Adverse) Effect Level (NOAEL) of the most relevant study. According to the toxicological profile of a compound, long-term studies are usually considered the most appropriate since the ADI is intended to cover the potential risks arising from life-time exposure. By convention, a safety factor of 100 is normally used to allow for both inter and intra-species variations and to provide an adequate margin of safety.

Overview of long-term studies on M01 (AE C653711) for ADI setting

Study Type	Year/ Study design	Results	Reference
2-year oral rat (dietary) *	**1967** **0, 60, 100, 180, or 500 ppm** **[equal to 0/0, 2.2/2.8, 3.6/4.7, 6.5/8.5 or 19/25 mg/kg/day (M/F)]**	**NOAEL = 6.5 mg/kg/day (M) and 4.7 mg/kg/day (F)** **LOAEL = 500 ppm = 19 mg/kg/day (M) and 180 ppm = 8.5 mg/kg/day (F) based on decreased body weights and histological liver changes in females**	**Wheldon, G.H**
	1996	**Re-assessment of liver lesions/tumours**	**Connick, H.,Crome,S.J. and Gopinath,**

			C.
	1996	**Homogeneity/Stability data addendum to report**	**Johnson, S.F.**
	2006	**Re-Assessment of liver lesions/tumours – complimentary statistical analysis**	**Pallen, C.**
	2007	**Expert opinion on the carcinogenic potential of 2,6-dichlorobenzamide (BAM)**	**Gopinath, C.**
	2008	**Letter from the conducting laboratory on the carcinogenic potential of 2,6-dichlorobenzamide (BAM)**	**Pilling, A.**
2-year oral dog (dietary) **	**1971** **0, 60, 100, 180, or 500 ppm (equal to 0, 1.5, 2.5, 4.5, or 12.5 mg/kg/day)**	**NOAEL = 4.5 mg/kg/day LOAEL = 12.5 mg/kg/day based on decreased body weight and body weight gain**	**Wilson, A.B. and Thorpe, E.**
3-generation reproduction rat study (dietary) **	**1971** **0, 60, 100, or 180 ppm (equivalent 0, 4.5, 7.5, or 13.5 mg/kg/day)**	**Parental NOAEL = 13.5 mg/kg/day Parental LOAEL was not observed. Reproductive NOAEL = 13.5 mg/kg/day Reproductive LOAEL was not observed. Offspring NOAEL = 13.5 mg/kg/day Offspring LOAEL was not observed.**	**Hine, C.H., Eisenlord, G. and Loquvam, G.S.**

* = Studies submitted as part of the fluopicolide dossier
** = Studies not submitted as part of the fluopicolide dossier

The chronic toxicity study performed in CD rats with M01 (AE C653711) (Wheldon, G.H. 1971, with amendment and liver re-assessment Connick *et al.*, 1996) at doses up to 500 ppm showed the liver to be the target organ with a slightly higher incidence (not statistically significant) of hepatocellular adenoma in females at 500 ppm. No carcinogenicity was seen after 2 years of treatment with M01 (AE C653711). The NOEL was 180 ppm in the males, equivalent to 6.5 mg/kg/day in males and 100 ppm equivalent to 4.7 mg/kg/day in females. A more detailed evaluation and consideration of this study is given in Appendix II, in summary:

- The slightly higher incidence of liver tumours was only seen in females at the top dose level which exceeded the MTD
- The incidence of liver tumours did not reach statistical significance when analysed with appropriate methods
- Liver tumours were considered as incidental and not as a factor contributing to death
- There was no indication of progression towards malignancy
- M01 (AE C653711) was clearly not genotoxic, neither *in vitro* nor *in vivo*
- The study was not designed as an oncogenicity study
- A recent statement by Gopinath (Gopinath, C. 2007) the reviewing pathologist confirmed there was no evidence of carcinogenicity.

The chronic toxicity study performed in Beagle dogs with M01 (AE C653711) (Wilson, A.B. 1971) at doses up to 500 ppm evidenced systemic toxicity with decreases in body weight at the highest dose level. No target organs were identified after 2 years of treatment with M01

(AE C653711). The NOAEL was 180 ppm, equivalent to 4.5 mg/kg/day. A more detailed evaluation and consideration of this study is given in Appendix III.

The 3-generation study performed in Long Evans rats with M01 (AE C653711) (Hine, C.H., 1971) at doses up to 180 ppm evidenced no critical findings. The NOAEL for parental systemic and offspring toxicity and the NOAEL for reproduction was 180 ppm, equivalent to 13.5 mg/kg/day. A more detailed evaluation and consideration of this study is given in Appendix III.

The NOAELs determined in these three studies are essentially comparable and ranged from 4.5 mg/kg/day to 13.5 mg/kg/day in the 2-year dog and rat multi-generation studies, respectively. However taking the most conservative approach, Bayer CropScience considers that the most relevant study to set the ADI is the 2-year dog study, in which the lowest NOAEL was determined.

Hence the ADI from this study would be 0.045 mg/kg bw/day with a 100 fold safety factor.

ADI = 4.5 /100 = 0.045 mg/kg bw/day

The US EPA also used the dog study for setting the chronic reference dose for M01 (AE C653711). The NOEL in the rat two year study was 6.5 for males and 4.7 for females mg/kg bw/day and was therefore very similar to the dog study.

Despite the fact there were no critical findings in the 2-year dog study US EPA gave an additional 3-fold safety factor due to lack of details in the study report but concluded that a new study was not required, (see page 21 of the dichlobenil RED document). However this is not customary and Bayer CropScience does not believe this is necessary as the toxicology database available on M01 (AE C653711) is very extensive in comparison with most metabolites.

4.3.5 Comparative Toxicological Assessment

The guidance document for the assessment of the relevance of metabolites provides the scientific basis for the tests to be conducted to enable decision making on relevance.

There is a misconception that a metabolite must always be shown to be less toxic than the parent molecule in order that it can be considered as non-relevant. This thinking was discussed extensively during the preparation of the EU guidance document on relevant metabolites in groundwater and whilst this was part of the initial proposals, it was removed from later versions as it was obvious that it would mean that active substances which had more severe toxicological properties would more readily be shown to have less toxic metabolites and hence would be registerable. Whereas active substances which have no severe toxicological properties it would not so readily be shown that the metabolite was less toxic and so would not be so readily registerable. The major arguments for this decision are highlighted below and are still valid:

- if parent and metabolite are not tested under the same conditions a fair comparison is not possible
- in case parent shows more or less no toxicological findings and a very favorable profile, how to show the non relevance for metabolites?
- if two parent molecules have the same metabolite and one compound is very toxic and the other compound is very favourable, the metabolite would be not relevant in case of the toxic parent but relevant in case of the favourable parent.
- As a consequence of this comparison favourable parents would suffer and toxic parents would benefit. This is clearly not in the interest of the consumer.

It was for this reason that the logic was developed to use a classification system as given in EU Directive 67/548/EEC, based on absolute properties rather than a comparative assessment. This logic is well illustrated in the case of the M01 (AE C653711) metabolite coming from two different active substances.

Even so, if a comparison were to be made between ADI's, the ADI of fluopicolide is 0.08 mg/kg bw/day and that of M01 (AE C653711) is 0.045 mg/kg bw/day since the two values come from different species (mice 18 month chronic compared to two year dog) and within the dose setting variations the two ADI's can be regarded as comparable. In addition if one compares on a molar basis, since the molecular weight of M01 (AE C653711) is almost half that of fluopicolide and is formed on an equimolar basis from parent, then on a mole for mole basis the ADI's are virtually identical.

4.3.6 Summary

In conclusion, these data show that the toxicological profile of the metabolite M01 (AE C653711) is similar to that of fluopicolide and that M01 passes all the hazard assessment criteria of the guidance document.

Therefore from a toxicological perspective the metabolite M01 (AE C653711) passes the assessment of stages 2 and 3 of the EU guidance document and is considered non-relevant.

4.4 Step 4 Exposure assessment - threshold of concern approach.

The metabolite M01 (AE C653711) has been shown to potentially exceed 0.75 µg/L in groundwater. There are also small contributions coming from other dietary sources. A refined risk assessment as given in step 5 has therefore been conducted.

4.5 Step 5 Refined risk assessments for non-relevant metabolites.

Metabolites which have passed steps 1 to 3 and for which levels of estimated concentrations in groundwater are between 0.75 µg/L and 10 µg/L require a refined assessment.

Presence in Target Crops – Metabolism Studies

The metabolite M01 (AE C653711) has also been observed in plant metabolism studies.

