Asulam sodium

Annex B (Volume 3)

B.8 Environmental Fate and Behaviour

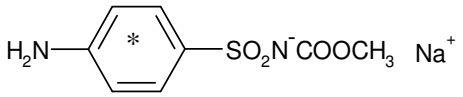
Version history

When	What
2016-04-18	Initial DAR

B.8 ENVIRONMENTAL FATE AND BEHAVIOUR

The majority of radiolabelled studies used ^{14}C-asulam sodium (Methyl sulfanilylcarbamate monosodium salt) labelled in the aromatic ring (see Figure B.8.1 for radiolabel position). Radiochemical purity and specific activity are reported in each radiolabelled study. Some studies have been conducted with asulam. In solution asulam sodium will dissociate (pKa1, 1.25; pKa2, 4.68) and the ionised and unionised forms will be in equilibrium which is dependant on the pH of the compound's environment. The amounts of asulam used in the tests were not sufficient to affect the pH and therefore would not affect the equilibrium, nor was the aqueous solubility of asulam exceeded in any of the tests. In the opinion of the Rapporteur, asulam and asulam sodium can therefore be considered equivalent and the form of the compound applied will not influence the results of the test. The recommended maximum field application rate notified for asulam is 2.4 kg a.s./ha applied once per crop in the spring. A maximum soil concentration of 2.56 mg a.s./kg, was calculated for a single season use, based on the assumptions given in Section B.8.3. The exposure assessment was performed using the application of asulam in the calculation of PEC's.

Figure B.8.1 Position of radiolabel (*) in ^{14}C-asulam sodium



Asulam was first reported in 1965 and was introduced by May and Baker. Asulam itself had the initial development code M&B 9057 (sometimes written as M&B-9057 or M&B009057). With changes in company name and ownership of the molecule, other codes were applied and these included RPA096125, RPA590048, Hoe 074383 and AE B106159 and finally AE F074383. As the great majority of the reports summarised under Point 7 use the names asulam or asulam sodium, these are used throughout this section.

B.8.1 Route and rate of degradation in soil (IIA 7.1.1, IIIA 9.1.1)

B.8.1.1 Aerobic and anaerobic studies (II 7.1.1, IIIA 9.1.1)

B.8.1.1.1 Soil microbial studies

a) **This study has previously been evaluated at EU level. The conclusions reached are consistent with the EFSA conclusion report. Following the review of CA 7.1.1/06 (P. Lowden ,2013b), raised in answer to a question of clarification, no concerns were raised at EU level.**

Report:

P. Yeomans (2000a) [^{14}C]-Asulam Sodium: Aerobic Route and Rate of Degradation. Covance Laboratories Ltd, Harrogate, North Yorkshire, England for Aventis CropScience, Unpublished report No.: 68/171-D2142

P. Yeomans, S. Swales (2003a) [^{14}C]-Asulam Sodium: Characterisation and Degradation Kinetics of a Putative Soil Metabolite. Covance Laboratories Ltd, Harrogate, North Yorkshire, England for Bayer CropScience SA, Unpublished report No.: 1849/20-D2149

Guideline:
EU 95/36/EC
Deviations: None

GLP: Yes

RMS opinion on study: fully acceptable

A laboratory soil degradation study was conducted in four soils. The study, which was conducted in accordance with EC Directive 95/36/EC and SETAC guidelines (1995) and to GLP, was considered acceptable by the Rapporteur.

The route of degradation was investigated in 1 soil at 20°C and 10°C (groups A and E), and 3 contrasting soils (groups B, C and D) at 20°C using radiolabelled asulam sodium (specific activity 5.617 MBq/mg; radiochemical purity >98%). The test soils (2mm sieved) were characterised as shown in Table B.8.1 overleaf.

Table B.8. 1 Characterisation of soils used to investigate the aerobic degradation of ^{14}C-asulam sodium.

Soil Code	PT102	PT103	SK15556090	SK961089
UK Particle size distribution:				
Sand % (2000 - 63 µm)	50	75	21	36
Silt % (63 - 2 µm)	38	12	60	32
Clay % (<2 µm)	12	13	19	32
Textural Class ADAS	Sandy loam	Sandy loam	Clay loam	Clay loam
Textural Class BBA	mittel lehmiger Sand	stark lehmiger Sand	schluffiger Lehm	schwach toniger Lehm
Textural Class USDA	Loam	Sandy loam	Silt loam	Clay loam
Organic Carbon (%)	2.9	1.3	5.4	5.1
Organic Matter (%)	5.0	2.2	9.3	8.8
pH in Water	7.3	5.5	7.0	7.9
pH in KCl (1 M)	6.7	5.1	6.1	7.3
Cation Exchange Capacity mEq/100 g	19.8	9.8	28.5	37.8
Water holding capacity at pF 0	58.9	37.5	110.5	86.1
Water holding capacity at pF 2.5	20.8	10.4	38.8	31.2
Biomass (µg C/g)				
Study start	522.3	126.3	1084.9	992.1
Study end	563.5	348.3	967.2	1017.3
	549.4 (10°C)			

The soil samples (50g dry weight equivalent) were purged with moist air. A series of traps were fitted, the first an empty security trap, and the second and third containing sodium hydroxide to collect CO_2. The soils were maintained under test conditions for 2 to 5 days prior to application of asulam. ^{14}C-asulam was applied in an equimolar solution of sodium hydroxide in order to form the sodium salt (asulam sodium) at a rate equivalent to 4.4 kg/ha. Soils were maintained at 45% of the maximum water holding capacity in the dark under the conditions summarised below.

Incubation group	Soil code	No. of replicates	Soil type	Temperature (°C)	Study type
A	PT102	2	Sandy loam	20	Route of degradation
B	PT103	1	Sandy loam	20	Rate of degradation
C	SK15556090	1	Clay loam	20	Rate of degradation
D	SK961089	1	Clay loam	20	Rate of degradation
E	PT102	1	Sandy loam	10	Rate of degradation

The soils were sampled at intervals of 0h, 1, 3, 7, 14, 28, 59 and 120 days after application. Radioactivity in the traps was quantified by LSC. The traps were analysed when the respective units were removed for analysis, or analysed and renewed every *ca* 30 to 40 days.

The soils were extracted twice with acetone/water (50/50 v/v), evaporated to near dryness and reconstituted in acetonitrile prior to HPLC analysis. Extracted soils were also Soxhlet extracted overnight using acidified water followed by acidified acetonitrile. The combined Soxhlet extracts were evaporated to near dryness and reconstituted in water acetonitrile. Extracts were then analysed by HPLC with UV and radioactivity detection and confirmation by TLC by preparation of a radioluminogram.

Radiolabelled samples and a standard mix of non-radiolabelled standards including non-radiolabelled asulam were co-injected for confirmation.

Selected samples and reference standards were analysed using LC-MS with atmospheric pressure chemical ionisation (APcI) to confirm the presence of asulam. Initial work confirmed the presence of asulam however attempts to confirm the presence of sulphanilamide using both positive and negative ion APcI was unsuccessful.

The Soxhlet extracted soils, following air drying, were combusted in oxygen and the resultant combustion products were adsorbed in Oxysolve C-350 and the radioactivity determined by LSC.

Samples incubated for 59 days after ^{14}C-asulam application were extracted with a range of additional extraction solvents as shown in Table B.8.12.

Soil samples previously subjected to room temperature and Soxhlet extraction from the 7, 28 and 120 day time points were also extracted with dilute hydrochloric acid and sodium hydroxide followed by more concentrated acid and alkali (see Table B.8.13).

Selected samples of extracted soil (after acetone/water extraction and 2 Soxhlet extractions) were subject to bound residue fractionation to recover humin, and fulvic and humic acids.

Recoveries of radioactivity from samples is shown in Tables B.8.2 to B.8.6. It is noted by the Rapporteur that the recovery of %AR is a little low particularly in soil PT103 (Table B.8. 3). The only volatile traps used were for CO_2. There may be a possibility of other volatile components present that were not trapped.

Levels of asulam and its degradation products are presented in Table B.8.7 to Table B.8.11. The results of additional extractions on selected samples are presented in Table B.8.12 to Table B.8. 13. The results of bound residue fractionation from 59 day samples are shown in Table B.8. 14.

An unidentified degradation product (unknown 1) recovered from the Soxhlet extractions showed levels of up to *ca* 20% applied radioactivity (see Tables B.8.7 to B.8.11). This was reported by the Notifier to be a cluster of polar components indicated by TLC to be more than 10 components. It is noted by the Rapporteur that the 'Max unknown' labelled in Table B.8.7 to Table B.8.11 exceeds 5% AR at 2 or more consecutive time points. It is stated by the Notifier however that these are areas of diffuse radioactivity, aggregated by a rapid change of gradient to allow quantification and the Rapporteur accepts that these may be mixtures of components.

A follow-up study (Yeomans and Swales, 2003a) was performed to generate and characterise this degradation product. Unknown 1 was shown to be an artefact produced by the liquid scintillant used in the original study. In the characterisation study, a different scintillant was used and unknown 1 was not present. Sulphanilamide was again shown to be the major metabolite and the short half-life of asulam in soil was confirmed.

Table B.8. 2 Recovery of applied radioactivity (%) from sandy loam soil (PT102) maintained at 20°C in the dark following a single application of ^{14}C-asulam sodium
Mean results.

Time (days)	Initial Extract	Soxhlet Extract 1	Soxhlet Extract 2	Un-extracted	Carbon Dioxide	Total
0	84.9	2.8	2.1	3.1	n/a	92.8
1	75.7	7.2	3.4	11.9	0.3	98.4
3	54.6	15.6	3.0	15.5	0.7	89.3
7	32.0	14.6	6.2	39.2	1.6	93.5
14	17.3	22.6	5.8	45.0	2.4	93.1
28	5.5	14.7	6.1	64.3	3.8	94.5
59*	2.7	8.1	2.7	67.8	5.3	86.7
120	2.4	1.5	4.9	76.0	6.1	90.8

n/a = not applicable *one sample only

Table B.8. 3 Recovery of applied radioactivity (%) from sandy loam soil (PT103) maintained at 20°C in the dark following a single application of ^{14}C-asulam sodium

Time (days)	Initial Extract	Soxhlet Extract 1	Soxhlet Extract 2	Un-extracted	Carbon Dioxide	Total
0	92.7	3.1	0.6	1.4	n/a	97.8
1	74.7	7.7	3.2	10.1	0.2	95.9
3	59.6	15.9	3.3	11.2	0.5	90.5
7	38.4	11.9	10.5	30.6	0.9	92.3
14	21.6	13.7	9.0	45.8	1.7	91.8
28	9.0	25.5	6.0	45.8	2.6	88.9
59	5.7	7.5	11.1	52.8	3.8	80.9
120	5.0	4.1	6.9	63.9	6.3	86.2

n/a = not applicable

Table B.8. 4 Recovery of applied radioactivity from clay loam soil (SK15556090) maintained at 20°C in the dark following a single application of ^{14}C-asulam sodium

Time (days)	Initial Extract	Soxhlet Extract 1	Soxhlet Extract 2	Un-extracted	Carbon Dioxide	Total
0	76.3	3.5	3.7	10.2	n/a	93.7
1	62.4	7.7	6.4	23.3	0.2	100.0
3	40.5	18.3	4.6	28.0	0.7	92.1
7	21.4	24.6	4.9	43.0	1.3	95.2

14	4.3	20.5	5.9	57.4	0.8	88.9
28	2.5	16.4	8.1	57.2	3.6	87.8
59	2.0	5.6	12.1	69.0	4.9	93.6
120	1.6	9.1	4.8	69.4	6.9	91.8

n/a = not applicable

Table B.8.5 Recovery of applied radioactivity from clay loam soil (SK961089) maintained at 20°C in the dark following a single application of ^{14}C-asulam sodium

Time (days)	Initial Extract	Soxhlet Extract 1	Soxhlet Extract 2	Un-extracted	Carbon Dioxide	Total
0	88.2	2.9	2.6	5.5	n/a	99.2
1	72.7	8.0	3.0	13.8	0.2	97.7
3	57.0	15.6	2.8	16.5	0.6	92.5
7	35.0	15.3	4.8	37.7	1.4	94.2
14	17.7	17.6	4.5	51.5	1.7	93.0
28	6.0	12.4	7.4	63.1	3.7	92.6
59	2.7	7.3	5.8	68.9	5.6	90.3
120	1.8	3.8	5.3	73.5	7.5	91.9

n/a = not applicable

Table B.8.6 Recovery of applied radioactivity (%) from sandy loam soil (PT102) maintained at 10°C in the dark following a single application of ^{14}C-asulam sodium

Time (days)	Initial Extract	Soxhlet Extract 1	Soxhlet Extract 2	Un-extracted	Carbon Dioxide	Total
0	91.9	2.8	1.4	2.8	n/a	98.9
1	75.9	4.9	3.2	6.5	0.1	90.6
3	68.3	12.0	1.7	9.0	0.3	91.3
7	57.6	13.0	3.6	20.8	0.8	95.8
14	41.7	17.7	4.9	31.2	1.3	96.8
28	17.7	6.2	10.0	52.9	2.2	89.0
59	7.3	7.2	15.5	59.2	3.1	92.4
120	3.9	5.6	8.3	68.5	4.3	90.6

n/a = not applicable

Table B.8.7 Percentage of applied radioactivity present as asulam and its degradation products in sandy loam soil (PT102) maintained at 20°C in the dark following a single application of ^{14}C-asulam sodium. Mean Results.