In lettuce (Rupprecht, J.K. 2004) quantities of M01 ranged from 0.009 mg/kg at day 0 to 0.112 mg/kg expressed in fluopicolide parent equivalents, which is 0.0045 to 0.055 mg/kg of M01.

In grapes (Rupprecht, J.K. 2004a) quantities of M01 ranged from 0.026 mg/kg parent equivalents following application of fluopicolide at the 1x rate to 0.133 mg/kg fluopicolide parent equivalents at the 10x rate, which is 0.013 and 0.066 mg/kg of M01.

In potatoes (Rupprecht, J.K, 2004b) the quantity of M01 ranged from 0.021 mg/kg parent equivalents at the 1x rate to 0.116 mg/kg fluopicolide parent equivalents at the 10x rate, which is 0.010 and 0.0575 mg/kg in terms of M01 (AE C653711).

Presence in Target Crops – Residue Studies

However in residue studies in grapes (Sonder, K. 2003) residue values were ≤ 0.01 mg/kg and in Sonder, K. 2003a values ranged from < 0.01 to 0.05 mg/kg. In potatoes the residue values were below the LOQ of 0.01 mg/kg.

Presence in Rotational Crops - Field

In field rotational crop residue studies the quantities of M01 (AE C653711) were found to be very low with most findings below 0.01 mg/kg.

Summary of residues in crops from EU field rotational crops with M01 (AE C653711)	
Crop description	**Range of residue values (mg/kg)**
EU field rotation study year 2000/2001 (Schuengel et.al. 2004 & Zietz and Klimmek, 2003 & 2003a)	
Target crop: potatoes (mature)	all < 0.01
Rotational crop: field beans (immature)	< 0.01 to 0.02
Rotational crop: field beans (mature)	all < 0.01
Rotational crop: cabbage (mature)	all < 0.01
Rotational crop: wheat (immature straw)	< 0.01 to 0.02
Rotational crop: wheat (mature straw)	< 0.01 to 0.03
Rotational crop: wheat (mature grain)	all < 0.01
EU field rotation study year 2001/2002 (Zietz and Klimmek 2003a)	
Target crop: potatoes (mature)	all < 0.01
Rotational crop: field beans (immature)	< 0.01 to 0.01
Rotational crop: field beans (mature)	all < 0.01
Rotational crop: cabbage (mature)	< 0.01 to 0.04
Rotational crop: wheat (immature straw)	all < 0.01
Rotational crop: wheat (mature straw)	< 0.01 to 0.01
Rotational crop: wheat (mature grain)	all < 0.01

Theoretical Maximum Daily Intake (TMDI) from residues of M01 (AE C653711 BAM) in drinking water.

A worst-case exposure assessment as recommended by the Rapporteur Member State, UK (PSD), was conducted based on the intake of M01 (AE C653711) by an infant weighing 8.7 kg and drinking 2 litres of water (*e.g.* in infant formula) with a worst-case value of 10 µg M01 (AE C653711)/litre water.

The worst-case TMDI from residues of M01 (AE C653711) for an UK infant is calculated at 0.00229 mg/kg bw/day.

Theoretical Maximum Daily Intake (TMDI) from residues of M01 (AE C653711 BAM) in commodities of plant origin.

For the calculation of the TMDI from residues of M01 (AE C653711), the EFSA model for chronic and acute risk assessment rev. 2.0 was used. This model was designed to be used for the risk assessment of proposed temporary MRLs (pTMRLs) according to Regulation 396/2005 and includes the national chronic diets from 22 EU member states including the WHO cluster diets for Europe and vulnerable sub-groups as the UK toddler and the German child.

Taking the highest residue values as those given by the Highest Residue Level of M01 (AE C653711) and including all the crops the Theoretical Maximum Daily Intake (TMDI) was calculated:

Crop	Highest Residue (mg/kg)
Table grapes	0.05
Wine grapes	0.05
Raisins	0.15
Potatoes	0.01
Cabbage	0.04
All other commodities of plant origin	0.01

The TMDI for M01 (AE C653711) for the worst-case European national chronic diet was calculated at 0.00048 mg/kg bw/day for the WHO cluster diet B and at 0.00045 mg/kg bw/day for the UK toddler.

Comparison of calculated TMDI for M01 (AE C653711) with ADI

The worst-case TMDIs for M01 (AE C653711) resulting from dietary intake of drinking water (worst case 10 µg M01 (AE C653711)/litre water) and plant commodities were compared in two ways;

firstly with the ADI derived for M01 (AE C653711) from the 2-year dog study (*i.e.* 4.5 mg/kg bw/day), or alternatively the 2-year rat study (*i.e.* 4.7 mg/kg bw/day)

secondly with the ADI for fluopicolide (0.08 mg/kg bw/day) in accordance with the guidance for setting health based drinking water levels.

Comparison of calculated TMDI with ADI derived from 2-year dog study

Source of Intake	Dietary intake model	TMDI (mg/kg bw/day)	ADI (mg/kg bw/day)	% of ADI
Drinking water	UK infant	0.00229	0.045	5.08
Plant commodities	UK toddler	0.00045	0.045	1.00
Total		**0.00274**	**0.045**	**6**

Comparison of calculated TMDI with ADI derived from 2-year rat study

Source of Intake	Dietary intake model	TMDI (mg/kg bw/day)	ADI (mg/kg bw/day)	% of ADI
Drinking water	UK infant	0.00229	0.047	4.87
Plant commodities	UK toddler	0.00045	0.047	0.96
Total		**0.00274**	**0.047**	**5.8**

The TMDI resulting from the worst-case uptake of M01 (AE C653711) via drinking water and plant commodities accounts for only 5.8 % of the proposed ADI, based on the rat study or 6 % of the ADI, based on the 2-year dog study.

Taking the second approach with ADI for fluopicolide (0.08 mg/kg bw/day) the TMDI resulting from the worst case uptake of M01 (AE C653711) via drinking water and plant commodities accounts for less than 3.5% of the ADI.

Comparison of calculated TMDI with 10% of the ADI of fluopicolide

Source of Intake	Dietary intake model	TMDI (mg/kg bw/day)	fluopicolide ADI (mg/kg bw/day)	% of fluopicolide ADI
Drinking water	UK infant	0.00229	0.08	2.86
Plant commodities	UK toddler	0.00045	0.08	0.56
Total		**0.00274**	**0.08**	**3.42**

Hence it is clear that neither will water fill 10% of the ADI of fluopicolide nor will the total, including plant commodities, reach 10% of the ADI of fluopicolide.

Fluopicolide -Theoretical Maximum Daily Intake (TMDI) from residues of fluopicolide in commodities of plant origin

For the calculation of the TMDI from residues of fluopicolide, the EFSA model for chronic and acute risk assessment rev. 2.0 was used. The model was designed to be used for the risk assessment of proposed temporary MRLs (pTMRLs) according to Regulation 396/2005 and combines the national food intake models from 12 EU member states including the WHO cluster diets for Europe.

The following residue levels of fluopicolide were used for the TMDI calculation:

Crop	Proposed EU MRL (mg/kg)
Table grapes	2.0
Wine grapes	2.0
Potatoes	0.02
All other commodities of plant origin	0.01

The worst-case TMDI for fluopicolide was calculated at 0.00832 mg/kg bw/day for the French general population. Compared to the proposed ADI of 0.08 mg/kg bw/day, the TMDI for fluopicolide accounts for only 10.4% of the ADI.

This shows that even the combined uptake of fluopicolide + M01 (AE C653711) residues via drinking water and plant commodities poses no risk for the consumer even when considering exaggerated very worst-case conditions such as:

a) **the assumption that an UK infant who weighs 8.7 kg drinks 2 litres of water every day while the UK chronic water consumption figures give a mean value of 336.15 g water per day for a toddler weighing 14.5 kg (no water consumption data are available for UK infants)**

b) **performing the TMDI calculation for M01 (AE C653711) with the highest residue levels found in the different crops and not with the median residue values**

c) **choosing the worst-case for M01 (AE C653711) diet out of 22 European national chronic diets for the TMDI calculation of intake and comparison with the ADI**

5 Assessment of ecotoxicological relevance

The assessment of ecotoxicological relevance of metabolites is given in the Guidance Document on Aquatic Ecotoxicology (Sanco/3268/2001/rev. 4 (final) 17 October 2002). Metabolites that might occur in groundwater could cause exposure to aquatic life where groundwater becomes surface water and a dilution factor of 10 can be taken to give a PECsw max of 0.63 µg/L. M01 (AE C653711) may be considered as a major metabolite in aquatic systems and has been tested on the main representative and sensitive aquatic species.