Time (days)	Asulam	Sulfanilic Acid	Sulfanil -amide	Acetyl Sulfanil -amide	Acetyl Asulam	Unk 1	Total Unks	Max Unk*	Un-resolved BKG**	Total
0	80.5	0.7	0.4	0.1	0.2	nd	9.5	6.6	0.4	91.7
1	68.8	0.4	2.6	0.7	0.2	0.5	10.6	4.3	0.5	84.2
3	48.6	0.1	4.5	0.1	0.3	0.7	18.0	7.9	0.4	72.5
7	26.6	nd	2.9	nd	0.1	1.0	21.1	7.0	0.4	52.0
14	11.2	nd	5.9	nd	nd	2.3	25.0	6.4	0.3	44.6
28	3.7	nd	6.5	1.4	nd	0.3	14.0	7.1	0.1	25.9
59	0.1	nd	1.0	0.6	nd	8.5	3.6	0.6	0.2	14.0
120	nd	nd	1.0	0.2	nd	1.4	3.3	0.7	0.1	5.9

*included in sum of unknowns in previous column
** BKG = background

Table B.8.8 Percentage of applied radioactivity present as asulam and its degradation products in sandy loam soil (PT103) maintained at 20°C in the dark following a single application of ^{14}C-asulam sodium

Time (days)	Asulam	Sulfanilic Acid	Sulfanil -amide	Acetyl Sulfanil -amide	Acetyl Asulam	Unk 1	Other Unks (Total)	Max Unk*	Un-resolved Bkg**	Total
0	84.7	0.4	1.5	<0.1	nd	nd	6.7	6.0	0.4	93.7
1	66.2	0.3	6.7	0.4	1.9	1.6	8.1	5.1	0.7	85.9
3	44.3	0.1	10.1	nd	1.6	2.7	14.8	7.6	0.4	74.0
7	27.6	nd	6.4	nd	0.7	5.7	19.7	8.4	0.3	60.4
14	11.5	nd	9.2	nd	nd	3.6	19.5	6.8	0.4	44.2
28	3.0	nd	12.0	2.3	nd	1.2	19.0	7.8	0.5	38.0
59	<0.1	nd	0.4	0.1	nd	14.2	0.2	0.1	0.1	15.0
120	nd	nd	1.9	nd	nd	4.3	4.1	2.2	<0.1	10.3

* included in sum of unknowns in previous column
** BKG = background

Table B.8.9 Percentage of applied radioactivity present as asulam and its degradation products in clay loam soil (SK15556090) maintained at 20°C in the dark following a single application of ^{14}C-asulam sodium

Time (days)	Asulam	Sulfanilic Acid	Sulfanil -amide	Acetyl Sulfanil -amide	Acetyl Asulam	Unk 1	Other Unks (Total)	Max Unk*	Un-resolved Bkg	Total
0	71.0	0.3	0.9	0.1	nd	nd	7.4	6.2	0.1	79.8
1	54.9	0.2	1.4	nd	0.2	0.6	15.9	6.1	0.3	73.5
3	35.0	nd	3.4	nd	0.2	0.3	23.6	8.5	0.5	63.0
7	15.6	nd	4.6	0.1	0.1	1.3	27.4	7.8	0.2	49.3
14	1.8	nd	3.6	nd	nd	3.5	21.4	6.8	0.2	30.5
28	0.5	nd	2.6	nd	nd	19.7	3.5	2.4	0.4	26.7
59	nd	nd	1.6	1.2	nd	2.8	11.5	2.1	<0.1	17.1
120	nd	nd	1.6	nd	nd	4.6	6.6	2.4	<0.1	12.8

*included in sum of unknowns in previous column
** BKG = background

Table B.8.10 Percentage of applied radioactivity present as asulam and its degradation products in clay loam soil (SK961089) maintained at 20°C in the dark following a single application of ^{14}C-asulam sodium

Time (days)	Asulam	Sulfanilic Acid	Sulfanil -amide	Acetyl Sulfanil -amide	Acetyl Asulam	Unk 1	Other Unks (Total)	Max Unk*	Un-resolved Bkg**	Total
0	63.3	0.7	0.4	0.1	nd	nd	5.7	4.0	0.3	70.5
1	68.7	0.5	3.6	nd	nd	0.7	9.2	5.1	0.3	83.0
3	51.4	0.2	7.9	nd	nd	1.0	12.8	5.6	0.5	73.8
7	29.1	nd	1.6	nd	nd	0.5	21.3	7.3	0.5	53.0
14	14.6	nd	13.9	nd	nd	0.5	9.5	3.4	0.7	39.2
28	3.4	nd	3.5	0.3	nd	11.9	5.5	2.3	0.2	24.8
59	nd	nd	3.3	0.2	nd	6.3	2.4	0.4	0.1	12.3
120	nd	nd	0.3	0.3	nd	nd	2.5	0.6	<0.1	3.1

*included in sum of unknowns in previous column
** BKG = background

Table B.8.11 Percentage of applied radioactivity present as asulam and its degradation products in sandy loam soil (PT102) maintained at 10°C in the dark following a single application of ^{14}C-asulam sodium.

Time (days)	Asulam	Sulfanilic Acid	Sulfanil -amide	Acetyl Sulfanil -amide	Acetyl Asulam	Unk 1	Other Unks (Total)	Max Unk*	Un-resolved Bkg**	Total
0	78.5	0.4	0.5	<0.1	0.2	nd	8.9	6.8	0.3	88.8
1	73.1	0.6	2.5	0.4	0.3	0.2	8.2	4.5	0.8	86.1
3	62.5	0.4	4.0	nd	0.4	0.6	13.0	6.1	0.8	81.7
7	49.3	0.1	2.4	0.1	0.3	2.9	17.7	5.9	0.3	73.1
14	33.6	0.1	7.1	nd	0.2	1.4	21.1	7.1	0.1	63.6
28	16.1	nd	6.8	0.6	nd	8.0	8.0	5.7	0.6	40.1
59	0.1	nd	1.6	nd	nd	18.5	1.3	0.3	0.4	21.9
120	nd	nd	3.2	0.9	nd	3.2	9.2	1.2	<0.1	16.5

*included in sum of unknowns in previous column
** BKG = background

Table B.8.12 Recovery of applied radioactivity in alternative extracts of soil maintained at 20°C or 10°C in the dark for 59 days following a single application of ^{14}C-asulam sodium

Dose Group Soil	B PT103	C SK15556090	D SK961089	E PT102 (10°C)
$CaC1_2$(0.01M)	4.1	1.5	2.5	10.4
ACN:H20(1:I v/v)	3.7	1.2	2.0	3.1
Acetonitrile	0.3	0.2	0.2	0.4
Methanol	0.3	0.2	0.1	0.4
Ethyl acetate	0.0	0.0	0.0	0.1
Diethyl ether	0.0	0.0	0.0	0.0
HCI (0.5M)	5.1	1.6	2.4	2.2
NaOH (0.5M)	27.6	8.6	3.1	14.3
Total	41.1	13.3	10.3	30.9

Table B.8. 13 Recovery of applied radioactiovity in additional extracts from soil previously cold and Soxhlet extracted maintained at 20 or 10°C in the dark following a single application of ^{14}C-asulam sodium

Extractant and Time Period	Sandy Loam Soil (PT102) @ 20°C	Sandy Loam Soil (PT103) @20°C	Clay Loam Soil (SK15556090) @ 20°C	Clay Loam Soil (SK961089) @ 20°C	Sandy Loam Soil (PT102) @ 10°C
HCl (0.1M) 7 day	1.1	1.3	0.6	1.5	0.6
NaOH (0.1M) 7 day	13.2	16.9	11.6	3.4	6.2
HCl (0.1M) 14 day	0.8	1.7	0.7	1.4	0.6
NaOH (0.1M) 14 day	15.3	26.9	20.9	4.5	11.7
HCl (0.1M) 28 day	1.2	1.2	0.6	1.5	1.0
NaOH (0.1M) 28 day	20.7	27.3	17.5	6.1	17.2
HCl (0.1M) 120 day	1.9	2.4	0.6	1.5	1.7
NaOH (0.1M) 120 day	31.0	41.5	14.6	14.3	27.9

Table B.8. 14 Fractionation of bound residues of soils maintained at 20°C in the dark for 59 days following a single application of ^{14}C-asulam sodium

Dose Group Soil	A PT102	B PT103	C SKI 5556090	D SK961089	E PT102 (10°C)
Fulvic acids	19.3 (11.3)	32.3 (14.8)	13.2 (8.8)	10.6 (7.0)	15.4 (8.5)
Humic acids	50.9 (29.9)	38.9 (17.8)	49.9 (33.3)	22.3 (14.7)	52.7 (29.1)
Humin	29.8 (17.5)	28.8 (13.2)	36.9 (24.6)	67.1 (44.3)	31.9 (17.6)
Total	100.0 (58.7)	100.0(45.8)	100.0(66.7)	100.0(66.0)	100.0(55.2)

Values quoted as a percentage of bound residue and as a percentage of applied radioactivity in parentheses

(Yeomans, 2000a)

This study has previously been evaluated at EU level. The conclusions reached are consistent with the EFSA conclusion report and no concerns were raised at EU level.

b) P. Yeomans, S. Swales (2003a) [^{14}C]-Asulam Sodium: Characterisation and Degradation Kinetics of a Putative Soil Metabolite. Covance Laboratories Ltd, Harrogate, North Yorkshire, England for Bayer CropScience SA, Unpublished report No.: 1849/20-D2149

Guideline:
EU 95/36/EC
Deviations: None

GLP: Yes

RMS opinion on study: fully acceptable

A follow-up study (Yeomans and Swales, 2003a) was performed to generate and characterise a degradation product designated 'Unknown 1' which was observed in certain extracts (soxhlet extracts) the appeared to represent > 10% AR. The study was conducted on three soils, two of which had been investigated in the previous study (Yeomans 2000a).

The application rate application rate and extraction procedure were the same as in the previous study. The characterisation of the soils is presented below in Table B.8. 15.

Table B.8. 15 Characterisation of soils used to investigate the artefact peak in the aerobic degradation of ^{14}C-asulam sodium.

Soil Code	PT103	SK15556090	SK960087
Particle size distribution:			
Sand (53–2000 µm, %)	74	24	38
Silt (2-53 µm, %)	13	56	31
Clay (<2 µm, %)	13	20	31
Textural Class USDA	Sandy loam	Silt loam	Clay loam
Textural Class ADAS	Sandy loam	Clay loam	Clay loam
Textural Class BBA	stark lehmiger Sand	schluffiger Lehm	schwach toniger Lehm
Organic Carbon (%)	1.0	4.2	2.8
Organic Matter (%)	1.7	7.2	4.9
pH in Water	5.5	6.8	7.9
pH in KCl (1 M)	4.9	6.1	7.3
Cation Exchange Capacity mEq/100 g	7.5	21.4	20.4
Water holding capacity at pF 0	42.7	86.8	62.1
Water holding capacity at pF 2.5	11.9	31.8	26.0
Biomass (µg C/g)			
Start of incubation	226.89	564.07	403.56
Study end	115.39	601.24	377.65

The soil samples (50 g dry weight) were dosed with asulam sodium at a rate equivalent to 4.4 kg as/ha (same as in Yeomans 2000). Since this study was performed to generate and if necessary characterise the product 'Unknown 1' no volatile organic or carbon dioxide traps were fitted to the flasks and no mass balance was attempted.

Soils were maintained at 45% of maximum water holding capacity in the dark. The soils were sampled immediately after dosing and at at intervals of 1, 3, 7, 14, 21, 28, 42, 62, 90 and 120 days after application. The soil extraction procedure and analysis was identical to the procedure used in the Yeomans (2000) study.

Extracts were analysed by HPLC with UV and radioactivity detection. The recovery and distribution of applied radioactivity for each soil is presented in Table B.8.16 to Table B.8.18.

Table B.8. 16 Recovery and distribution of radioactivity following treatment of sandy loam soil (PT 103) with [^{14}C]-asulam sodium at 4.5 mg/kg

Time (days)	% applied radioactivity in:			
	Cold Extract	Soxhlet Extract	Unextracted	Total
0	90.2	2.5	1.7	94.4
1	74.6	10.1	10.5	95.2
3	53.3	15.5	24.9	93.7
7	29.3	20.7	41.7	91.7
14	15.2	23.1	50.8	89.1
21	9.0	18.8	58.9	86.7
28	7.9	17.0	65.2	90.1
42	7.8	11.1	67.5	86.4
62	5.1	13.3	65.3	83.7
90	4.2	13.2	67.6	85.0
120	4.4	8.4	71.0	83.8

Table B.8. 17 Recovery and distribution of radioactivity following treatment of silt loam soil (SK15556090) with [^{14}C]-asulam sodium at 4.5 mg/kg

Time (days)	% applied radioactivity in:			
	Cold Extract	Soxhlet Extract	Unextracted	Total
0	84.2	3.5	8.0	95.7
1	49.8	10.1	21.1	81.0
3	45.7	13.3	34.2	93.2
7	20.3	14.1	55.9	90.3
14	12.6	20.4	57.0	90.0
21	11.2	15.8	62.3	89.3
28	4.9	12.8	67.3	85.0
42	4.6	10.3	73.0	87.9
62	3.4	12.3	70.7	86.7
90	2.3	7.9	75.3	85.5
120	2.2	5.1	75.3	82.6

Table B.8. 18 Recovery and distribution of radioactivity following treatment of clay loam soil (SK960087) with [^{14}C]-asulam sodium at 4.5 mg/kg

Time (days)	% applied radioactivity in:			
	Cold Extract	Soxhlet Extract	Unextracted	Total
0	91.8	2.7	2.5	97.0
1	72.2	9.0	14.1	95.3
3	48.4	11.8	33.0	93.2
7	22.9	14.2	49.5	86.6
14	8.7	18.6	62.7	90.0
21	5.8	14.6	66.5	87.2
28	3.5	9.2	71.7	84.4
42	3.9	10.3	69.3	83.5
62	2.4	10.8	71.4	84.6
90	2.1	7.6	76.9	86.6
120	1.9	3.9	76.2	82.0

The HPLC of the soxhlet extracts (in which the unknown had been seen) conducted on the system used in the original study that used Monoflow 3 scintillant indicated the presence of a significant very sharp polar peak at a retention time between 3 to 4 minutes as presented in Figure B.8.2. When a different scintillant (Quickzint Flow 302) was used this peak disappeared. Because this peak was the largest in the chromatogram it had effectively suppressed the minor peaks. When it did not appear, the peaks for sulphanilamide and the aggregated diffuse peak became the largest but a number of other minor peaks also became significant as illustrated in Figure B.8.2 and Figure B.8.3. These are chromatograms obtained with the two different scintillants. The spiked peak at 3.1 to 4.0 minutes on the first chromatogram has disappeared, leaving a small normal shaped polar peak at 2.8 to 3.6 minutes. This effect was replicated in samples from the soxhlet extracts of all soils and was considered by the applicant that the polar peak was an artefact caused by the reaction of the original scintillant with the acidic extracts. The RMS agrees with the opinion of the applicant that the sharp polar peak is an artefact of the Monoflow 3 scintillant. It is noted that the percentage represented by sulphanilamide did not change greatly but the amount accounted for, in total, by the minor peaks increased (see comparison sulphanilamide in Table B.8.8 and Table B.8. 19) which indicates that figures obtained for sulphanilamide in the original study are reliable. The results of the characterisation of extractable radioactivity by HPLC for each of the soils are presented in
Table B.8. 19) which indicates that figures obtained for sulphanilamide in the original study are reliable. The results of the characterisation of extractable radioactivity by HPLC for each of the soils are presented in Table B.8. 19 to Table B.8. 21.

Figure B.8.2 HPLC Radiochromatogram of Soxhlet Extract of Asulam Sodium (SK960087, day 62) treated Soil using Original (MonoFlow 3) Scintillant

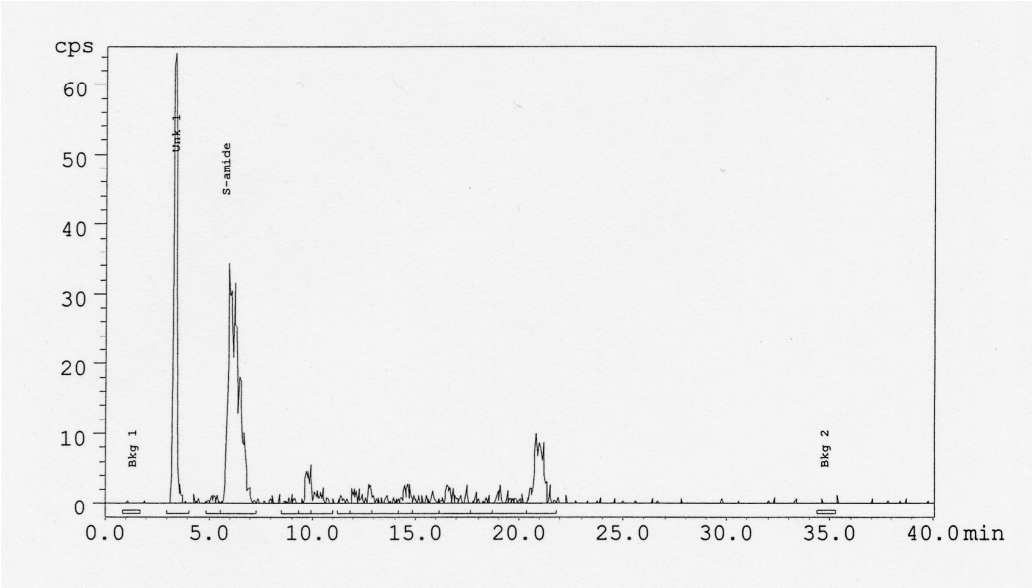


Figure B.8.3 HPLC Radiochromatogram of Soxhlet Extract of Asulam Sodium (SK960087, day 62) treated Soil using New (Quickzint Flow 302) Scintillant

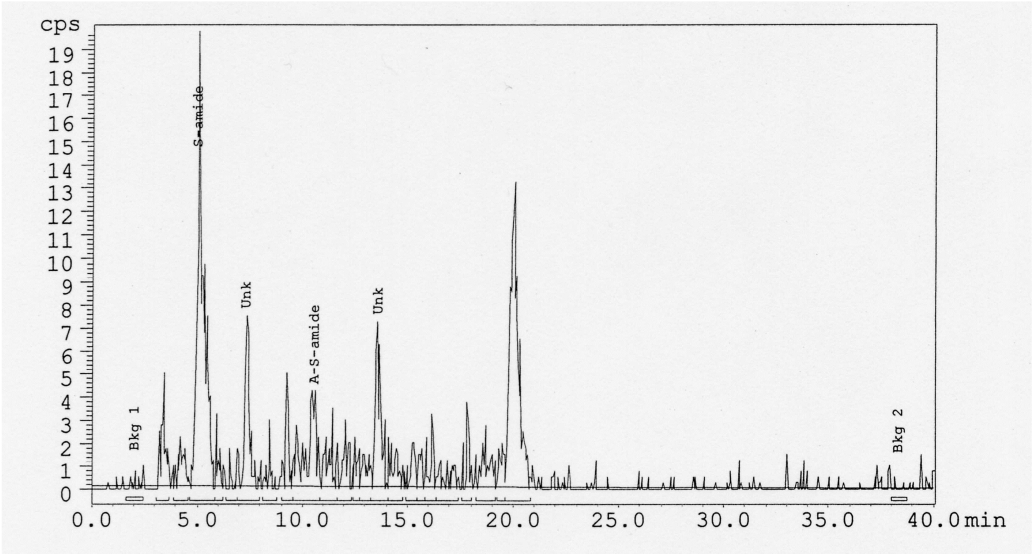


Table B.8. 19 Characterisation (by HPLC) of extractable radioactivity following treatment of sandy loam soil (PT 103) with [^{14}C]-asulam sodium at 4.5 mg/kg

Time (days)	% applied radioactivity as:								
	Asulam	Sulphanilic Acid	Sulphanil -amide	Acetyl Sulphanil -amide	Acetyl Asulam	Largest Unknown*	Total Unknowns	Un-resolved Bkg	Total
0	78.2	nd	2.1	nd	nd	7.0	9.3	0.5	90.1
1	61.2	nd	6.2	nd	1.7	6.6	15.1	0.2	84.4
3	36.4	0.2	10.4	nd	1.5	6.2	19.0	0.7	68.2
7	17.2	nd	9.7	nd	nd	5.7	22.4	0.4	49.7
14	8.9	nd	10.4	nd	nd	4.1	17.9	0.3	37.4
21	2.7	nd	9.6	nd	nd	5.3	15.6	0.4	28.2
28	1.6	nd	6.4	0.1	nd	5.2	13.5	0.3	21.8
42	1.0	0.1	3.0	nd	nd	5.0	11.5	0.1	15.7
62	0.5	0.1	3.7	nd	nd	5.0	13.8	0.2	18.3
90	0.3	0.1	2.1	nd	nd	6.1	14.6	0.2	17.3
120	0.4	nd	1.3	0.2	nd	4.5	9.9	0.2	12.0

Table B.8. 20 Characterisation (by HPLC) of extractable radioactivity following treatment of silt loam soil (SK15556090) with [^{14}C]-asulam sodium at 4.5 mg/kg

Time (days)	% applied radioactivity as:								
	Asulam	Sulphanilic Acid	Sulphanil -amide	Acetyl Sulphanil -amide	Acetyl Asulam	Largest Unknown*	Total Unknowns	Un-resolved Bkg	Total
0	75.6	0.4	1.6	nd	nd	4.5	5.6	1.0	84.2
1	36.5	nd	2.0	nd	nd	11.3	20.5	0.3	59.3
3	35.8	0.2	6.6	nd	0.4	5.3	15.1	0.5	58.6
7	13.7	0.2	3.7	0.3	nd	5.0	16.2	0.4	34.4
14	4.6	0.4	6.0	0.4	nd	6.1	20.9	0.4	32.7
21	3.5	nd	6.5	nd	nd	4.8	13.3	0.1	23.4
28	1.1	nd	5.7	0.1	nd	4.4	12.6	0.2	17.6
42	0.5	nd	3.4	nd	nd	3.3	10.8	0.1	14.8
62	0.4	0.0	2.3	nd	nd	4.0	12.9	0.0	15.6
90	0.1	nd	0.9	nd	nd	3.8	9.1	0.1	10.2
120	nd	nd	0.6	nd	0.1	2.5	7.3	0.0	8.0

Table B.8. 21 Characterisation (by HPLC) of extractable radioactivity following treatment of clay loam (SK960087) soil with [^{14}C]-asulam sodium at 4.5 mg/kg

Time (days)	% applied radioactivity as:								
	Asulam	Sulphanilic Acid	Sulphanil -amide	Acetyl Sulphanil -amide	Acetyl Asulam	Largest Unknown*	Total Unknowns	Un-resolved Bkg	Total
0	85.0	nd	0.2	nd	nd	4.9	4.9	1.6	91.7
1	65.8	nd	1.0	nd	nd	6.3	13.5	0.7	81.0
3	41.1	nd	2.0	nd	nd	5.1	16.0	0.4	59.4
7	18.3	nd	1.0	nd	nd	8.6	17.6	0.3	37.1
14	3.1	nd	4.6	nd	nd	6.7	19.2	0.2	27.1
21	1.6	nd	4.6	nd	nd	4.7	13.8	0.1	20.1
28	1.7	nd	3.1	nd	nd	3.3	8.3	0.1	13.2
42	1.3	0.0	1.9	nd	nd	3.8	11.2	0.1	14.5
62	0.5	0.1	2.6	nd	nd	4.4	9.9	0.1	13.2
90	0.3	nd	1.5	nd	nd	3.0	7.7	0.1	9.6
120	0.2	nd	0.2	nd	nd	2.0	4.7	0.1	5.1

* in all cases the aggregated area of diffuse radioactivity, included in sum of unknowns in following column.

(Yeomans and Swales, 2003a)

This study has not previously been evaluated at EU level. It was evaluated at national Level. The conclusions are in agreement with the EFSA conclusions.

A Roohi and E Mackenzie (2009a) [^{14}C]-Asulam Sodium: Route and Rate of Degradation in Soil at 20°C. Battelle UK Ltd, Ongar, Essex, England for AgriChem BV, Unpublished report No.: OU/07/010

Guidelines:

EU 95/36/EC

Deviations: No

GLP:

Yes

RMS opinion on study: fully acceptable. Please note the summary below is the applicant's. It is an accurate account of the report and considered acceptable by the RMS. The RMS has provided comments at the end of the study summary.

Executive Summary:

The route and rate of degradation of asulam was been investigated under aerobic conditions at 20 ± 2°C in four soils in the dark. Asulam was applied to the surface of individual soil samples at an application rate of 1064 µg/100g, equivalent to a field rate of 4.0 kg/ha, and the treated samples were incubated at a soil moisture content of between pF 2.0 and pF 2.5 for 119 days.

At intervals of up to 119 days duplicate samples were removed from the incubation system and subjected to three successive extractions procedures. The extracted soil samples were air-dried, ground to a fine powder and the residual radioactivity quantified by combustion. At each sampling interval the radioactivity in the trap solutions associated with each sample was quantified by liquid scintillation counting (LSC). Traps were also changed between sampling intervals and the radioactivity quantified by liquid scintillation counting.

The solvent extracts from each soil sample were concentrated (if necessary), and analysed by reverse phase high performance liquid chromatography (HPLC). Liquid Chromatography-Mass Spectrometry-Mass Spectrometry (LC-MS/MS) of representative soil extracts produced structural confirmation of the species present.

The overall recoveries were good for all soils and the distribution of radioactivity was similar in all four soils. The level of extractable radioactivity ranged from 96.5 to 98.1% at time zero but rapidly decreased with time, with a corresponding increase in the levels of unextractable radioactivity. Levels of volatile radioactivity in each of the soils reached maximum values of between 2.9 and 6.2% by the end of the incubation period.

Asulam very rapidly degraded in all of the soils, declining from between 91.4 and 93.9% at time zero to between 1.2 and 5.8% by 119 days. The asulam was degraded to sulphanilamide and a large number of less abundant metabolites, with up to 15 individual metabolites detected. Sulphanilamide reached maximum values of 17.3, 7.0, 1.8 and 13.1%. Other identified components were sulphanilic acid, acetyl asulam and desamino asulam, none of these, however, exceeded 10% at any time-point or 5% at two consecutive time-points.

Materials and Methods

Test Material:

[^{14}C]-Asulam sodium, purity 97.3%, Batch no.: 2414MFO004-2

Test Design:

The study used four soils, the characteristics of which are tabulated below.

Soil Reference Batch ID	LUFA Speyer 2.2 07/022	Further Field 07/024	Flint Hall 07/028	Warsop A1 07/030
Source Geographic Location	LUFA, Grober Striet, Hanhofen, Rheinland-Pfalz, Germany	Boarded Barns Farm, Ongar, Essex, UK	Flint Hall, Royston, Herts., UK	Site A1, Warsop, Nottinghamshire, UK
OS Map Reference	Not available	TL557047	TL365397	SK566696
Textural classification (USDA) Sand % (50-2000 µm) Silt % (2-50 µm) Clay % (< 2 µm)	Loamy sand 83 8 9	Clay loam 22 47 31	Clay loam 26 40 34	Loamy sand 87 6 7
Textural classification (ADAS) Sand % (63-2000 µm) Silt % (2-63 µm)	Loamy sand 84 8	Silty clay loam 19 50	Clay / Clay loam 23 42	Loamy sand 86 6

Clay % (< 2 µm)	8	31	35	8
pH Deionised Water 0.01M CaCl2 1M KCl	5.8 5.2 5.2	6.7 6.1 5.8	8.2 7.3 7.3	5.1 4.2 3.9
Organic Carbon %	1.9	1.6	2.5	0.9
Organic Matter %	3.3	2.7	4.4	1.5
Cation Exchange Capacity (meq/100g)	7.7	17.1	15.8	5.3
% Soil Moisture during incubation	15.1	28.2	29.6	6.6
Water holding capacity pF 2.0 – WHC 0.1 bar pF 2.5 – WHC 0.33 bar	 19.7 10.4	 28.5 27.8	 31.3 27.8	 6.5 6.7
Bulk Density (fresh g/L)	980	854	802	1176
Microbial Biomass initial (µg C/g soil) final	292.7 337.5	300.7 226.2	620.9 468.0	138.2 142.2

The soil samples were dispensed (100 g oven-dried equivalent) into glass sample flasks and their water content adjusted to pF 2 to pF 2.5. They were allowed to acclimatise under study conditions for approximately one week prior to treatment. Twenty flasks for each soil were prepared and treated with [^{14}C]-asulam, allowing duplicate samples to be collected at eight specified time-points with four spare flasks. Eight untreated flasks for biomass determination (four for initial biomass and four for final biomass) were also prepared.

Moist carbon dioxide-free air was passed across the soil surface and out through trapping solutions consisting of three calibrated 50 mL tubes, the first containing ethylene glycol followed by two containing 2M potassium hydroxide, were connected in series to the incubation flasks. The use of flow restrictors before each flask regulated the airflow and allowed flasks to be removed without disruption of the airflow to the remaining flasks.

Radiolabelled and non-radiolabelled asulam (Batch 357-150A) were mixed and dissolved in water (10 mL) with an equimolar amount of sodium hydroxide to give a treatment solution with a nominal concentration of 11.85 mg/mL. The soil samples were each treated with the [^{14}C]-treatment solution using a positive displacement pipette, adding the solution drop-wise onto the soil surface, ensuring even distribution. Treatment checks for each soil were made before, during and after treatment by dispensing an equal volume of treatment solution directly into a 100 mL volumetric flask. At intervals of 0, 1, 3, 7, 14, 21, 28, 57 and 119 days duplicate flasks, along with their associated traps, were removed from the incubation system for analysis.

At each sampling interval three ambient solvent extractions using acetone/water (1:1 v/v) were employed. Further extraction of the soil residues by soxhlet apparatus first under acidic conditions with water and then under acidic conditions with acetonitrile was conducted for all time-points after time zero. The soil residues were then air dried and the radioactive content of the extracted soil quantified by the combustion of multiple aliquots. For selected samples the remaining non-extractable soil residue was

also subjected to organic matter fractionation. Soil samples were extracted on the day that they were collected. Ambient extracts were typically analysed on the day they were generated and then stored at -15°C. Extracted soil residues were stored at 5°C before further extraction by soxhlet.

Extracts and trap solutions were radioassayed by LSC. Post-extraction soil residues were radioassayed by combustion and the LSC if trapped combustion gases. The radioactivity still associated with the soil was further characterised by organic matter fractionation (into fulvic acid, humic acid and humin fractions).
Extracts were qualitatively analyzed by HPLC. The system used utilized a Synergy Hydro-RP 4um 80A, (250 x 4.6mm i.d) connected to a UV detector and a radiodetector. The mobile phase was a gradient of water/formic acid (100+0.2, v/v) against acetonitrile/formic acid (100+0.2, v/v). Standards of asulam and four possible metabolites were also run.

The test item and selected soil extracts were also analysed by liquid chromatography / tandem mass spectroscopic analysis (LC/MS/MS) to confirm the identity of asulam and its degradates. The HPLC system was connected to a MICROMASS QUATTRO-LC Triple Quadrupole mass spectroscope that was run in Positive Ion Electrospray Ionisation (ESP+) mode. A mixture of asulam reference item and four possible degradation products: sulphanilamide, sulphanilic acid, acetyl asulam and methylbenzenesulphonyl carbamate (desamino asulam), was analysed with the samples. An additional reference item, 4-formylamino-benzene-sulphonamide, was used in separate analytical runs to confirm the identity of an additional component found in study samples.

Results and Discussion:
Prior to use the purity of the test item was determined to be 97.3% (mean value) by HPLC. LC/MS/MS confirmed the identity of the test item. The temperature was maintained at the intended temperature of 20 ± 2°C at all times. The results of the soil biomass determinations confirmed that all of the soils were microbiologically active throughout the course of the study.

The extraction efficiency, using three cycles of ambient temperature solvent extraction, ranged from 96 to 98% of applied radioactivity for the zero time samples, indicating the extraction method was suitable for the extraction of [^{14}C]-asulam from the test soil matrices.

Selected ambient extracts and all soxhlet extracts were concentrated prior to analysis by HPLC. The mean recovery of radioactivity on concentration was 92.4% for the ambient extracts and 96.0% for the soxhlet extracts. HPLC column recovery was found to be quantitative with a mean of 98.5% of injected radioactivity being recovered post-column.

The overall recoveries were good for all soils with overall mean values for each soil ranging from 94.6 to 97.1%. Material balances are shown in Tables B.8.22 to B.8.25 below. Observed trends for mass balance did not suggest loss during a specific analytical technique or indeed the loss of any specific metabolite.