Test organism	Study type	Test duration	LC/EC_{50} (mg/L)
Acute toxicity to fish			
Oncorhynchus mykiss (rainbow trout)	static acute	96 h	240
Acute toxicity to aquatic invertebrates			
Daphnia magna (water flea)	static acute	48 h	180
Effects on algal growth			
Pseudokirchneriella subcapitata (green alga)	growth inhibition test	72 h	E_bC_{50} = 60 E_rC_{50} = 120 Algistatic
Navicula pelliculosa (freshwater diatom)	growth inhibition test	72 h	E_bC_{50} > 10 E_rC_{50} > 10
Effects on aquatic plants			
Lemna gibba (duck weed)	growth inhibition test	7 d	E_bC_{50} = 80 E_rC_{50} = 97

The above studies indicate that the metabolite M01 (AE C653711) is not toxic to any of the tested representative aquatic organisms and certainly not more toxic than the parent compound to aquatic organisms (especially with regard to freshwater diatoms). In order to confirm the ecotoxicological non-relevance of M01 (AE C653711) for surface waters also Toxicity/Exposure ratio's (TER's) were calculated, using the PECsw max of 0.63 µg/L.

Taxonomic group	Lowest tox. value (µg/l)	Initial PECsw (µg/l)	TER sw	EU Trigger value
Fish	96h LC_{50}: 240,000	0.63	380,952	100
Daphnia	48h EC_{50}: 180,000	0.63	285,714	100
Diatoms	72h EbC_{50}: > 10,000	0.63	> 15,873	10
Aquatic Plants	7 d EbC_{50}: 80,000	0.63	126,984	10

Therefore it can be concluded that M01 (AE C653711) is not ecotoxicologically relevant in aquatic systems.

6 Overall conclusions

The metabolite of fluopicolide M01 (AE C653711), also known as 2,6-dichlorobenzamide (BAM), has been thoroughly investigated and a comprehensive data package is available.

A full and comprehensive risk assessment has been conducted, taking account of all sources of dietary input from fluopicolide.

Exposure Assessment

The metabolite M01 (AE C653711) has been shown to have the potential to reach maximum concentrations between 2 and 6.3 µg/L in shallow groundwater at 1 metre depth, following application of fluopicolide to potatoes and vines. A worst-case value of 10µg/L has been taken for the risk assessment.

- **Pesticidal /biological assessment** – M01 has been shown to have no fungicidal or herbicidal activity.

- **Toxicological assessment** - M01 has been shown:

  - not to be genotoxic in an Ames, HPRT and UDS tests *in-vitro*, and in micronucleus test *in-vivo*.
  - that the majority is excreted via urine, both unchanged and following biotransformation, small quantities were excreted via the faeces and very low quantities were retained, showing that it is not subject to bioaccumulation.
  - to have a LD_{50} is in the range between 500 and 2330 mg/kg and therefore not toxic (T) or very toxic (T+).
  - not to be carcinogenic
  - not to be a reproductive toxicant
  - therefore to be non-toxicologically relevant

- **Total Dietary Risk Assessment** considering all sources of the diet. It has been shown that when all sources of the diet are included; primary crops, rotational crops and water, M01 will contribute, **as a worst-case,** no more than 6% of the acceptable daily intake in total. The worst case contribution from water is only 5% of the ADI.

- **Ecotoxicological assessment –** M01 (AE C653711) has been shown not to be toxic to any of the tested aquatic organisms. Therefore it can be considered as not ecotoxicologically relevant in aquatic systems.

M01 (AE C653711) has been shown clearly and comprehensively to be non-relevant.

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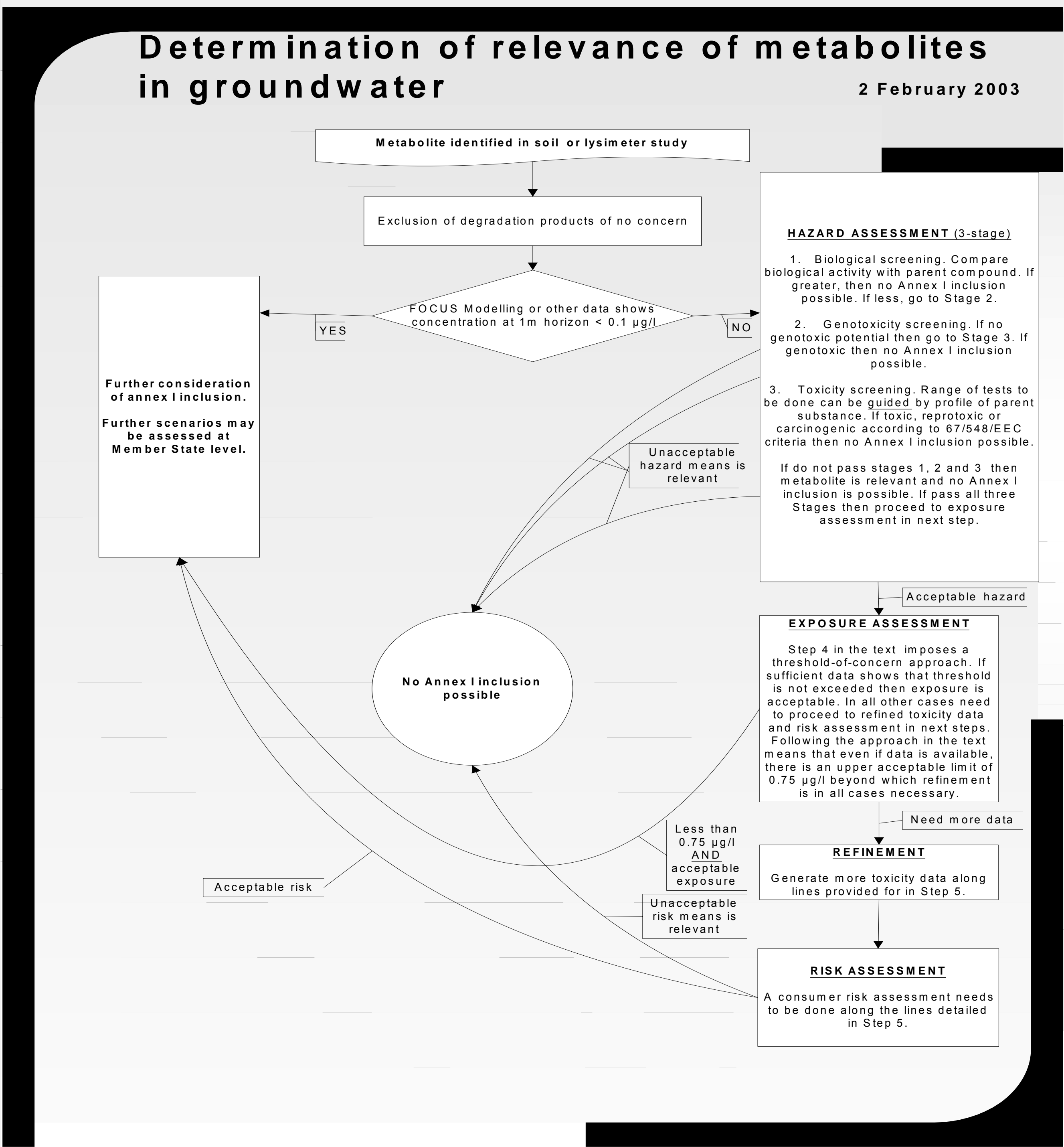
Appendix I: Flow diagram of testing for relevance



Appendix II: Evaluation and Consideration of the 2-year rat study conducted with M01 (AE C653711)

In this study, which was run in 1968 before the introduction of GLP, groups of 35 male and 35 female CD rats received M01 (AE C653711) in the diet at concentrations of either 0, 60, 100, 180 or 500 ppm (equal to 0/0, 2.2/2.8, 3.6/4.7, 6.5/8.5 or 19/25 mg/kg/day in males and females) for 106 weeks (Wheldon, G.H., 1971).

The continuous administration of M01 (AE C653711) produced clear systemic toxicity at 500 ppm and at 180 ppm to a lesser extent. Severe effects on body weights were observed in both sexes at 500 ppm. At Week 106 body weight gains were decreased by 16% in males and by 26% in females at this dose level when compared to controls showing that the Maximum Tolerated Dose (MTD) had been exceeded. Haematological changes that consisted of minor reduction in the red blood cell parameters (haemoglobin concentration, red blood cell count and haematocrit) were observed at 500 ppm in both sexes. Blood chemistry and urinalysis did not show any treatment-related effects. No changes in organ weight were noted at any dose level. Macroscopic examination did not reveal any treatment-related abnormalities.

In the original histopathological examination, the liver was identified to be the target organ. This evaluation, which was conducted on a very limited number of animals, showed that liver changes were characterised by hepatocyte vacuolation and degeneration as well as fat deposition. As a consequence of this, additional livers from high dose level animals of both sexes were examined. However, as no additional livers from control animals were evaluated, many more livers were examined in the high dose group than in the control group. Histopathology of females dosed at 500 ppm indicated a higher incidence of liver tumours diagnosed as hepatoma. No malignant tumours were observed. However, it must be noted that all liver tumour bearing high dose females were terminal kill and less than half of terminal control females were examined. Therefore the two groups were not treated in the same manner.

A re-assessment of the liver pathology was conducted in 1996 by Huntingdon Research Laboratory in order to bring the terminology up to date (Connick,H., Crome, S.J. and Gopinath., C. 1996).

As shown below, non-neoplastic changes were detected in both sexes at 500 ppm and to a lesser extent at 180 ppm in females. Males treated at 500 ppm displayed eosinophilic foci and hepatocytes vacuolation. The slightly higher incidence noted at 100 ppm was considered not to be toxicologically significant since the incidence at the next higher dose was not statistically significant. Females treated at 500 ppm showed eosinophilic foci (focal and diffuse), basophilic foci (focal) whereas females treated at 180 ppm also displayed an increased incidence of eosinophilic foci (focal).

Incidence of liver non-neoplastic changes upon re-analysis

	Male					Female				
Dose (ppm)	**0**	**60**	**100**	**180**	**500**	**0**	**60**	**100**	**180**	**500**
Total of animals examined	26	28	32	25	34	24	28	27	32	35
Eosinophilic hepatocytes focal	5	12	17 b	11	21 b	5	4	7	16 a	23 b
Eosinophilic hepatocytes diffuse	1	3	0	2	4	2	2	1	5	18 b
Basophilic hepatocytes focal	7	11	5	6	9	9	10	6	14	23 a
Basophilic hepatocytes diffuse	1	0	1	0	1	3	3	0	2	5
Hepatocyte centrilobular vacuolation	5	7	10	5	16 a	5	7	5	8	11

a: p<0.05, b: p<0.01 with Fischer Exact test

The re-assessment of the liver sections available revealed a slightly higher incidence of benign hepatocellular adenoma in females treated at 500 ppm. Four out of the five hepatocellular adenomas were observed in terminal animals showing that they were not contributing to the death of animals. No hepatocellular carcinoma was noted in either sex. Using a one-tailed pairwise comparison against control, the original statistical analysis showed that the slight increase of benign hepatocellular adenoma noted in females at 500 ppm was of borderline significance (p=0.049).

Incidence of liver neoplastic changes upon re-analysis

		Male					Female				
Dose (ppm)		**0**	**60**	**100**	**180**	**500**	**0**	**60**	**100**	**180**	**500**
Total of animals examined		26	28	32	25	34	24	28	27	32	35
Hepatocellular adenoma	D	0	0	1	0	0	0	1	0	0	1
	T	1	0	0	0	1	0	0	0	0	4
Hepatocellular carcinoma	D	1	1	1	1	0	0	0	0	0	0
	T	1	0	1	0	0	0	0	0	0	0

Since the hepatocellular adenoma were considered as incidental and not a factor contributing to the death of the animals, a complementary statistical analysis, to current standards, was carried out and showed that the slightly higher incidence of benign hepatocellular adenoma noted in females treated at 500 ppm was not statistically significant (with a p value of 0.14) (Pallen, C. 2006).

The pathologist, who conducted the re-assessment of liver section, concluded in a recent document that "the weight of evidence indicates that M01 (AE C653711) resulted in a minimally higher incidence of hepatocellular adenomas at the highest dose level, affecting only females. In the absence of any hepatocellular carcinomas amongst the liver tumours, the test substance has not shown any evidence of carcinogenic potential under the test conditions employed" (Gopinath C., 2007).

However it should be noted that:

- o Only 35 animals were allocated per group instead of a minimum of 50 to comply with current guidelines for carcinogenicity study
- o Only 24 out 35 control animals were subjected to histopathological examination whereas all

high dose group females were evaluated

- o Only 8 out 17 terminal control animals were subjected to histopathological examination whereas all terminal high dose group females were evaluated

The performing laboratory concluded in a recent statement that "in view of the above limitations" caution should be exercised when drawing "firm conclusions regarding the tumorigenic potential of BAM". "The absence of examination of liver tissue from the terminal control females precludes the possibility of judging whether the slightly higher incidence of liver cell adenoma seen at the high dose level in females was treatment-related" (Pilling A., 2008). Furthermore, the laboratory noted that the original statistical analysis would not have been significant if it had been conducted according to current standards.

Taking into account the critical deficiencies of the study and the fact that:

- o The slightly higher incidence of liver tumours was only seen in females at the top dose level which exceeded the MTD
- o The incidence of liver tumours did not reach statistical significance when analysed with appropriate methods
- o Liver tumours were considered as incidental and not as a factor contributing to death
- o There was no indication of progression towards malignancy
- o M01 (AE C653711) was clearly not genotoxic, neither *in vitro* nor *in vivo*
- o The study was not designed as an oncogenicity study had some deficiencies.

The rapporteur Member State (UK PSD) considered in the Draft Assessment Report that "**no carcinogenic effect was seen after a 2-year treatment period with M01 (AE C653711)**" (see page 475). In the Addendum 1 to the Draft Assessment Report the RMS further concluded that "**there was no evidence of substance related carcinogenicity and the weight of evidence suggests that M01 (AE C653711) is unlikely to pose a carcinogenic risk to humans and does not meet the EC criteria for classification for carcinogenicity**." (see page 45).

These conclusions are in line with those drawn by EPA in 1998 in the Reregistration Eligibility Decision (RED) of dichlobenil where **M01 (AE C653711) was considered not to pose any concerns with regard to carcinogenicity** and those very recently reached in November 2007 by the Health Effects Division of EPA which concluded that "**there was no evidence of carcinogenicity in the 2-year combined chronic toxicity/carcinogencity study of M01 (AE C653711) in rats**" (see pages 13 and 22).

On the basis of body weight effects and liver changes noted in males at 500 ppm (19 mg/kg/day) and the liver alteration reported in females at 180 ppm (8.5 mg/kg/day), the dose levels of 180 ppm (6.5 mg/kg/day) and 100 ppm (4.7 mg/kg/day) were considered to be the No Adverse Effect Levels (NOAEL) in males and females, respectively.

Appendix III: Summaries of the 2 year Dog Study, the Rat Multigeneration Study and the Rabbit developmental study conducted on M01 (AE C653711)

The following section specifically summarises those studies that were considered relevant to evaluate the chronic/long-term and reproductive toxicity of M01 (AE C653711).

- **2-year dog study**

In a non-GLP study conducted in 1971, groups of 4 male and 4 female Beagle dogs received M01 (AE C653711) in the diet at concentrations of either 0, 60, 100, 180 and 500 ppm for 2 years (equal to 0, 1.5, 2.5, 4.5 or 12.5 mg/kg/day) (Wilson, A.B. and Thorpe, E. 1971, Toxicity studies on the "Prefix" residue 2,6-dichlorobenzamide : two year oral experiment with dogs).

The continuous dietary administration of M01 (AE C653711) at 500 ppm induced clear evidence of systemic toxicity as shown by effects on body weight. Mean body weights of male were decreased by 14 % on Week 54 and by 13% at the end of dosing. Mean body weights of female were reduced by 12% on Week 15 ($p<0.01$), 21% on Week 54 ($p<0.01$) and 23% ($p<0.01$) at the end of dosing. Similar changes were noted with body weight gains. Haematology, blood chemistry and urinalysis did not reveal any treatment-related effects. No changes in organ weight were noted at any dose level. Macroscopic and histopathological examination did not show any treatment-related abnormalities.

On the basis of the body weight effects noted at 500 ppm (12.5 mg/kg/day), the dose level of 180 ppm (4.5 mg/kg/day in both sexes) was considered to be the No Adverse Effect Level (NOAEL) of the study.