Table B.8. 22 Distribution of Radioactivity in LUFA Speyer 2.2 Loamy Sand (07/022) Incubated at 20ºC, following Application of [^{14}C]-Asulam at 4 kg/ha

Time (days)	% applied radioactivity in				
	Initial Extract	Soxhlet Extract 1	Un-extracted	Carbon Dioxide	Total
0	96.96	n/a	5.45	0.00	102.42
1	84.07	3.95	6.13	0.00	94.14
3	82.57	5.44	10.65	0.13	98.79
7	70.20	8.23	18.11	0.17	96.70
14	55.62	7.88	33.15	0.50	97.16
21	44.26	8.15	44.84	0.87	98.12
28	32.28	9.62	52.94	1.37	96.20
57	13.15	8.96	73.92	2.01	98.05
119	7.08	6.82	75.20	3.15	92.25

Table B.8. 23 Distribution of Radioactivity in Further Field Clay Loam (07/024) Incubated at 20ºC, following Application of [^{14}C]-Asulam at 4 kg/ha

Time (days)	% applied radioactivity in				
	Initial Extract	Soxhlet Extract 1	Un-extracted	Carbon Dioxide	Total
0	96.97	n/a	6.04	0.00	103.01
1	83.52	3.19	11.30	0.06	98.07
3	72.64	4.24	21.13	0.17	98.17
7	52.37	7.28	35.30	0.38	95.32
14	29.25	6.26	53.96	1.69	91.16
21	21.15	5.19	63.55	1.62	91.51
28	14.87	6.41	66.75	3.14	91.16
57	6.06	4.43	77.15	2.97	90.61
119	3.56	3.92	79.12	6.17	92.78

Table B.8. 24 Distribution of Radioactivity in Flint Hall Clay Loam (07/028) Incubated at 20ºC, following Application of [^{14}C]-Asulam at 4 kg/ha

Time (days)	% applied radioactivity in				
	Initial Extract	Soxhlet Extract 1	Un-extracted	Carbon Dioxide	Total
0	96.52	n/a	6.95	0.00	103.47
1	87.47	2.92	7.92	0.08	98.39
3	75.34	4.13	16.87	0.52	96.86
7	61.60	4.08	30.56	0.47	96.71
14	43.65	4.56	45.00	0.69	93.90

Time (days)	% applied radioactivity in				
	Initial Extract	Soxhlet Extract 1	Un-extracted	Carbon Dioxide	Total
21	27.20	4.06	61.17	1.73	94.16
28	15.29	4.50	70.43	1.45	91.67
57	5.04	3.20	82.63	3.43	94.29
119	3.01	1.91	82.49	4.70	92.10

Table B.8. 25 Distribution of Radioactivity in Warsop A1 Loamy Sand (07/030) Incubated at 20ºC, following Application of [^{14}C]-Asulam at 4 kg/ha

Time (days)	% applied radioactivity in				
	Initial Extract	Soxhlet Extract 1	Un-extracted	Carbon Dioxide	Total
0	98.09	n/a	3.06	0.00	101.15
1	87.59	5.22	4.95	0.02	97.78
3	77.28	7.08	11.15	0.13	95.64
7	68.18	10.49	18.52	0.21	97.40
14	51.49	10.23	33.60	0.72	96.04
21	33.65	13.14	44.93	0.94	92.65
28	29.20	9.82	52.78	1.66	93.46
57	21.89	14.59	56.02	3.02	95.51
119	11.30	9.10	69.08	2.92	92.39

The results from the characterisation of radioactivity in ambient and soxhlet extracts showed that asulam very rapidly degraded in the soils to form a number of metabolites and, despite repeated extraction of soil samples, rapidly formed significant quantities of unextractable soil bound residues. The levels of asulam declined from between 91.4 and 93.9% at time zero to between 1.2 and 5.8% by 119 days. The identification of asulam in soil extracts was confirmed by mass spectrometry.

Asulam was degraded to sulphanilamide and a large number of less abundant metabolites. Up to 15 individual metabolites were detected, most of which were classified as being minor metabolites. One compound, 4-formyl-amino-benzene-sulphonamide, was suggested to be an artefact of the extraction method used (soxhlet extraction with acetonitrile and with water, both acidified with formic acid) and was therefore considered to be sulphanilamide, released from the soil as the formyl derivative due to the formic acid present in the extraction solvent. Total levels of sulphanilamide reached maximum values of 17.3, 7.0, 1.8 and 13.1% in soils 07/022, 07/024, 07/028 and 07/030, respectively. In all soils total amounts of sulphanilamide reached maximum levels at day 21-28 before declining to ≤ 5% in all soils by day 119.

Other identified components were sulphanilic acid, acetyl asulam and desamino-asulam, none of these, however, exceeded 10% at any time-point or 5% at two consecutive time-points.

Numerous other metabolites were detected. For clarity the very minor metabolites were grouped by their retention time in the HPLC system into three diffuse regions of radioactivity, Regions 1, 2 and 3. Region 1 reached a maximum of 10.56% at 14 days in the Speyer 2.2 loamy sand (soil 07/022) before declining to 0.2% by the end of the incubation period. This region comprised up to five unidentified polar metabolites which eluted ahead of sulphanilamide with relative retention times (RRT) of between 0.27 and 0.6. Region 2 reached a maximum of 1.7% by 57 days in the Flint Hall clay loam (soil 07/028) before declining to 0.4% by the end of the incubation period. This region comprised up to two unidentified metabolites which eluted after sulphanilamide with RRT of between 0.93 and 0.98. Region 3 increased to a maximum of 4.3% by 28 days in the Warsop A1 loamy sand (soil 07/030) before declining to 0.4 % by the end of the incubation period. This region comprised two unidentified metabolites which eluted after asulam with RRT of between 1.19 and 1.3.

In the LUFA Speyer 2.2 soil the unextracted radioactivity was principally associated with the humic acid and fulvic acid fractions (*ca* 44 and 37% of total, respectively) with < 20% in the humin fraction. In the Further Field soil the unextracted radioactivity was principally associated with the fulvic acid and humin fractions (*ca* 40 and 39% of total, respectively) with *ca* 21% in the humic acid fraction. In the Flint Hall soil the unextracted radioactivity was principally associated with the humin and fulvic acid fractions (*ca* 44 and 37% of total, respectively) with < 20% in the humic acid fraction. Finally, in the Warsop A1 soil the unextracted radioactivity was principally associated with the fulvic acid fractions (*ca* 62% of total) with only *ca* 25% and 13% in the humic acid and humin fractions.

The composition of extractable radioactivity at various times after application in each soil type is summarized in Tables B.8.26 to B.8.29 below.

Table B.8. 26 Composition of Extractable Radioactivity by HPLC in LUFA Speyer 2.2 Loamy Sand (07/022) Incubated at 20ºC, following Application of [^{14}C]-Asulam at 4 kg/ha

	Percent of Applied												
Incubation Time [days]	% of Applied in extract	sulphanilic acid	unknown Region 1 RRT 0.27-0.6	Sulphanilamide RRT 0.72	Unknown RRT 0.82	Unknown RRT 0.85	4-Formylamino-benzenesulphonamide RRT 0.85*	Unknown Region 2 RRT 0.93-0.98	Asulam RRT 1.0	Acetyl Asulam RRT 1.04	unknown RRT 1.1	desamino RRT 1.15	unknown Region 3 RRT 1.19-1.3
0	96.96	1.13	0.00	0.00	0.00	0.00	0.00	0.00	93.12	0.00	1.24	0.96	0.51
1	88.01	1.05	0.20	0.08	0.00	0.00	1.53	0.00	80.78	1.36	0.86	1.41	0.76
3	88.01	0.84	1.28	0.20	0.00	0.00	3.22	0.00	77.65	0.00	0.00	3.12	1.71
7	78.43	0.43	2.70	0.31	0.00	0.00	4.36	0.00	63.97	0.00	0.00	4.59	2.05
14	63.51	0.00	10.56	0.26	0.00	0.00	4.57	0.00	41.62	0.22	0.06	6.00	0.23
21	52.41	0.00	0.97	10.89	0.06	6.07	3.01	0.32	23.73	1.14	0.10	4.54	1.57
28	41.90	0.00	0.71	12.19	2.24	0.00	5.14	0.99	16.01	0.29	0.17	2.17	1.99
57	22.11	0.12	1.13	4.60	1.82	0.00	5.18	0.37	6.42	0.00	0.00	2.44	0.04
119	13.90	0.12	0.20	1.71	0.57	0.20	2.83	1.82	3.39	1.03	0.87	0.89	0.25

* artefact derived from sulphanilamide – values added to those for sulphanilamide for maximum formation determination

Note: Regions 1, 2 and 3 contain multiple minor components all at <5% throughout.

Table B.8. 27 Composition of Extractable Radioactivity by HPLC in Further Field Clay Loam (07/024) Incubated at 20ºC, following Application of [^{14}C]-Asulam at 4 kg/ha

	Percent of Applied												
Incubation Time [days]	% of Applied in extract	sulphanilic acid	unknown Region 1 RRT 0.27-0.6	Sulphanilamide RRT 0.72	Unknown RRT 0.82	Unknown RRT 0.85	4-Formylamino-benzenesulphonamide RRT 0.85*	Unknown Region 2 RRT 0.93-0.98	Asulam RRT 1.0	Acetyl Asulam RRT 1.04	unknown RRT 1.1	desamino RRT 1.15	unknown Region 3 RRT 1.19-1.3
0	96.97	1.54	0.00	0.00	0.00	0.00	0.00	0.00	91.41	0.00	0.00	1.11	2.91
1	86.71	0.97	0.00	0.00	0.00	0.00	0.46	0.08	80.22	1.80	0.07	1.79	1.32
3	76.88	0.00	0.86	0.03	0.00	0.00	1.91	0.04	69.52	0.11	0.11	2.18	2.11
7	59.65	0.02	2.20	0.24	0.00	0.00	2.98	0.21	50.14	0.28	0.00	1.10	2.46
14	35.51	0.00	4.19	0.09	0.00	0.00	3.80	0.07	25.17	0.11	0.00	1.84	0.25
21	26.34	0.00	0.51	4.55	0.10	3.82	2.12	0.86	11.63	0.23	0.09	0.94	1.49
28	21.27	0.24	0.12	3.67	0.11	2.74	3.32	0.75	8.59	0.14	0.05	0.32	1.22
57	10.49	0.13	0.36	0.91	0.01	0.00	2.37	1.64	4.04	0.00	0.00	1.03	0.00
119	7.50	0.13	0.26	0.52	0.27	0.21	1.70	1.00	2.27	0.30	0.51	0.30	0.02

* artefact derived from sulphanilamide – values added to those for sulphanilamide for maximum formation determination

Note: Regions 1, 2 and 3 contain multiple minor components all at <5% throughout.

Table B.8. 28 Composition of Extractable Radioactivity by HPLC in Flint Hall Clay Loam (07/028) Incubated at 20ºC, following Application of [^{14}C]-Asulam at 4 kg/ha

	Percent of Applied												
Incubation Time [days]	% of Applied in extract	sulphanilic acid	unknown Region 1 RRT 0.27-0.6	Sulphanilamide RRT 0.72	Unknown RRT 0.82	Unknown RRT 0.85	4-Formylamino-benzenesulphonamide RRT 0.85*	Unknown Region 2 RRT 0.93-0.98	Asulam RRT 1.0	Acetyl Asulam RRT 1.04	unknown RRT 1.1	desamino RRT 1.15	unknown Region 3 RRT 1.19-1.3
0	96.52	1.16	0.00	0.00	0.00	0.00	0.00	0.00	92.70	0.30	0.00	1.32	1.03
1	90.39	0.81	0.00	0.00	0.00	0.00	0.26	0.04	83.39	1.67	0.04	1.44	2.74
3	79.47	0.00	0.00	0.00	0.12	0.00	0.54	0.81	73.23	0.42	0.08	1.08	3.18
7	65.68	0.00	0.27	0.00	0.00	0.00	0.64	0.10	60.86	0.56	0.00	0.62	2.62
14	48.21	0.04	0.00	0.00	0.17	0.00	0.77	0.09	44.03	0.46	0.52	0.75	1.38
21	31.26	0.00	0.00	0.60	0.47	0.47	0.58	1.15	25.30	0.56	0.05	1.67	0.40
28	19.79	0.00	0.00	0.51	0.48	0.46	1.29	0.63	12.52	0.40	0.43	1.44	1.64
57	8.23	0.08	0.25	0.12	0.01	0.00	0.00	1.73	4.11	0.00	0.00	1.42	0.52
119	4.92	0.15	0.26	0.26	0.10	0.14	0.23	0.35	1.22	0.26	0.44	0.78	0.72

* artefact derived from sulphanilamide – values added to those for sulphanilamide for maximum formation determination

Note: Regions 1, 2 and 3 contain multiple minor components all at <5% throughout.

Table B.8. 29 Composition of Extractable Radioactivity by HPLC in Warsop A1 Loamy Sand (07/030) Incubated at 20ºC, following Application of [^{14}C]-Asulam at 4 kg/ha

	Percent of Applied												
Incubation Time [days]	% of Applied in extract	sulphanilic acid	unknown Region 1 RRT 0.27-0.6	Sulphanilamide RRT 0.72	Unknown RRT 0.82	Unknown RRT 0.85	4-Formylamino-benzenesulphonamide RRT 0.85*	Unknown Region 2 RRT 0.93-0.98	Asulam RRT 1.0	Acetyl Asulam RRT 1.04	unknown RRT 1.1	desamino RRT 1.15	unknown Region 3 RRT 1.19-1.3
0	98.09	1.26	0.00	0.00	0.00	0.00	0.00	0.00	93.92	0.00	0.61	1.24	1.05
1	92.81	1.12	0.35	0.24	1.04	0.00	0.94	0.06	84.66	1.10	0.00	1.46	1.85
3	84.36	0.79	0.80	0.13	2.09	0.00	1.82	0.05	73.69	1.52	0.00	2.23	1.25
7	78.67	0.26	1.83	0.33	2.57	0.00	2.88	0.00	63.48	0.24	0.00	4.56	2.51
14	61.72	0.04	5.56	0.38	1.97	0.00	3.08	0.20	38.95	4.00	0.23	4.42	2.91
21	46.79	0.00	0.00	6.97	1.54	0.28	6.14	1.24	22.76	0.50	0.36	4.33	2.68
28	39.02	0.00	0.00	5.37	0.88	0.67	4.52	1.44	16.70	1.36	0.51	3.24	4.33
57	36.48	0.21	0.15	3.89	0.00	3.13	8.35	0.00	15.16	0.00	0.00	5.47	0.10
119	20.39	0.31	0.09	1.82	1.01	0.75	3.57	1.04	5.77	1.17	1.85	2.57	0.44

* artefact derived from sulphanilamide – values added to those for sulphanilamide for maximum formation determination

Note: Regions 1, 2 and 3 contain multiple minor components all at <5% throughout.

Conclusions:

[^{14}C]-Asulam rapidly dissipated in aerobic soil *via* a large number of intermediate metabolites to form soil bound residues and, to a lesser extent, by mineralisation to carbon dioxide. The major metabolite was sulphanilamide (consistent with other lab soil degradation studies) reaching 12.19% after 28 days (Speyer 2.2 soil).

(Roohi and Mackenzie, 2009a)

RMS opinion on study

The overall recoveries were good for all soils with overall mean material balances for each soil ranging from 94.6 to 97.1%. The minimum recovery was 92.1% (Flint Hall soil) and considered acceptable by the RMS. The major metabolite was sulphanilamide (consistent with other lab soil degradation studies) reaching 12.19% after 28 days (Speyer 2.2 soil). Numerous other minor metabolites were detected, sulfanilic acid, acetyl asulam and desamino asulam (also known as MBSC), none of these exceeded 10% at any timepoint or 5% at two consecutive timepoints. Three unknown regions were detected labelled Region 1, 2 and 3. Unknown Region 1 was detected at RRT 0.27-0.6 reaching max 10.56% considered to be composed of 5 unidentified polar metabolites each individual metabolite being considered less than 5%. Two other unknowns Region 2 and 3 were detected at max 1.7% and 4.3% AR respectively. A further unknown (RRT0.85) was detected at max 8.4% which also exceeded 5% on two consecutive occasions was identified by LC/MS/MS as 4-formylaminobenzenesulfonamide (by comparison with a certified reference standard) and believed by the applicant to be an artefact of the extraction method used (soxhlet extraction with acetonitrile/water, both acidified with formic acid) and considered to be sulphanilamide, released from the soil as the formyl derivative due to the formic

acid present in the extraction solvent. Investigation by the applicant, by refluxing sulphanilamide with formic acid under the conditions of the Soxhlet extractions demonstrated the formation of 4-formylaminobenzenesulfonamide. The RMS considers that this is acceptable.

The kinetics for the rate of degradation of asulam in this study were re-evaluated in the study of Hardy (2011a) below.

Overall the study of Roohi and Mackenzie, (2009a) was well reported and considered acceptable by the RMS.

This study has not previously been evaluated at EU level. It was evaluated at member-state level. The conclusions are in line with the EFSA conclusions.

Report:
W. Völkel (2000a) Degradation of [^{14}C]-Asulam in One Soil Incubated under Aerobic Conditions. RCC Ltd., Itingen, Switzerland for AgriChem BV, Unpublished report No.: 689940
Guidelines:
EU 95/36/EC
Deviations: No
GLP:
Yes
Executive Summary:
The aerobic degradation of asulam was studied in Borstel soil over a period of 118 days. Soil samples, treated at a rate equivalent to 4.8 kg/ha were incubated at 20°C and 40% MWHC. Samples were taken for analysis at 0, 1, 4, 7, 14, 35, 56 and 118 days after treatment.

These samples were subjected to exhaustive solvent extractions, and the concentrated extracts were analysed by HPLC and selected extracts were further analysed by TLC. A total balance of radioactivity and pattern of degradation products were established for each sampling day.

The amounts of non-extractable radioactivity were 1.7% on day 0. They increased to 20.7% and 62.7% after 4 and 35 days of incubation, respectively. After 118 days of incubation, the non-extractable radioactivity amounted to 68.1% of the applied radioactivity. The radioactivity associated with the fulvic acids fraction amounted to 22.8% of the applied radioactivity. Amounts of 45.3% of the applied radioactivity were bound to the humic acids and humin fractions. No significant formation of volatiles was observed. The amount of $^{14}CO_2$ reached a maximum of 2.9% of applied radioactivity. Other organic volatiles did not exceed 0.1% of the applied radioactivity.

Asulam rapidly decreased from 94.4% of the applied radioactivity on day 0 to 37.6% of the applied radioactivity, after 1 day of incubation. It further decreased to 3.6% of the applied radioactivity, after 118 days of incubation. The chromatographic results showed that asulam rapidly degraded to many compounds, a number of which chromatographed close together in the HPLC system that was ultimately used, and were termed ‘M2’. This group included methylbenzenesulphonyl carbamate (desamino-asulam) and benzenesulphonamide. M2 reached its maximum concentration

within one day, accounting for 47.7% of the applied radioactivity. Development HPLC systems showed that there were in excess of 30 compounds that had been aggregated to form M2. The amount of M2 further decreased steadily to 14.4% of the applied radioactivity on day 118. This decrease was concomitant with an increase in bound residues.

In addition, up to five minor degradation products (M1 and M3-M6) were detected, individually not exceeding 5.6% of the applied radioactivity. Two of these were tentatively identified as sulphanilamide (M1) and sulphanilic acid (M4).

Materials and Methods

Test Material:

[^{14}C]-Asulam sodium, purity: 99.3%, Batch no.: CFQ 10616

Test Design:

The metabolism of asulam in Borstel soil was investigated. The characteristics of the soil are shown below.

Parameter	Borstel 98
Soil Type (USDA)	sandy loam
Batch	6/98
pH (KCl)	5.01
Organic carbon (g/100 g dry soil)	1.30
Carbonate (%$CaCO_3$)	0.25
Cation Exchange capacity (meq/100 g dry soil)	7.75
Total nitrogen (%)	0.1
Particle size (mm)	
< 0.002 (clay)	6.6
0.002 – 0.05 (silt)	17.4
> 0.05 (sand)	76.0
Max water holding capacity (g/100 g dry soil)	36.33
40% MWHC (g/100 g dry soil)	14.53
Field capacity: FC: pF 1.8 (g/100 g soil)	22.75
Field capacity: FC: pF 2.5 (g/100 g soil)	10.13
Biomass (mg C/100 g soil) at start of incubation	24.7
Biomass (mg C/100 g soil) at start of incubation	25.8

The study was carried out in glass metabolism flasks connected to an open air-flow-through system. Soil samples (100 g) based on the dry weight of the soil were incubated under aerobic conditions in all-glass metabolism flasks in the dark at 20 ± 1 °C. The flasks were equipped with an air inlet and outlet. The system was continuously ventilated with moistened air. The outgoing air was passed through a trapping system,

equipped with absorption traps containing 50 ml of ethylene glycol and 50 ml of 2N NaOH, in this order, to trap organic volatiles and $^{14}CO_2$, respectively. For sampling day 0, no absorption traps were set up. The application solution (570 µL of an aqueous solution containing 480 µg compound) was added drop-wise to each 100g of the soil (dry-weight basis) by means of a microsyringe and the soil mixed thoroughly. The moisture content of the soil samples was controlled by weighing and adjusting to 40% of the soil maximum water holding capacity with bi-distilled water, if necessary.

Soil samples were taken for analysis at 0, 1, 4, 7, 14, 28, 35, 56 and 118 days after the treatment. At each sampling interval, one whole sample was analysed. Ethylene glycol and sodium hydroxide solutions were exchanged at each sampling interval and monthly after day 56. LSC analyses of these solutions were performed accordingly. The water content of the soils was adjusted weekly until day 42. Thereafter the water was adjusted every two weeks. Control samples were used for determination of the microbial biomass by the method suggested by Anderson and Domsch. The microbial biomass was determined at the beginning and at the end of incubation.
At each sampling interval, the whole soil sample was subjected to the following extractions:

a) Acetonitrile/water (8:2, v/v), 2-3 times at ambient temperature
b) Water, once at ambient temperature
c) Soxhlet with acetone/water (9:1, v/v), for at least four hours (beginning with1 day sample)
d) Reflux with methanol/0.1 M HCI (4:1, v/v) for about two hours (beginning with 1 day sample).

Extractions at ambient temperature were performed in a shaker at about 200-250 strokes per minute for about 30 minutes. The amount of solvent used was in general about 1 ml per g of soil. The individual extracts were quantified by LSC.
The extracts a), b) and c) were combined and analysed separately from the reflux extracts except for days 56 and 118 where all extracts were combined. The combined extracts were concentrated under reduced pressure (at about 35 °C) in a rotary evaporator and chromatographically profiled by HPLC. The determination of the content and nature of the reflux extracts (so called "harsh" extracts) was performed separately except for day 118.

The residual radioactivity remaining in soil after the extraction procedures [a) to c) or a) to d), respectively, was quantified by LSC after combustion of 3 aliquots of the air-dried and homogenised soil. Thereafter, the exhaustively extracted soil sample of sampling day 118 was submitted to organic matter fractionation to determine the percentage of the applied radioactivity which remained bound to humic and fulvic acids and the humin fraction of the soils.

The HPLC system comprised a LiChrospher 100 C18 pre-column leading to a 25 cm x 4.0 mm, 5 Mm, Hypersil 120 ODS column connected to a Merck-Hitachi L4000 UV detector (238 nm) and a Packard FLO-ONE, Beta A500 TR radiodetector fitted with a liquid cell. The mobile phase was a gradient of tetrabutyl ammoniumhydrogensulphate (2 g/l) adjusted to pH 2.3 against acetonitrile. Parent and reference samples were run along with the sample extracts.

TLC was used to confirm residual parent and degradates initially identified by HPLC, based on retention-time comparison with authentic reference standards. One- and two-dimensional TLC (1D and 2D-TLC) was performed on precoated plates (5 cm x 20 cm or 20 cm x 20 cm, layer thickness 0.25 mm) of silica gel 60 F254 (normal phase) and of RP-18 (reversed phase). The plates were developed without chamber saturation. Eighteen different solvent systems were used.

Non-labelled reference compounds were visualised by quenching of UV-light at a wavelength of 254 nm. The radioactive zones on TLC plates were detected by scanning the plates with an Automatic TLC Linear Analyser (Dr. Berthold) equipped with a Data Processing System for one-dimensional TLC.

Results and Discussion:
The radiopurity of the [14C]-asulam was found to be 99.3% by TLC. The total mean recovery of the applied radioactivity was 92.0% ± 5.4% (4.467 ± 0.260 ppm) of the applied radioactivity. The individual recoveries ranged from 90.0% to 100.1%. The values are summarized in Table B.8.30 below.

Table B.8. 30 **Distribution of Radioactivity in Borstel Soil Incubated at 20ºC, following Application of [^{14}C]-Asulam at 4.8 kg/ha**

Fraction	% applied radioactivity at							
	0 d	1 d	4 d	7 d	14 d	35 d	56 d	118 d
Extractables I	92.4	79.9	55.5	39.2	25.9	7.7	4.7	4.1
Extractables II	2.0	5.1	3.2	4.0	4.0	3.8	4.1	3.1
Soxhlet extract	np	4.8	5.6	5.6	4.3	3.9	3.4	1.4
Reflux extract	np	2.6	8.3	9.1	10.9	14.4	12.2	12.4
Total extractable	94.4	92.5	72.5	57.9	45.1	29.8	24.3	21.0
Non-extractable	1.7	7.5	20.7	31.6	44.3	62.7	64.2	68.1
CO_2	np	0.1	0.3	0.5	0.9	2.2	2.7	2.9
Volatiles	np	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Recovery	96.2	100.1	93.5	90.0	90.2	94.7	91.2	91.9
Mean (sd)	92.0 (5.4)							

Note: recoveries from 28-day samples were < 90% so a replacement sampling time of 35 days was added.

Immediately after treatment 94.4% of the applied radioactivity could be extracted. With increasing incubation time, the amount of extractable radioactivity decreased continuously to values of 29.8 and 21.0% of the applied radioactivity after 35 and 118 days of incubation, respectively. The amounts of non-extractable radioactivity were 1.7% of the applied radioactivity on day 0 and 20.7% after 4 days of incubation. After 35 days of incubation the non-extractable radioactivity increased to 62.7% of the applied radioactivity and increased thereafter slowly to only 68.1% after 118 days of incubation.

Table B.8.31 below summarises the pattern of parent decline and degradates formation/decline found in all soil extracts in terms of percent of the applied radioactivity.

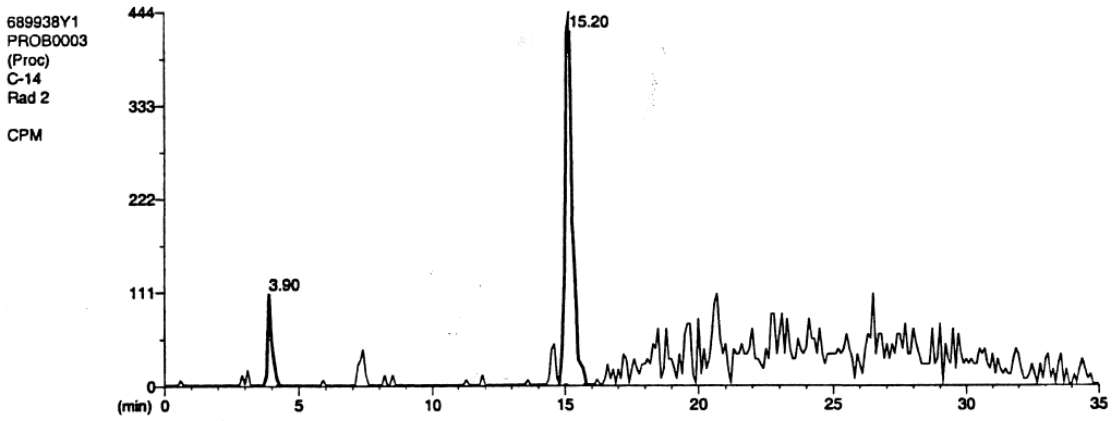
Table B.8. 31 Distribution of Radioactivity in Borstel Soil Incubated at 20ºC, following Application of [^{14}C]-Asulam at 4.8 kg/ha

Compound	% applied radioactivity at							
	0 d	1 d	4 d	7 d	14 d	35 d	56 d	118 d
Parent	94.4	37.6	26.0	16.3	8.9	7.1	3.5	3.6
M1 sulphanilamide	-	1.3	0.8	1.5	0.7	1.4	-	0.9
M2 (unknown)*	-	47.7	42.3	39.0	33.4	19.1	20.8	14.4
M3 (unknown)	-	5.6	2.8	1.2	0.8	-	-	-
M4 (sulphanilic acid)	-	0.3	0.6	-	0.1	1.1	-	-
M5 (unknown)	-	-	-	-	0.6	0.5	-	1.1
M6 (unknown)	-	-	-	-	0.6	0.6	-	1.0
Non-extractable	1.7	7.5	20.7	31.6	44.3	62.7	64.2	68.1
CO_2	np	0.1	0.3	0.5	0.9	2.2	2.7	2.9
Total	96.2	100.1	93.5	90.0	90.2	94.7	91.2	91.9

* Consisting of a number of components

The tabulated results showed that asulam was rapidly degraded to a number of metabolites. Sulphanilamide and sulphanilic acid were present at low levels in extracts from most time-points. Radioactive fraction M2 was eluting as one fraction but development chromatography had shown that it consisted of large number fractions. Beside several unknown components the compounds methyl-benzene-sulphonylcarbamate (MBSC, desamino asulam), 4-acetyl-asulam and benzenesulphonamide were shown to be present in the region designated M2. The large number of peaks that M2 comprised is shown in the HPLC chromatogram of soil extract shown in Figure B.8.4 below.

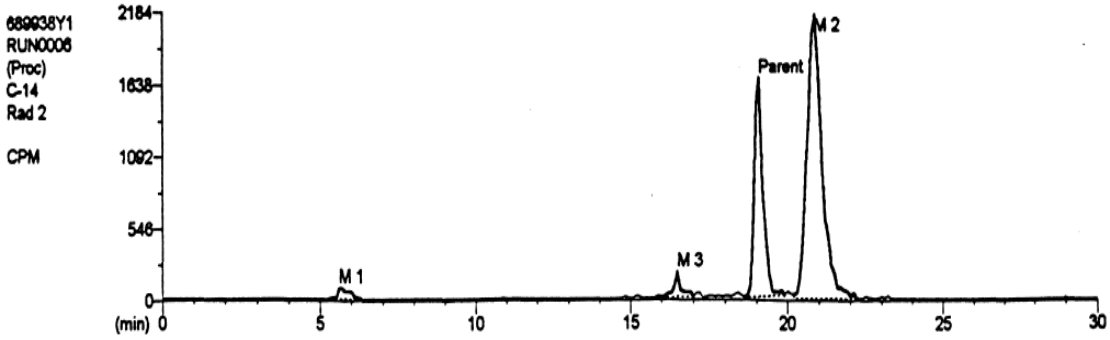
Figure B.8.4 **HPLC Radiochromatogram of Extract of Borstel Soil Incubated at 20ºC, following Application of [^{14}C]-Asulam at 4.8 kg/ha (Preliminary HPLC System)**



Column: 25 cm x 4 mm, 5 µm Hypersil 120 ODS
Mobile phase :A = Water/acetonitrile (pH 2.3, 95+5, v/v) B = Acetonitrile/water (95+5, v/v)
Gradient: 5 min. 0%B, 30 min 60%B

It can be seen that the peak which was aggregated by the HPLC used for quantification (shown in Figure B.8.5 below) came from about 40 small peaks and although together these represented 47.7% of applied radioactivity no peak is dominant and none could individually account for 5% AR. Accurate integration of such a forest of peaks would not be feasible. This is analogous to the 'maximum unknown' found in Yeomans 2000a.