- **Rat multigeneration study**

In a non-GLP study conducted in 1971, groups of 10 male and 20 female Long Evans rats received M01 (AE C653711) in the diet at concentrations of either 0, 60, 100 or 180 ppm (equal to 0, 4.5, 7.5 or 13.5 mg/kg/day) for 3 consecutive generations (Hine, C.H, et al. 1971, Results of Reproduction Study of Rats Fed Diets Containing 2,6 dichlorobenzamide (BAM) over three generations). Two litters were produced in each generation. The number of pups per litter was calculated on Postnatal Day (PND) 1, 5 and 21 and pups were weighed. Litters were culled to 10 animals on PND 5. Parents, F1 and F2 generation parental animals were weighed and examined for gross pathology on the day of sacrifice. Organ weights were measured and histopathology was performed on selected F3 weanling pups.

The continuous administration of M01 (AE C653711) throughout 3 generations produced no evidence of parental toxicity up to a dose level of 180 ppm. There were no mortalities, no effects on body weight and no changes at necropsy. Reproductive performance was not affected by treatment at any dose level in any generation. No changes in fertility and gestation indices were observed throughout 3 generations. M01 (AE C653711) produced no evidence of pup toxicity up to a dose level of 180 ppm. There were no changes in the number of pups per litter and survival. No changes in viability and lactation indices were observed throughout 3 generations. There were no consistent effects on pup body weight, changes at necropsy and after histopathological examination.

On that basis, the dose level of 180 ppm (13.5 mg/kg/day) was considered to be a No Observed Adverse Effect Level (NOAEL) for parental systemic and offspring toxicity. In the absence of any treatment-related effects on reproductive performance throughout the study, the dose level of 180 ppm (13.5 mg/kg/day) was considered to be a No Observed Adverse Effect Level (NOAEL) for reproduction.

- **Rabbit developmental study**

In a GLP study conducted in 1987, groups of 16 mated female New Zealand White rabbits were given M01 (AE C653711) orally at dose levels of either 0, 10, 30 or 90 mg/kg/day daily from day 7 to 19 of gestation inclusive (McIntyre, M., 1987, 2,6-dichlorobenzamide: Oral (Gavage) Teratology Study in the Rabbit)

Three females at 90 mg/kg/day were killed following abortion on days 19, 21 or 22 of gestation. A further two females at this dose level were killed following deterioration in physical condition on days 21 or 22 of gestation. These deaths were considered to be treatment-related following bodyweight loss, thin appearance and reduced food intake in the majority of these animals during the dosing period. No macroscopic changes were observed at examination post-mortem. Two females at 30 mg/kg/day were killed following deterioration in physical condition on days 12 and 14 of gestation and one female at 10 mg/kg/day was killed following abortion on day 23 of gestation. One control female was killed following abortion on day 20 of gestation and a further female was killed following deterioration in physical condition on day 24 of gestation. All these mortalities were considered unlikely to be related to treatment.

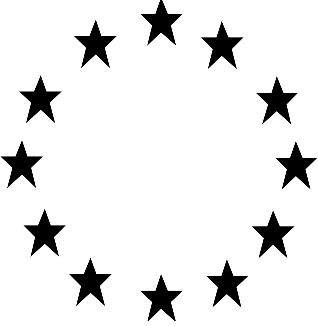
The majority of females at 90 mg/kg/day had a thin appearance and fur staining. No clinical signs were seen at 10 or 30 mg/kg/day. There was a marked decrease in group mean bodyweight and food consumption throughout the dosing period of females at 90 mg/kg/day. No change in bodyweight or food consumption was seen in females at 10 or 30 mg/kg/day.

No effect was seen on pregnancy rate, implantation rate, pre or post-implantation loss or litter size.

Mean foetal weight was slightly reduced at 90 mg/kg/day but was not affected at 10 or 30 mg/kg/day. The overall incidences of major malformations, minor external and visceral and skeletal defects and variants showed no evidence of a treatment-related effect.

In conclusion, administration of M01 (AE C653711) at a dose level of 90 mg/kg/day elicited maternal toxicity characterized by maternal deaths and bodyweight loss, and foetal weight was slightly reduced. No evidence of teratogenicity was seen whatsoever. On that basis, the No Adverse Effect level was 30 mg/kg/day in both the dam and the foetus.

Council Directive 91/414/EEC



Fluopicolide (AE C638206)

Volume 4
ADDENDUM 2

Annex C

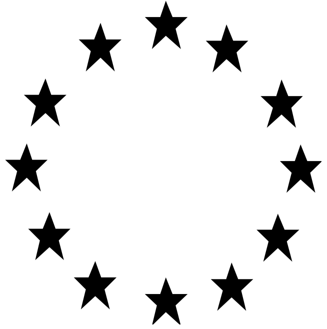
to the Report and Proposed Decision of the United Kingdom made to the European Commission under Article 8(1) of 91/414/EEC

Confidential Information

December 2008

CONFIDENTIAL INFORMATION AVAILABLE WITH RMS

Council Directive 91/414/EEC



Fluopicolide (AE C638206)

ADDENDUM 3
TO THE DRAFT ASSESSMENT REPORT PREPARED BY THE UNITED KINGDOM

February 2009

PESTICIDES SAFETY DIRECTORATE
Mallard House, Kings Pool,
3 Peasholme Green,
York YO1 7PX, UK
Website: www.pesticides.gov.uk

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B.7 RESIDUES

2.3 Data gap identified at PRAPeR 39:

Notifier to provide further information on M01 if deemed necessary.

PRAPeR 64 (19 -23 01.2009):

Data gap obsolete.

M-01 is not relevant according to the guidance document on groundwater metabolites, however a consumer risk assessment is needed as its concentration in groundwater can exceed 0.75 µg/L and an ADI of 0.05 mg/kg bw/day is set for this metabolite.

Intakes by humans

a) Metabolite M-01 (BAM)

i) Chronic exposure

NEDIs of BAM – UK Model

Commodity	Residue (mg/kg)	NEDI for Adult (mg/kg bw/day)	NEDI for Infant (mg/kg bw/day)	NEDI for Toddlers (mg/kg bw/day)	NEDI for Children Years 4-6 (mg/kg bw/day)	NEDI for Children Years 7-10 (mg/kg bw/day)	NEDI for Children Years 11-14 (mg/kg bw/day)	NEDI for Children Years 15-18 (mg/kg bw/day)	NEDI for Vegeta-rian (mg/kg bw/day)	NEDI for Elderly (Own home) (mg/kg bw/day)	NEDI for Elderly (Reside ntial) (mg/kg bw/day)
Potato	0.01	0.00004	0.00011	0.00009	0.00008	0.00007	0.00005	0.00005	0.00004	0.00003	0.00003
Grape-table	0.02	0.00003	0.00003	0.00009	0.00004	0.00005	0.00002	0.00001	0.00004	0.00003	0.00001
Wine*	0.01*	0.00006	L/C	L/C	L/C	L/C	0.00001	0.00002	0.00006	0.00004	0.00001
Cabbage	0.01	0.00001	0.00002	0.00002	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
Wheat	0.01	0.00004	0.00003	0.00008	0.00009	0.00007	0.00005	0.00004	0.00004	0.00003	0.00003
Barley	0.01	L/C	L/C	L/C	L/C	0.00001	L/C	L/C	L/C	L/C	L/C
Oats	0.01	L/C	0.00002	0.00001	0.00001	L/C	L/C	0.00001	0.00001	0.00001	0.00001
Rye	0.01	0.00001	0.00001	L/C	L/C	L/C	L/C	L/C	0.00001	L/C	L/C
Water**	0.0067	0.00017	0.0015	0.00094	0.00066	0.00044	0.00028	0.00021	0.00020	0.00019	0.00021

*the STMR from the grapes trials was 0.02, applying a processing factor of 0.5 gives a residue of 0.01 mg/kg in wine

**Water consumption taken to be 2 litres for each consumer group

L/C = Low Consumption

The NEDIs for grape, wine, potato, cabbage, wheat, barley, rye and oats (and drinking water) are all below (less than 4%) the ADI of 0.05 mg/kg bw/day.

The total NEDIs from the combined consumption of all raw commodities have been calculated using the Rees/Day model and are presented below:

Consumer groups	**Total NEDI (mg/kg bw/day)**
ADULT	0.00031
INFANT	0.0018
TODDLER	0.0011
CHILDREN (Years 4-6)	0.00087
CHILDREN (Years 7-10)	0.00057
CHILDREN (Years 11-14)	0.00039
CHILDREN (Years 15-18)	0.00043
VEGETARIAN	0.00029
ELDERLY (FREE LIVING)	0.00028
ELDERLY (INSTITUTIONAL)	0.00029

The total NEDIs for adults, children, toddlers, infants, vegetarians and the elderly are all well below (less than 4%) the ADI of 0.05 mg/kg bw/day.