Figure B.8.5 **HPLC Radiochromatogram of Extract of Borstel Soil Incubated at 20ºC, following Application of [^{14}C]-Asulam at 4.8 kg/ha (Final HPLC System)**



Conclusions:
[^{14}C]-Asulam rapidly degraded to a very large number of minor metabolites the disappearance of which was concomitant with the increase in unextractable residues. No metabolite represented > 10% AR or > 5% AR at two consecutive time-points. The minor metabolites that were identified included sulphanilamide and sulphanilic acid. Methyl-benzene-sulphonylcarbamate, 4-acetyl-asulam and benzenesulphonamide were shown to be among the compounds eluting close together as M2.

(Völkel, 2000a)

RMS opinion on study

The overall recoveries were acceptable with overall mean material balance ranging between 90.0 to 100.1. The major fraction in the distribution of radioacvtivity was M2 reaching a maximum of 47.7%AR on day 1 falling to 14.4% AR at day 118. Development chromatography showed this fraction to be composed of a large number of components 47.7%AR (up to 40 small peaks presented in Figure 7.1.1-3) methyl-benzene-sulphonylcarbamate (MBSC, desamino asulam), 4 acetyl-asulam and benzenesulphonamide plus several unidentified components each individual component being less than 5% AR. Numerous other minor metabolites were detected which included sulphanilamide and sulfanilic acid, none of these exceeded 5% at two consecutive timepoints.

The kinetics for the rate of degradation of asulam in this study was re-evaluated in the study of Hardy (2011a) below.

Overall the study of Völkel, (2000a) was well reported and considered acceptable by the RMS.

This paper has not previously been evaluated at EU level. It was raised to clarify the results of CA 7.1.1/01 (Yeomans, 2000a) following the study of CA 7.1.1/02 (Yeomans and Swales) that showed one of the peaks in the former study was an artefact.

Report:

P. Lowden (2013a) Recalculation of the Distribution of Extractable Radioactivity in An Asulam Soil Study following the Demonstration that One HPLC Peak is an Artefact. Battelle UK Ltd, Ongar Essex for United Phosphorus Ltd, Unpublished report No.: NZ/10/003A

Guidelines:
EU 95/36/EC
Deviations: Not applicable

GLP:
Not relevant

Executive Summary:
In the report on the laboratory soil degradation of asulam the tables summarising the percentages of asulam and its metabolites in each soil at various times of incubation contained a column with the heading 'Unknown 1'. This label was applied to a peak that appeared only in the soxhlet extracts. It was demonstrated in a subsequent study that this peak was an artefact of the HPLC system and resulted from the reaction of the acidified soxhlet extract with the liquid scintillant being used at that time (since replaced). The result of that peak being found to be an artefact, which had been integrated, was that the allocation of radioactivity to the various component compounds in the soxhlet extracts was incorrect, albeit by small amounts. This may have had a marginal effect on the kinetics of degradation of asulam.

The percentage of the artefactual peak in the soxhlet extracts was therefore redistributed between the other peaks, proportionally to their abundance. The new values were then added to the original values obtained from the HPLC of ambient extracts in which the artefactual peak did not appear (as they were not acidic and therefore were unaffected).

The changes in the concentrations of asulam were particularly small but, for accuracy, it is these recalculated values that should be used for kinetic analysis. The changes in concentrations of other compounds did not affect the conclusions originally drawn from this study.

Materials and Methods
The percentage of the applied radioactivity allocated to the artefactual peak was redistributed to the remaining peaks according to their abundance by use of an Excel spreadsheet. The values of each peak were taken from the tables in the report and that for the artefact was added to each of the others based on their value within the total.

Findings
The effect of this exercise was limited. In the early time-point samples most of the radioactivity was extracted by the initial extraction procedures and so little was present in the soxhlet extracts. In the middle and some of the later time-points there was a significant amount of radioactivity in these extracts but very little (and often no) asulam and so the correction made little difference to the asulam concentration in those extracts. The majority of the asulam having been extracted in the initial extracts the total amount of asulam recovered at each time-point was either unchanged or only marginally increased. There were increases (generally small) in the concentrations of sulphanilamide and acetyl sulphanilamide and the total unknowns.

Conclusions
The redistribution of the values obtained by integration for the HPLC radiopeak, later demonstrated to be an artefact, resulted in modest changes in the values for other compounds. The changes in the concentrations of asulam were particularly small but, for accuracy, it is these recalculated values that should be used for kinetic analysis. The changes in concentrations of other compounds did not affect the conclusions originally drawn from this study.

This paper has not been previously evaluated at EU level. A previous paper with the same title and conclusions was evaluated and no concerns were raised at EU level. This new paper covers the soil studies originally submitted and, in addition, soil studies not originally included in the original submission.

Report:
P. Lowden (2013b) Composition of an 'unknown' soil metabolite in Asulam soil degradation studies. Battelle UK Ltd, Ongar Essex, England for United Phosphorus Ltd, Unpublished report No.: NZ/10/003B

Guidelines:
EU 95/36/EC
Deviations: Not applicable
GLP: Not relevant

Executive Summary:
In the report on the laboratory soil degradation of asulam (Yeomans, 2000a) the tables summarising the percentages of asulam and its metabolites in each soil at various times of incubation contained a column with the heading 'Largest Unknown*'. The asterisk linked to the column heading a footnote to the table that stated 'Unless indicated this was an area of diffuse radioactivity (RT of ca 19 min) aggregated by a rapid change of gradient to allow quantification'. This area accounted for > 5% of applied radioactivity at more than one time-point in all soils incubated at 20°C with a maximum level of 8.4%. That gave rise to the question as to whether or not there could be a constituent within this area that accounted for >5% applied radioactivity at two consecutive time-points. In order to clarify the position and resolve the issues, the report was carefully re-examined and the raw data from the study were retrieved and examined. A review of the initial chromatography available from the raw data showed clearly that this unknown consists of not just a few but a large number of small peaks that were deliberately aggregated (in the knowledge that they were all very minor) for convenience. The initial chromatography revealed that none of the peaks in that region

was sufficiently dominant to account for the majority of the radioactivity in the region. This indicates that no individual compound would have represented > 5% AR at any time in the study.

Similarly a region designated 'M2' in the report of a laboratory soil study of asulam in a single soil (Völkel, 2000a) can be seen to be the same group of compounds as the 'largest unknown' of Yeomans, in greater concentration but still without any individual component accounting for > 5% AR. Some of the compounds detected in amongst this group were also found in the study of Roohi and Mackenzie, along with a number of unknowns. Neither the identified compounds nor the unknowns represented 5% AR at consecutive time-points.
This review shows that asulam degrades to a very large number of metabolites which in turn form bound residues. It is likely that most, if not all, of these, coming from a molecule that is small to begin with, are actually incorporated into the soil constituents. Sulphanilamide is the only metabolite that reaches the guideline trigger levels.

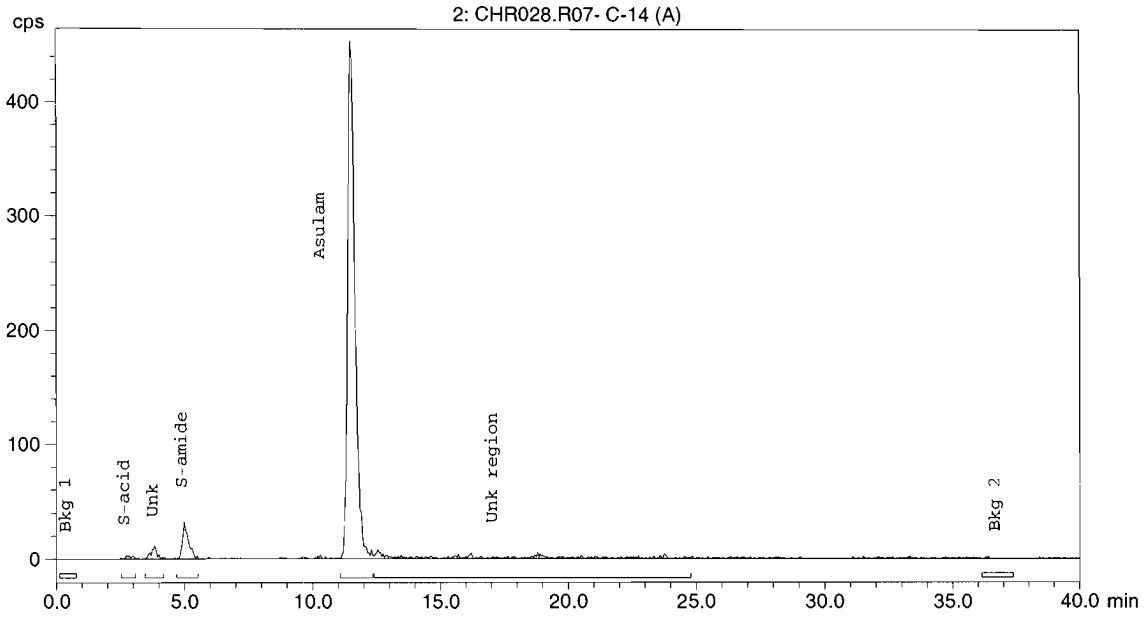
Materials and Methods
The raw data from the original Yeomans study were retrieved and examined. The appendices of the report on laboratory soil study of asulam in a single soil (Völkel, 2000a) were scrutinized for additional chromatographic data pertinent to the region of diffuse radioactivity and the report on the study of Roohi and Mackenzie was examined to see if it contained any information relevant to the multiplicity of peaks following that of parent.

Findings
The raw data from the Yeomans study showed that a total of four HPLC methods were used. The initial method employed an isocratic mobile phase of water/acetonitrile/formic acid and a run time of 30 minutes. Method 2 used a gradient of 0.2% formic acid in water against 0.2% formic acid in acetonitrile with a solid cell and a run time of 40 minutes. Method 3 was the same as method 2 except that the solid cell was replaced by a liquid cell. These methods were used for determining the radiopurity of the test compound. In addition a number of samples from preliminary incubations were examined by these methods as part of the method development for the main study. The radiochromatograms of sample extracts obtained by these methods showed that asulam had a retention time of about 10 minutes and that there were earlier-eluting peaks. They also showed that there were a very large number of very small peaks which followed that of asulam. In some chromatograms not all of these were clearly separated and the area was described as 'a region of diffuse radioactivity'. The integration of such a large number of small peaks (some close to limit of detection) was unlikely to give accurate quantification.

The gradient of the mobile phase was altered so that the retention time of asulam was increased (allowing better separation of peaks with shorter retention time) and so that an abrupt gradient change caused the forest of small peaks which followed that of asulam to be aggregated into a much smaller period of time. This allowed the run time to be reduced from 40 minutes to 25 minutes and the total area represented by all the small peaks following that of asulam to be accurately quantified. This was HPLC method 4 that was used for the analysis of sample extracts that were presented in the report.

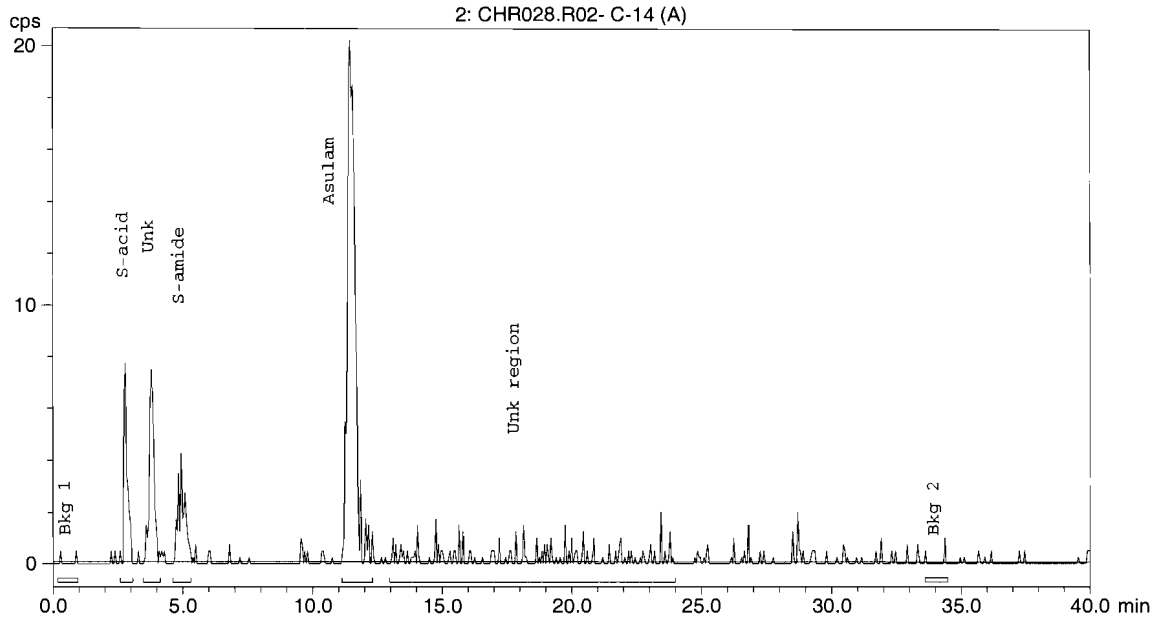
Extracts were examined by HPLC method 2 but were not presented in the report. These were, however, present in the raw data. Examination of these chromatograms shows that there is no dominant peak but a multiplicity of very small peaks. The chromatograms selected for the report covered a range of time-points from one soil type to demonstrate that this was the case over a range of time that the 'unknown' represented a significant amount of applied radioactivity. In chromatograms from early time-points (see Figure B.8.6) about 20 peaks can be distinguished (upon magnification) and none of these is large enough to account for anything close to 5% AR.

Figure B.8.6 HPLC radiochromatogram Method 2 Radiochromatogram of Extract of Soil PT102 at 7 Days of Incubation Flask 1 of 2 (Preliminary Experiment)



With time the number of peaks increases and become more obvious as the height of the asulam peak decreases. By the time-point of Figure B.8.7 there is clearly a forest of peaks, between 40 and 50 in number.

Figure B.8.7 **HPLC radiochromatogram Method 2 Radiochromatogram of Extract of Soil PT102 at 7 Days of Incubation Flask 1 of 2 (Preliminary Experiment)**



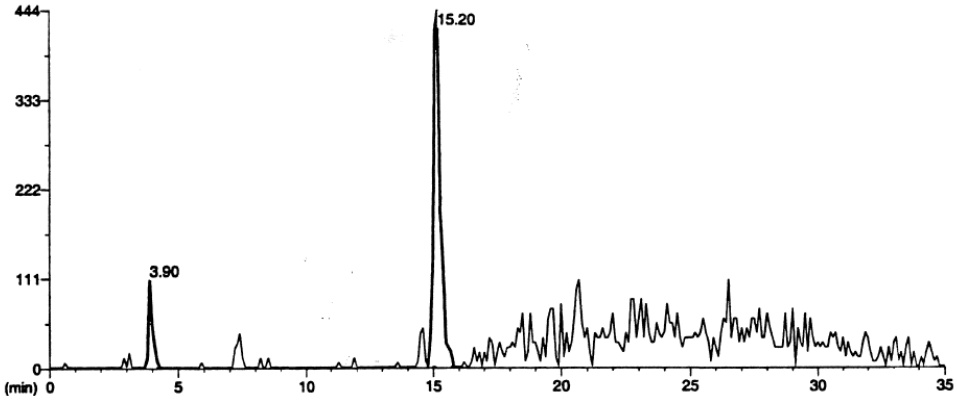
In the chromatograms presented in the report the peak shape suggests that it is an amalgam of peaks. It would have been difficult, and was probably not considered necessary in the light of the information gained during method development, to accurately distribute this peak amongst the number of compounds that it comprised. The omission of that information from the final report created the impression that the unknown area might include a dominant peak which would represent the majority of the radioactivity that area. In turn that would have meant that it could represent > 5% of applied radioactivity at two consecutive time-points. This resulted in the requirement for clarification.