TMDIs of BAM – EFSA Model (highest intake group for children and adults)

Crop	HR (mg/kg)	TMDI children (% of ADI)	TMDI adults (% of ADI)
Potato	0.01	0.049	0.11
Grape-table	0.02	0.007	0.011
Grape-wine	0.02	-	0.1
Cabbage	0.01	0.001	-
Wheat	0.01	0.11	0.078
Barley	0.01	-	0.001
Oats	0.01	0.006	0.001
Rye	0.01	0.088	0.003
Water**	0.0067	0.88	0.34
Total		1.1 (DK child)	0.64 (PT)

**Water consumption taken to be 2 litres for each consumer group

The TMDIs for adults and children are all well below (less than 2%) the ADI of 0.05 mg/kg bw/day.

ii) Acute exposure

NESTIs of BAM – UK Model

Commodity	Residue (mg/kg)	NESTI for Adult (mg/kg bw/day)	NESTI for Infant (mg/kg bw/day)	NESTI for Toddlers (mg/kg bw/day)	NESTI for Children Years 4-6 (mg/kg bw/day)	NESTI for Children Years 7-10 (mg/kg bw/day)	NESTI for Children Years 11-14 (mg/kg bw/day)	NESTI for Children Years 15-18 (mg/kg bw/day)	NESTI for Vegeta-rian (mg/kg bw/day)	NESTI for Elderly (Own home) (mg/kg bw/day)	NESTI for Elderly (Reside ntial) (mg/kg bw/day)
Potato	0.01	0.00024	0.0015	0.0011	0.0008	0.00055	0.00039	0.00029	0.0003	0.00024	0.00026
Grape-table	0.05	0.00099	0.0014	0.0031	0.0025	0.0023	0.0018	0.0009	0.0015	0.00056	0.0004
Wine	0.03	0.00044	0.00014	0.00009	0.00012	0.00003	0.00013	0.00035	0.00039	0.00024	0.00006
Cabbage	0.04	0.00049	0.0017	0.001	0.0013	0.0007	0.00067	0.00048	0.00068	0.00053	0.00039
Wheat	0.01	0.00006	0.00013	0.00013	0.00014	0.00011	0.00009	0.00008	0.00008	0.00005	0.00005
Barley	0.01	0.00001	L/C	0.00001	0.00002	0.00006	L/C	0.00001	0.00001	L/C	L/C
Oats	0.01	0.00001	0.00003	0.00003	0.00002	0.00006	0.00001	0.00001	0.00001	0.00001	0.00001
Rye	0.01	0.00001	0.00006	0.00001	0.00002	0.00001	0.00001	0.00001	0.00002	0.00001	L/C
Water**	0.0067	0.00017	0.0015	0.00094	0.00066	0.00044	0.00028	0.00021	0.00020	0.00019	0.00021

*the HR from the grapes trials was 0.05, applying a processing factor of 0.5 gives a residue of 0.03 mg/kg in wine

**Water consumption taken to be 2 litres for each consumer group

L/C = Low Consumption

The NESTIs for grape, wine, potato, cabbage, wheat, barley, rye and oats are all below (less than 2%) the ARfD of 0.3 mg/kg bw/day.

IESTIs of BAM – EFSA Model

Crop	HR (mg/kg)	IESTI children (% of ARfD)	IESTI adults (% of ARfD)
Potato	0.01	0.5 (UK infant)	0.1 (UK)
Grape-table	0.05	1.1 (DE child)	0.5 (NL)
Grape-wine	0.05	0.1 (UK infant)	0.4 (UK)
Cabbage	0.04	0.7 (NL child)	0.4 (NL)
Wheat	0.01	<0.1 (UK 4-6 year old)	<0.1 (UK)
Barley	0.01	<0.1 (UK 4-6 year old)	<0.1 (NL)
Oats	0.01	<0.1 (DE child)	<0.1 (LT)
Rye	0.01	<0.1 (UK Infant)	<0.1 (LT)
Water**	0.0067	0.9	0.3

**Water consumption taken to be 2 litres for each consumer group

The IESTIs for grape (table and wine), potato, cabbage, wheat, barley, rye and oats are all below (less than 2%) the ARfD of 0.3 mg/kg bw/day.

b) Metabolite M-05

i) Chronic exposure

NEDIs of M-05 – UK Model

Commodity	Residue (mg/kg)	NEDI for Adult (mg/kg bw/day)	NEDI for Infant (mg/kg bw/day)	NEDI for Toddlers (mg/kg bw/day)	NEDI for Children Years 4-6 (mg/kg bw/day)	NEDI for Children Years 7-10 (mg/kg bw/day)	NEDI for Children Years 11-14 (mg/kg bw/day)	NEDI for Children Years 15-18 (mg/kg bw/day)	NEDI for Vegeta-rian (mg/kg bw/day)	NEDI for Elderly (Own home) (mg/kg bw/day)	NEDI for Elderly (Reside ntial) (mg/kg bw/day)
Water**	0.0009	0.00003	0.00014	0.00009	0.00009	0.00006	0.00004	0.00003	0.00003	0.00003	0.00003

**Water consumption taken to be 2 litres for each consumer group

The NEDIs for drinking water are all below (less than 0.3%) the ADI (set for BAM) of 0.05 mg/kg bw/day.

ii) Acute exposure

NESTIs of M-05 – UK Model

Commodity	Residue (mg/kg)	NESTI for Adult (mg/kg bw/day)	NESTI for Infant (mg/kg bw/day)	NESTI for Toddlers (mg/kg bw/day)	NESTI for Children Years 4-6 (mg/kg bw/day)	NESTI for Children Years 7-10 (mg/kg bw/day)	NESTI for Children Years 11-14 (mg/kg bw/day)	NESTI for Children Years 15-18 (mg/kg bw/day)	NESTI for Vegeta-rian (mg/kg bw/day)	NESTI for Elderly (Own home) (mg/kg bw/day)	NESTI for Elderly (Reside ntial) (mg/kg bw/day)
Water**	0.0009	0.00003	0.00014	0.00009	0.00009	0.00006	0.00004	0.00003	0.00003	0.00003	0.00003

**Water consumption taken to be 2 litres for each consumer group

The NESTIs for drinking water are all below (less than 0.05%) the ARfD (set for BAM) of 0.3 mg/kg bw/day.

c) Metabolite M-10

i) Chronic exposure

NEDIs of M-10 – UK Model

Commodity	Residue (mg/kg)	NEDI for Adult (mg/kg bw/day)	NEDI for Infant (mg/kg bw/day)	NEDI for Toddlers (mg/kg bw/day)	NEDI for Children Years 4-6 (mg/kg bw/day)	NEDI for Children Years 7-10 (mg/kg bw/day)	NEDI for Children Years 11-14 (mg/kg bw/day)	NEDI for Children Years 15-18 (mg/kg bw/day)	NEDI for Vegeta-rian (mg/kg bw/day)	NEDI for Elderly (Own home) (mg/kg bw/day)	NEDI for Elderly (Reside ntial) (mg/kg bw/day)
Water**	0.00083	0.00003	0.00014	0.00009	0.00009	0.00006	0.00004	0.00003	0.00003	0.00003	0.00003

**Water consumption taken to be 2 litres for each consumer group

The NEDIs for drinking water are all below (less than 0.3%) the ADI (set for BAM) of 0.05 mg/kg bw/day.

ii) Acute exposure

NESTIs of M-10 – UK Model

Commod ity	Residue (mg/kg)	NESTI for Adult (mg/kg bw/day)	NESTI for Infant (mg/kg bw/day)	NESTI for Toddlers (mg/kg bw/day)	NESTI for Children Years 4-6 (mg/kg bw/day)	NESTI for Children Years 7-10 (mg/kg bw/day)	NESTI for Children Years 11-14 (mg/kg bw/day)	NESTI for Children Years 15-18 (mg/kg bw/day)	NESTI for Vegeta-rian (mg/kg bw/day)	NESTI for Elderly (Own home) (mg/kg bw/day)	NESTI for Elderly (Reside ntial) (mg/kg bw/day)
Water**	0.00083	0.00003	0.00014	0.00009	0.00009	0.00006	0.00004	0.00003	0.00003	0.00003	0.00003

**Water consumption taken to be 2 litres for each consumer group

The NESTIs for drinking water are all below (less than 0.05%) the ARfD (set for BAM) of 0.3 mg/kg bw/day.