In the study of Völkel there were a large number of HPLC systems used during the method development phase. One of these demonstrated that, as in the study of Yeomans, the radioactivity eluting after parent asulam comprised a very large number of metabolites/degradates, each of which accounted for a very small percentage of applied radioactivity. Unlike Yeomans some of these are presented in the report, in an appendix. The example selected is shown as Figure B.8.8. It can be seen that there is a large number of peaks following that of parent in that chromatogram as in the corresponding chromatograms from Yeomans. The difference in magnitude of the multiple peaks results from the more rapid and greater degradation of asulam in the study of Völkel, in which the aggregation of these peaks accounted for nearly 48% AR at its maximum. Despite that, the high number of peaks that are discernible (at least forty) with none dominant shows that no peak could represent in excess of 5% AR. The compounds that were detected in the aggregated region were methyl-benzene-sulphonylcarbamate (MBSC, desamino asulam), acetyl-asulam and benzenesulphonamide.

The compounds detected in the group eluting after parent compound in the HPLC system used in Roohi & Mackenzie were acetyl asulam and methyl-benzene-sulphonylcarbamate. These, with the unknowns that also eluted after parent, together represented similar amounts of applied radioactivity as the ‘largest unknown’ in the

study of Yeomans, which was an aggregation of compound eluting after parent. Neither of the identified compounds accounted for > 5% at consecutive time-points and nor did the groups of unknowns.

Figure B.8.8 **HPLC Radiochromatograms of Extract of Borstel Soil at 14 Days of Incubation (Völkel, Main Experiment)**



The composition of what was labelled as the 'largest unknown' in the asulam soil degradation study report would have been clearer if the terminology ('unknown area') used during initial chromatographic investigation had been applied in the report and if some of the initial chromatography had been presented. A review of the initial chromatography available from the raw data shows clearly that this unknown consists of not just a few but a large number (at least 30) of small peaks that were deliberately aggregated (in the knowledge that they were all very minor) for convenience. The area aggregated was described as that with a retention time of about 19 minutes. The initial chromatography reveals that the run time covered was extensive at 12 to 13 minutes long (i.e. from about 12 minutes to about 25 minutes, Figure B.8.7) and that none of the peaks in that region was sufficiently dominant to account for the majority of the radioactivity in the region. This indicates that no individual compound would have represented > 5% AR at any time in the study.

Examination of the HPLC radiochromatograms from Yeomans and from Völkel shows that the 'largest unknown' of the former and the 'M2' of the latter comprise the same group of minor and very minor metabolites that in both cases were deliberately aggregated by the HPLC system used for quantification. The system used in the study of Roohi & Mackenzie produced four regions two of which were multi-component. It is clear that asulam degrades to a very large number of metabolites which in turn form bound residues. It is likely that most, if not all, of these, coming from a molecule that is small to begin with, are actually incorporated into the soil constituents. Sulphanilamide is the only metabolite that reaches the guideline trigger levels.

This report has not previously been evaluated at EU level. It was requested by the RMS to provide firther information for the statement in the study of Willems (1997) that MBSC was a major metabolite of asulam in soil.

Report:

Edwards, R.A. (1972) Metabolic studies in soil with Asulam-ring ^{14}C, May & Baker Ltd Report No. RG/1433

Please note the summary below is the applicant's. It is an accurate account of the report and considered acceptable by the RMS. The RMS has provided comments at the end of the study summary.

Guidelines:

Not to any EU guideline

GLP:

No (pre-dates GLP)

Justification:

Not considered robust for further assessment

Notifier comments:

The study has been reviewed and is not considered robust for further evaluation due to a number of deficiencies:

- Not performed to any EU guideline (current or former)
- Non-GLP (predates GLP)
- Test item characterisation/purity not reported and no day 0 analysis
- No traps to collect CO_2 or organic volatiles
- No mass balance
- Only four datapoints (day 2 to 28)
- Soil collection/storage/handling information lacking
- TLC quantitation not reliable – Peak II clearly multiple components shown in 7/10 solvent systems
- Extraction method and analysis not evaluated

The report authors conclude that Metabolite A (Peak II) is tentatively identified as methyl benzenesulfonyl carbamate based on the TLC evidence. However, the identification was not confirmed by spectroscopic evaluation and the quantitative aspects of the study, *viz* assignment of activity to Peak II, are questioned based on multiple components shown to be present.
UPL therefore consider the study not robust, the quantitative results unreliable and not suitable for further evaluation. Methyl benzenesulfonyl carbamate is found as a minor (<10% and not >5% at two timepoints) metabolite in all other soil studies and does therefore not need to be considered further.

Executive Summary

The metabolism of asulam in a sandy clay loam soil was studied by radiochemical techniques using asulam-ring ^{14}C. The soil was spiked with asulam-ring ^{14}C at 20 ppm and stored for 28 days. Samples were taken for analysis 2, 7, 14 and 28 days after treatment.
Isotopically-labelled products were extracted from the soil with water, partitioned between various solvents and investigated by thin layer chromatography. 50-95% of the applied radioactivity was taken into ether by continuous extraction at pH 2.5-3.0 and most of the remainder into ethyl acetate at the same pH. In addition to asulam two metabolites were recovered from the soil. Metabolite A corresponded in several TLC systems with methyl benzenesulphonylcarbamate [MBSC, tentatively identified] and Metabolite B with benzenesulphonamide.

In moist soil after two days, 47.7% of the applied asulam remained, while 10.8% had been converted to metabolite A (methyl benzenesulphonylcarbamate) and 5.3% to metabolite B (benzenesulphonamide). The remainder of that extracted was contained in fine aggregates (5.0%) or was not analysed (9.2%). After 28 days only 7.9% of asulam remained, 8.4% of metabolite A, 3.4% as metabolite B, 5.1% in fine aggregates and 2.4% not analysed.
Within 28 days on moist soil less than 10% of radioactivity remained as asulam or either of the metabolites.

In flooded soil after two days 67.2% of the applied remained as asulam, 0% of metabolite A,· 6.2% as metabolite B, 22.4% as fine aggregates and 3.9'% not analysed. After 28 days 39.1% of asulam remained, 14.3% of metabolite A, 3.5% of metabolite B, 17.1% in fine aggregates and 4.0% was not analysed.

Materials and methods

Asulam-ring ^{14}C with a specific activity of 7.27 mCi/mM was used. No other details, e.g. purity, for the test substance were reported and no day 0 analysis was performed.

The study was carried out on Kettering Loam, a sandy clay loam [Table B.8. 32]. No collection / storage details were reported.

Table B.8. 32 **Soil characterisation**

	Kettering Loam
Texture	Sandy clay loam
Sand 0.2-2mm (%)	53.5
Silt 0.02-0.002mm (%)	17.0
Clay <0.002mm (%)	23.0
Organic matter (%)	4.3
pH [-]	6.9
MWHC (%w/w)	50

Analysis was by TLC on silica gel (Eastman 6060) or alumina (Eastman 6063) plates of 5x20cm [Table B.8. 33]. Principle analysis/quantitation was performed on silica gel

using Solvent System A: isopropanol/0.88 ammonia (10:1). Additional analyses were conducted with Solvent Systems B-J on silica gel and Solvent System K on alumina:

Table B.8. 33 **TLC solvent systems**

Solvent System	
A – silica gel	Isopropanol/0.88 ammonia (10:1)
B – silica gel	Acetonitrile
C – silica gel	Toluene/ethanol (3:1)
D – silica gel	1,2-Dichloroethane/toluene/glacial acetic acid (10:10:1)
E – silica gel	n-Butanol/conc. hydrochloric acid (10:1)
F – silica gel	n-Butanol/diethylketone (1:1)
G – silica gel	Acetone
H – silica gel	Chloroform/methanol (30:1)
J – silica gel	Ethyl acetate
K – alumina	Isopropanol/0.88 ammonia (10:1)

Study design

10g (dry weight) samples of air-died, sieved Kettering Loam in conical flasks were dosed at 20ppm with asulam-ring ^{14}C and the moisture contents made up to 25% and 200% (as a percentage of dry weight). They were stored in darkness at 25 ± 1°C for 2, 7, 14 and 28 days.

The samples were each mechanically shaken with 100 ml of distilled water for one hour. After decanting off the liquid a second extraction was performed. The two extracts were bulked. 10 ml of 12% aluminium sulphate solution was added and, after being allowed to stand for one hour, the solution was centrifuged at 2000 rpm for 20 minutes. The clear supernatant was made acid to pH 2.5-3.0 and continuously extracted, in darkness, with 50 ml diethyl ether for four hours. The aqueous phase was further continuously extracted with ethyl acetate at pH 2.5-3.0 for four hours. The residual aqueous phase was then taken to dryness at 45°C on a rotary vacuum evaporator and mechanically shaken with three 25 ml portions of 10% acetone in ethanol before filtering.
The ether, ethyl acetate and ethanol solutions were all concentrated to about 0.75 ml on a rotary vacuum evaporator and stored in a refrigerator ready for analysis.

The soil residue was placed in a Soxhlet thimble and extracted in darkness for eight hours with 50 ml of 5% benzene in ethanol. Aliquots of the solution were assayed for radioactivity. The soil residue was further Soxhlet extracted for eight hours with distilled water and the aqueous extract partitioned by continuous extraction with ether and ethyl acetate at pH 2.5-3.0. The benzene/ethanol, diethyl ether, and ethyl acetate fractions were taken down to about 0.75 ml on a rotary vacuum evaporator and the samples stored in a refrigerator.

The fractions obtained by partition of the soil extract between water and various organic solvents were subjected to a preliminary TLC examination in isopropanol/0.88 ammonia (10:1 v/v).
Samples were bulked and the whole spotted onto prepared Merck silica gel plates and developed in isopropanol/0.88 ammonia (10:1) before scanning. The activity-containing sections were removed and eluted with four portions of 25 ml acetone by shaking for two hours each. The acetone solutions were concentrated and spotted onto

fresh plates. These were developed in diethyl ketone/n-butanol (1:1) and the elution of activity repeated. The acetone solutions were again evaporated to small volume and stored in a freezer unit.
Small amounts of the solutions were spotted onto Eastman Chromagram silica gel sheets (6060) and developed again in isopropanol/ammonia (10:1) to assure purity of activity.

Results and Discussion
Table B.8. 34 summarises the reported quantitation results. The origin material appears to be associated with fine aggregates and Peak I is assigned as asulam. Peak II (also described as Metabolite A) is tentatively identified as methyl benzenesulfonyl carbamate. Peak III (also described as Metabolite B) is tentatively identified as benzenesulfonamide.

Table B.8. 34 **TLC solvent system A: %AR attributed to each peak number**

Sample	%Applied radioactivity						
	O	i	I	II	III	IV	Not analysed
25%MC, day 2	5.0	0	47.7	10.8	5.3	0	9.2
25%MC, day 7	12.4	0	41.8	11.6	5.9	0.3	2.0
25%MC, day 14	9.4	0.6	29.9	11.4	5.8	0	4.1
25%MC, day 28	5.1	0	7.9	8.4	3.4	0.5	2.4
200%MC, day 2	22.4	0	67.2	0	6.2	0.3	3.9
200%MC, day 28	17.1	1.3	39.1	14.3	3.5	0	4.0

Figure B.8.9 to Figure B.8.12 show the corresponding TLC scans for the 25%MC day 2, 7, 14 and 28 samples. These show asulam as a broad peak centred around R_f 0.27, with additional peaks at R_f 0.37-0.40 (Peak II) and 0.53-0.56 (Peak III). No details of the integration methods used to assign ^{14}C activity to each peak is described in the report, thus the robustness of the evaluation and assignments in Table B.8. 34 cannot be assessed. Assignment of activity for Peak II in sample (3) [Figure B.8.11] would clearly be problematic and questionable. The results in Table B.8. 34 are the consolidated data across the various extracts and partitions. The ether extracts of the water partition represent the major fraction of the extracted radioactivity (>65%) and thus it is difficult to explain the results for day 7 of Peak II representing 11.6%AR (ca 25% of the asulam peak) when considering Figure B.8.10.