d) Metabolite M-11

i) Chronic exposure

NEDIs of M-11 – UK Model

Commodity	Residue (mg/kg)	NEDI for Adult (mg/kg bw/day)	NEDI for Infant (mg/kg bw/day)	NEDI for Toddlers (mg/kg bw/day)	NEDI for Children Years 4-6 (mg/kg bw/day)	NEDI for Children Years 7-10 (mg/kg bw/day)	NEDI for Children Years 11-14 (mg/kg bw/day)	NEDI for Children Years 15-18 (mg/kg bw/day)	NEDI for Vegeta-rian (mg/kg bw/day)	NEDI for Elderly (Own home) (mg/kg bw/day)	NEDI for Elderly (Reside ntial) (mg/kg bw/day)
Water**	0.00081	0.00003	0.00014	0.00009	0.00009	0.00006	0.00004	0.00003	0.00003	0.00003	0.00003

**Water consumption taken to be 2 litres for each consumer group

The NEDIs for drinking water are all below (less than 0.3%) the ADI (set for BAM) of 0.05 mg/kg bw/day.

ii) Acute exposure

NESTIs of M-11 – UK Model

Commod ity	Residue (mg/kg)	NESTI for Adult (mg/kg bw/day)	NESTI for Infant (mg/kg bw/day)	NESTI for Toddlers (mg/kg bw/day)	NESTI for Children Years 4-6 (mg/kg bw/day)	NESTI for Children Years 7-10 (mg/kg bw/day)	NESTI for Children Years 11-14 (mg/kg bw/day)	NESTI for Children Years 15-18 (mg/kg bw/day)	NESTI for Vegeta-rian (mg/kg bw/day)	NESTI for Elderly (Own home) (mg/kg bw/day)	NESTI for Elderly (Reside ntial) (mg/kg bw/day)
Water**	0.00081	0.00003	0.00014	0.00009	0.00009	0.00006	0.00004	0.00003	0.00003	0.00003	0.00003

**Water consumption taken to be 2 litres for each consumer group

The NESTIs for drinking water are all below (less than 0.05%) the ARfD (set for BAM) of 0.3 mg/kg bw/day.

e) Conclusion

Acute and chronic exposure has been modelled for all the metabolites that exceed 0.75 μg/L.

For M-01, the NEDIs for grape, wine, potato, cabbage, wheat, barley, rye and oats (and drinking water) are less than 4% the ADI of 0.05 mg/kg bw/day. The total NEDIs for adults, children, toddlers, infants, vegetarians and the elderly are also less than 4% of the ADI. The TMDIs for adults and children are less than 1% of the ADI. The NESTIs for grape, wine, potato, cabbage, wheat, barley, rye and oats are all below (less than 2%) the ARfD of 0.3 mg/kg bw/day. The IESTIs for grape (table and wine), potato, cabbage, wheat, barley, rye and oats are also less than 2% of the ARfD.

For the Metabolites M-05, M-10 and M-11 the acute and chronic exposure are all less than 1% of the ADI and ARfD.

B.9 ECOTOXICOLOGY

Following discussion at EFSA PRAPeR 63 (Jan 2009) meeting, it was concluded that some ecotoxicological issues needed further clarification and amendment toLOEPs. These were identified as 5 Open Points (5.13, 5.5, 5.7, 5.15) in the Evaluation Table (Evaluation Table, fluopicolide, Rev 2-1, (300-01-2009)). The RMS has addressed these issues in this Addendum (3) and where appropriate as amended the LOEPs.

Open point 5.13 (new) – long term risk to herbivorous mammals in vineyards

RMS to include a note in the LoEP for the long-term risk assessment for herbivours mammals with the explanations, that the current risk assessment of mammals covers only one out of three applications in vineyards during early growth stages (up to BBCH 57).

For clarification, the long term risk to herbivorous mammals in vineyard has been re-presented in this addendum. The proposed vineyard GAP for fluopicolide (product 'EXP11074B') is a maximum individual dose of 0.133 kg fluopicolide/ha applied 3x per annum with a 10d spray interval at vine growth stages between BBCH 53-81. In accordance with SANCO 4145/2000 a dietary risk assessment is required for small herbivorous mammals feeding on sub-canopy ground vegetation (see Table 9.1).

Table 9.1 Long term mammalian risk assessment

Vineyard: 3x 0.133 kg a.s./ha, 10d spray interval, BBCH 53-81

Applic. rate (kg/ha)	FIR bw	MAF_{LT}	Deposition factor	f_{twa}	RUD (mean)	ETE $_{LT}$ (mg a.s./kg bw/d)	NOEC (mg a.s.kg bw/d)	TER	Annex VI
Small herbivore - SANCO 4145/2000 Tier I long term risk assessment									
0.133	1.39	1.8[4]	0.6[1]	0.53	76	8.04	20.0	***2.5***	5
Small herbivore - Refined long term risk assessment:									
0.133	1.39	1.8[4]	0.3[2]	0.53	76	4.02	20.0	5.0	5
0.133	1.39	1.8[4]	0.4[3]	0.53	76	5.36	20.0	***3.7***[4]	5
0.133	1.39	1.5[5]	0.4[3]	0.53	76	4.47	20.0	***4.5***[5]	5
0.133	1.39	1.0[6]	0.4[3]	0.53	76	2.98	20.0	6.7[6]	5

[1] SANCO 4145/2000 Tier 1 default based on 40% canopy interception
[2] based on ≥70% canopy interception BBCH53-81 (environmental exposure modelling assumption)
[3] based on ≥60% canopy interception assumed for BBCH53-57 (10d spray interval)
[4] based on 3 applications for BBCH53-57 (10d spray interval)
[5] based on 2 applications for BBCH53-57 (10d spray interval)
[6] based on 1 application for BBCH53-57

A TER<Annex VI trigger at Tier 1 of the SANCO 4145/2000 long term risk assessment, which assumes a 40% canopy interception (see Table 9.1), indicated that the chronic risk to herbivorous mammals required further consideration. A revised risk

assessment refining canopy spray interception and its effect on deposition on ground vegetation was undertaken.

Initially a 70% canopy spray interception was assessed (in line with all environmental exposure modelling assumptions). This derived a TER of 4.98 for the long term risk to herbivorous, i.e. close to the Annex VI trigger of 5. The RMS concluded that this was sufficient to demonstrate a low long term risk to herbivorous mammals in vineyards.

However, at PRAPeR 38 (Dec 2007) it was concluded that the canopy at early growth stages (BBCH53- 57) may not be fully developed, consequently, an interception of 60% was regarded as more appropriate at these growth stages. A revised refined long term risk assessment for herbivorous mammals was prepared which indicated that TER was now less that Annex VI trigger (see Table 9.1). However, a reduction in the number of applications during BBCH53-57 generated TERs approaching, or above, the Annex VI trigger value (Table 9.1).

Conclusion

For GAP applications at all growth stages (BBCH53-81), assuming ≥70% canopy interception, a low chronic risk to small herbivorous mammals consuming sub-canopy ground vegetation in vineyards is indicated. However, for early applications (BBCH53-57), assuming lower canopy interception (≥60%), the TERs indicate that for this period further risk refinement or mitigation (e.g. reduced number of applications) may need to be considered if the canopy is not fully developed.

Open point 5.5 – ecotoxicological relevance of GW metabolites

RMS to include the information and argumentation regarding the ecotoxicological relevance of GW metabolites presented in column 3 in an addendum for the sake of completeness.

The aquatic risk posed by fluopicolide ground water metabolites (PECgw >0.0001 mg/L) was assessed by deriving TERs for the most sensitive aquatic species (*Navicula pelliculosa* - FW diatom) with the worse case FOCUS groundwater PECgws from use vines (Table 9.2) and potato (Table 9.3).

Table 9.2 Aquatic risk posed by fluopicolide GW metabolites from vine use.

EXP 11074B: vine (3 x 0.133 kg fluopicolide/ha; 10d spray interval; BBCH 53-81) SEU

GW Metab.	Scenario	Test organism	Time scale	72h E_bC50 (mg/L)	PEC_{sw}[2] (mg/L)	TER	Annex VI trigger
Worse case PECgws from PEARL model							
M-01	Hamburg	*N. pelliculosa*	Chronic	>10.0	0.0006075	>16461	10
M-03	Hamburg	*N. pelliculosa*	Chronic	0.0029[1]	0.0000517	56	10
M-05	Hamburg	*N. pelliculosa*	Chronic	>10.0	0.000723	>138313	10
M-10	Hamburg	*N. pelliculosa*	Chronic	0.0029[1]	0.0000446	65	10
M-11	Hamburg	*N. pelliculosa*	Chronic	0.0029[1]	0.0000348	83	10
M-12	Hamburg	*N. pelliculosa*	Chronic	0.0029[1]	0.0000232	125	10
M-13	Hamburg	*N. pelliculosa*	Chronic	0.0029[1]	0.0000184	158	10
Worse case PECgws from PELMO model							
M-01	Hamburg	*N. pelliculosa*	Chronic	>10.0	0.0006265	>15962	10
M-03	Hamburg	*N. pelliculosa*	Chronic	0.0029[1]	0.0000525	55	10
M-05	Hamburg	*N. pelliculosa*	Chronic	>10.0	0.0000715	>139860	10
M-10	Hamburg	*N. pelliculosa*	Chronic	0.0029[1]	0.0000586	49	10
M-11	Hamburg	*N. pelliculosa*	Chronic	0.0029[1]	0.0000516	56	10
M-12	Hamburg	*N. pelliculosa*	Chronic	0.0029[1]	0.0000344	84	10
M-13	Hamburg	*N. pelliculosa*	Chronic	0.0029[1]	0.000216	134	10

[1] based on parent fluopicolide end point (0.029 mg/L) with 10x assessment factor
[2] based on PECgw x 0.1 (PECgw to PECsw dilution correction – SANCO 3268/2001)

Table 9.3 Aquatic risk posed by fluopicolide GW metabolites from potato use.

EXP 11120A: potato (4x 0.1 kg fluopicolide/ha; 5d spray interval; BBCH 20-91)

GW Metab.	Scenario	Test organism	Time scale	72h E_bC50 (mg/L)	$PEC_{sw}^{2,3}$ (mg/L)	TER	Annex VI trigger
Worse case PECgws from PEARL model							
M-01	Hamburg	*N. pelliculosa*	Chronic	>10.0	0.0006743	>14830	10
M-03	Hamburg	*N. pelliculosa*	Chronic	0.0029[1]	0.0000477	61	10
M-05	Hamburg	*N. pelliculosa*	Chronic	>10.0	0.0000749	>133511	10
M-10	Hamburg	*N. pelliculosa*	Chronic	0.0029[1]	0.0000492	59	10
M-11	Hamburg	*N. pelliculosa*	Chronic	0.0029[1]	0.0000502	58	10
M-12	Jokioinen	*N. pelliculosa*	Chronic	0.0029[1]	0.0000335	87	10
M-13	Jokioinen	*N. pelliculosa*	Chronic	0.0029[1]	0.0000272	107	10
Worse case PECgws from PELMO model							
M-01	Hamburg	*N. pelliculosa*	Chronic	>10.0	0.0006733	>14852	10
M-03	Hamburg	*N. pelliculosa*	Chronic	0.0029[1]	0.0000275	105	10
M-05	Hamburg	*N. pelliculosa*	Chronic	>10.0	0.0000592	>168919	10
M-10	Jokioinen	*N. pelliculosa*	Chronic	0.0029[1]	0.0000534	54	10
M-11	Jokioinen	*N. pelliculosa*	Chronic	0.0029[1]	0.0000813	36	10
M-12	Jokioinen	*N. pelliculosa*	Chronic	0.0029[1]	0.0000542	54	10
M-13	Jokioinen	*N. pelliculosa*	Chronic	0.0029[1]	0.0000369	79	10

[1] based on parent fluopicolide end point (0.029 mg/L) with 10x assessment factor
[2] based on PECgw x 0.1 (PECgw to PECsw dilution correction – SANCO 3268/2001)
[3] based on repeat annual applications (worse case)

Fluopicolide GW metabolite TERs are greater than the Annex VI trigger in all worse case PECgw scenarios (Table 9.2 & 9.3). Thus low risk to aquatic organisms via exposure from fluopicolide metabolites formed in groundwater can be concluded.

Conclusion

Low risk to aquatic organisms is indicated from fluopicolide metabolites formed in groundwater following proposed uses on vine and potato.

Open point 5.7 – update earthworm LOEPs

RMS to update the LoE with the endpoint for earthworm in mg a.s./kg soil. A clarification on the endpoint for earthworm reported in the LoE is also nessessary

The LoEPs have been amended as necessary.

Open point 5.15 (new) – Ecotox relevance of toluene in technical material

Notifier to address the ecotoxicological relevance of toluene in the technical material. RMS to include in an addendum a summary of the applicant.

The Notifier submitted a position paper with respect to above issue and the Notifier's summary is reproduced below.

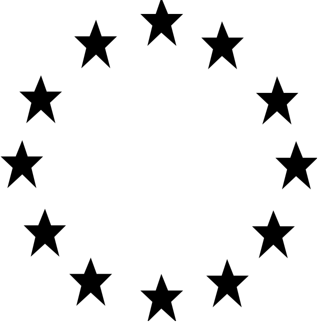
Title:	**Ecotoxicological relevance of toluene as impurity in Fluopicolide technical material**
Author:	Simone Pross
Date:	23 April 2008
Test:	Bayer CropScience AG Ecotoxicology Alfred-Nobel-Str. 50 D-40789 Monheim am Rhein Germany M-300968-01-1
Summary	Fluopicolide is currently in the review process in Europe for annex I inclusion. A question arose on the presence and the ecotoxicological relevance of the solvent toluene as an impurity in the Technical Grade Active Substance (TGAS). In chemical analyses of technical fluopicolide, the maximum specified limit for toluene in fluopicolide technical material proposed by BCS is 0.5% (5g/kg). Since toluene was present (1.0 - 4.06 g/kg which is 0.1 - 0.406 %) in the fluopicolide batches used for the ecotoxicological studies it is considered to have been adequately tested for its ecotoxicological effects. It is also covered by the risk assessment for fluopicolide up to the specified concentration limit of 0.5%. Thus the ecotoxicity of toluene at the level present in the fluopicolide TGAS has been adequately investigated and the occurrence of toluene did not affect the overall ecotoxicity profile of fluopicolide. A review of the literature shows that the toxicology and ecotoxicology of "pure" toluene is well described and documented. The ecotoxicological profile of toluene as evaluated in the EU Risk Assessment demonstrates than it is not more toxic than the TGAS. This resulted in a "no classification" for the environment within the EU legally binding classification and labelling system. From a risk assessment for toluene using a worst case PECi approach the TERs are well in excess of EU 91/414 Annex VI triggers for all species. Therefore, it can be concluded that the presence of toluene at the specified level does not lead to an unacceptable risk. In an overall conclusion the impurity toluene, at the specified maximum concentration limit of 0.5% in technical fluopicolide is considered not of ecotoxicological relevance.

The RMS has considered the case with respect to the ecotoxicological relevance of toluene as impurity in fluopicolide technical material as proposed by the Notifier (Pross, 2008). Ecotoxicological testing was undertaken using fluopicolide technical material (batches OP2050046, OP2050190, OP2350005, R001737, OP20500045) containing 0.1-0.4% w/w toluene (AEF125577) (see DAR Vol 4,Table C.1). Therefore the ecotoxicological risk assessment for technical fluopicolide essentially encompasses the risk from toluene in technical material (max. <0.5%w/w pilot plant; <0.3%w/w manufacturing plant – Volume 4, Addendum 2, C 2.2). Furthermore, the ecotoxicological profile of "pure" toluene shows it not to be more toxic than fluopicolide technical. A risk assessment using worse case toluene PECsoil (0.0009 mg/kg) and PECsw (0.000046 mg/L) initial values based on theoretical toluene content in fluopicolide PECs generate respective TERs of 16667, 16087 and 76087 with worse toxic toluene endpoints for worm(28dNOEC=15 mg/kg d.wt soil), Daphnia (96hEC50=3.5 mg/L) and Ceriodaphnia (7dNOEC=0.74 mg/L). The TERs clearly exceed relevant Annex VI EU 91/414 thresholds indicating low risk. Toluene also has low bioaccumulation potential (BCF=90). Thus all evidence indicates that environmental toluene derived from fluopicolide technical use in PPPs will not cause concern from an ecotoxicological perspective.

Conclusion

Environmental toluene derived from fluopicolide technical material used in PPPs for treatment of vine and potato is not relevant from an ecotoxicological perspective.

Council Directive 91/414/EEC



Fluopicolide (AE C638206)

Volume 4
ADDENDUM 3

Annex C

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

Confidential Information

February 2009

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