Figure B.8.9 **TLC solvent system A: 25%MC day2 sample**

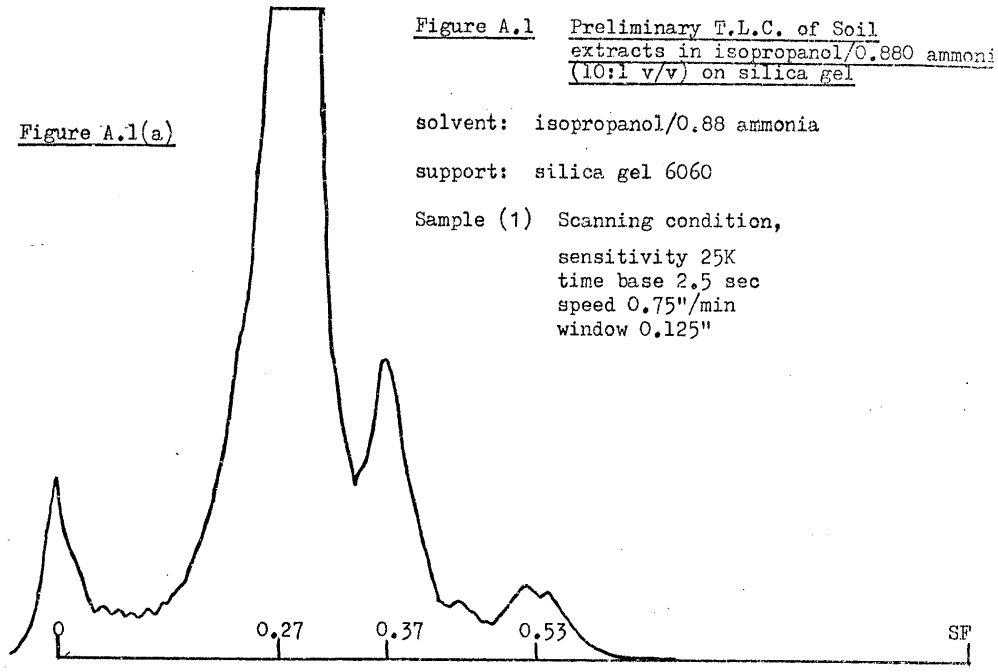


Figure B.8.10 **TLC solvent system A: 25%MC day7 sample**

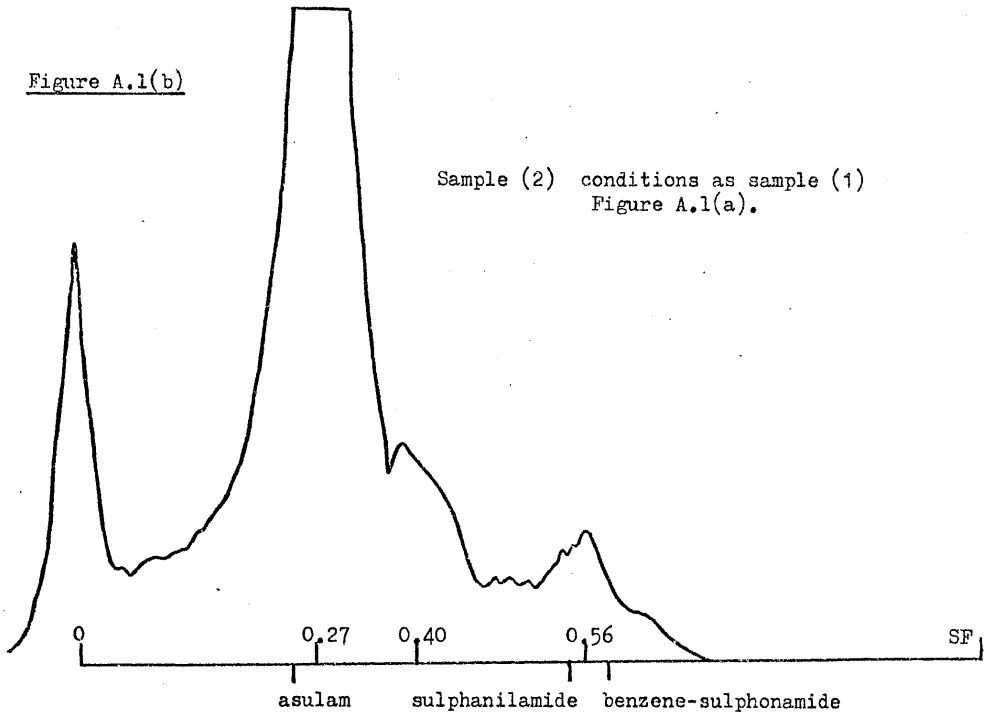


Figure B.8.11 **TLC solvent system A: 25%MC day14 sample**

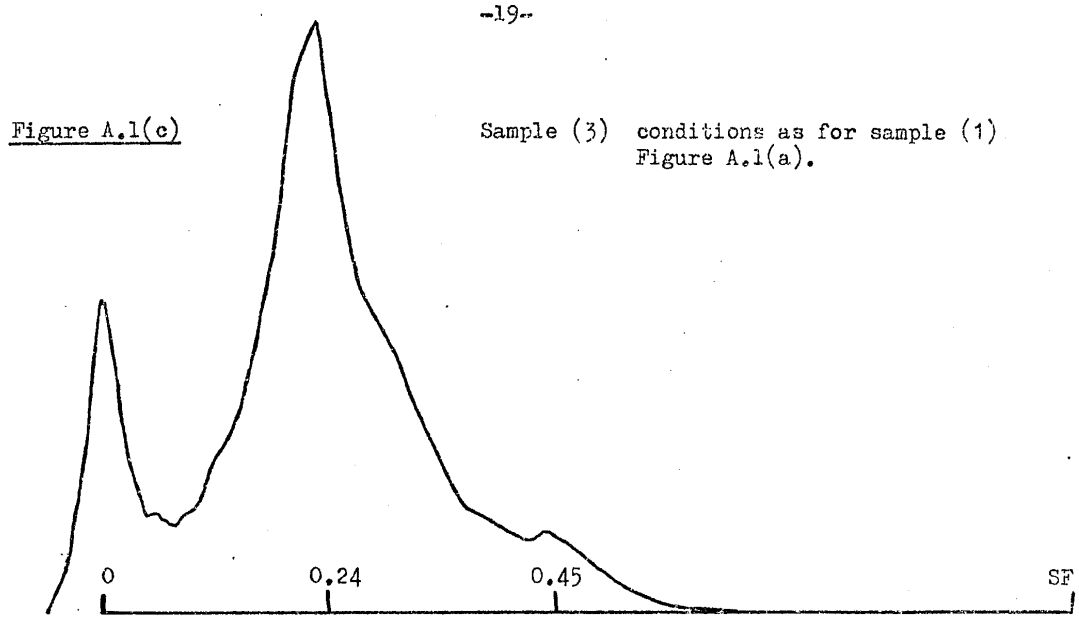
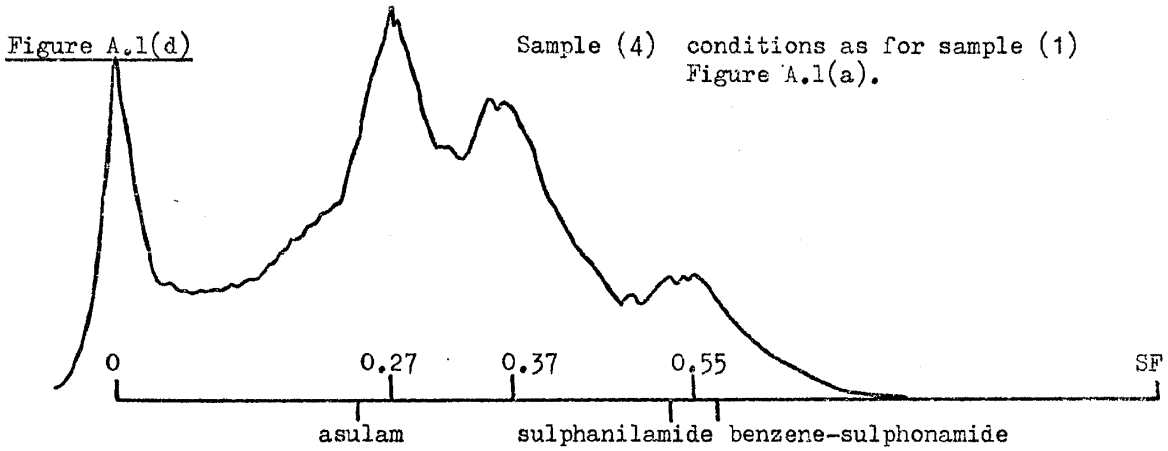


Figure B.8.12 **TLC solvent system A: 25%MC day28 sample**



Extraction of Peak II from the TLC plates followed by further analysis in additional solvent systems (B-K) shows that Peak II is composed of multiple [at least two] separate components. The extracted Peak II was evaluated in a total of 10 TLC systems [Table B.8. 33], resulting in multiple components in 7 of them [Table B.8. 35].

Table B.8. 35 **Comparison of R_f values for Peak II with methyl-benzenesulfonyl carbamate standard**

Sample	R_f Comparison									
	A	B	C	D	E	F	G	H	J	K

Methyl benzenesulfonyl carbamate	0.35	0.50	0.34	0.34	0.78	0.75	0.59	-	0.42	0.18
Peak II	0.37	0.15 0.54	0.11 0.36	0 0.37	0.78	0.19 0.75	0.23 0.67	0 0.08	0.07 0.48	0.14

Figure B.8.13 and

Figure B.8.14 show the TLC analysis of the concentrated Peak II extract in solvent systems A and G.

Figure B.8.13 **TLC solvent system A: Extracted Peak II concentrate**

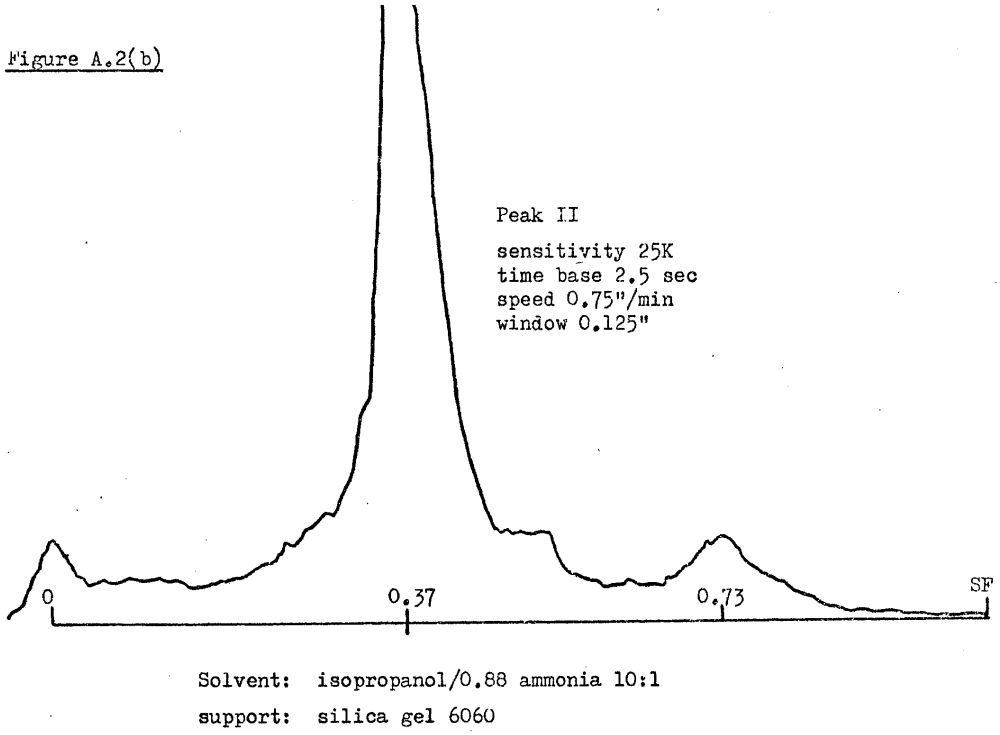
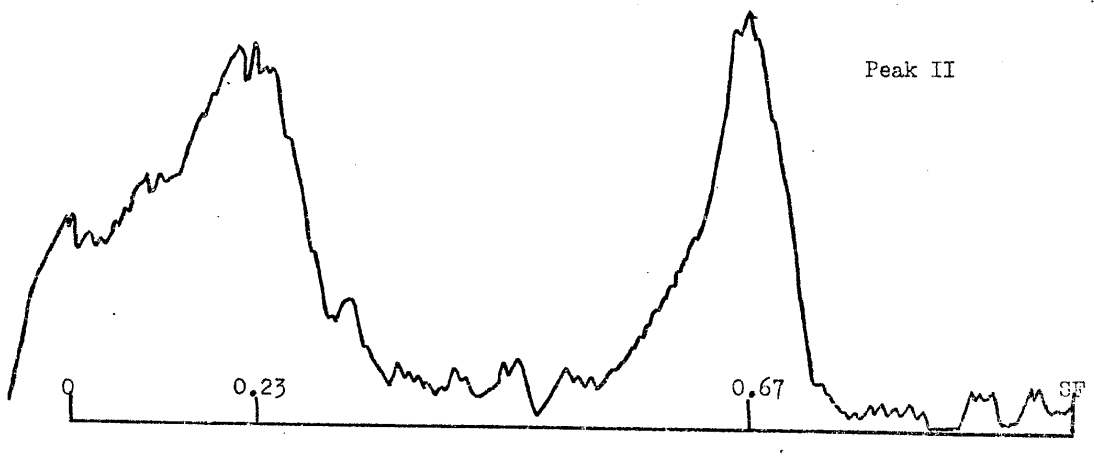


Figure B.8.14 **TLC solvent system G: Extracted Peak II concentrate**



conditions

sensitivity 1K
time base 10 sec
speed 0.75"/min
window 0.125"

solvent: acetone

support: silica gel 6060

Whilst Figure B.8.13 shows good extractability and concentration of Peak II,

Figure B.8.14 clearly illustrates that 'Peak II' is composed of at least two separate components (one of which may be tentatively identified as methyl benzensulfonyl carbamate [no spectroscopic confirmation]). The multiple and broad nature of the peaks in

Figure B.8.14 and the TLC analyses in general indicate that the radioactivity quantified as Peak II is composed of multiple components and thus the quantitation is no considered robust and cannot be relied upon.

The position paper of Lowden (2013b) summarised under CA 7.1.1/06 shows that asulam degrades in soil to yield a large number of minor components which are found to co-chromatograph. With the low resolving power of the TLC systems used in the Edwards (1972) report (as evidenced with asulam itself being a broad peak across an R_f range of ca 0.2 to 0.4) it is highly likely that the regions of radioactivity assigned to Peaks II and III are in fact constituted of multiple minor components.

Conclusions

The report authors conclude that Metabolite A (Peak II) is tentatively identified as methyl benzenesulfonyl carbamate based on the TLC evidence. However, the identification was not confirmed by spectroscopic evaluation and the quantitative aspects of the study, *viz* assignment of activity to Peak II, are questioned based on multiple components shown to be present.

UPL therefore consider the study not robust, the quantitative results unreliable and not suitable for further evaluation. Methyl benzenesulfonyl carbamate is found as a minor (<10% and not >5% at two timepoints) metabolite in all other soil studies and does therefore not need to be considered further.

RMS comments

Whilst the Edwards 1972 study was probably conducted to the standards accepted and using the technology available at that time, it does not meet the requirements of modern EU regulatory studies. The RMS has noted a number of concerns regarding the study. One point in favour of the study is that it was performed on radiolabelled material. However there are a number of deficiencies which are listed below:

- Not performed to any EU guideline
- Non-GLP (predates GLP)
- No traps to collect CO_2 or organic volatiles
- No mass balance therefore uncertainty regarding recovery of radioactivity
- Radiochemical purity determined but not reported
- No time zero samples taken
- Only four data points from day 2 to day 28
- Extraction method and analysis not evaluated

In addition to the points listed above, since there are no time zero samples and only four data points from day 2 to day 28, this does not comply with the current kinetics guidance (FOCUS 2006) since the minimum of six data points is recommended for reliable DT50 calculation, including a time zero value which in this case is not available. For these reasons, the RMS proposes not to calculate a soil DT50 from the data in the Edwards 1972 study.

The RMS notes that the asulam residue decreases rapidly to 47.7 AR% after 2 days which is consistent with the later studies (DT50 range 2.1 to 11.1, geomean 4.7 days).

Radioactivity from soil after treatment was investigated by TLC in order to characterise the components of the extracts.
Based on Rf values Peak I and Peak III (metabolite B) were identified as asulam and benzene sulphonamide respectively in all ten solvent systems.

TLC solvent systems

Solvent System	
A – silica gel	Isopropanol/0.88 ammonia (10:1)
B – silica gel	Acetonitrile
C – silica gel	Toluene/ethanol (3:1)
D – silica gel	1,2-Dichloroethane/toluene/glacial acetic acid (10:10:1)
E – silica gel	n-Butanol/conc. hydrochloric acid (10:1)
F – silica gel	n-Butanol/diethylketone (1:1)
G – silica gel	Acetone
H – silica gel	Chloroform/methanol (30:1)
J – silica gel	Ethyl acetate
K – alumina	Isopropanol/0.88 ammonia (10:1)

The study authors considered that Peak II did not correspond closely to the reference compounds. The study authors postulated that Peak II could be methyl benzene sulphonylcarbamate although at this point a reference standard was not available. When a reference standard became available, it was run on the various solvent systems to determine the Rf values. The Rf values from the reference standard were then compared with the Rf values of Peak II in the various solvent systems. The study authors concluded that Peak II appeared to correspond to methyl-benzene sulphonamide. It is noted by the RMS (and in the study) that the Rf values of the reference standard were determined at a later date than the extracts (not determined at the same time/simultaneously) and not under strictly identical conditions. This casts doubt on the validity of the tentative identification of Peak II. In addition, it is also noted by the RMS (and the notifier) that in 7 out of 10 solvent systems, Peak II is present as multiple peaks in the TLC indicating that Peak II was actually composed of a number of components. Furthermore, on inspection of other reference standards run simultaneously with the extracts, Peak II could possibly correspond to a number of the other reference standards for example, (in addition to methyl benzene sulphonyl carbamate):

Comparison of R_f values for Peak II with methyl-benzenesulfonyl carbamate standard

Sample	R_f Comparison									
	A	B	C	D	E	F	G	H	J	K
Asulam	0.21	0.19	0.06	0.06	0.73	0.30	0.25	0	0.08	0.04
Sulphanilamide	0.60	0.57	0.28	0.06	0.52	0.74	0.65	0.12	0.38	0.50
2-methoxyasulam	0.38	0.19	0.10	0.14	0.68	0.41	0.49	0.21	0.13	0.01
Benzene sulphonamide	0.75	0.63	0.51	0.22	-	0.74	0.67	-	0.65	-

n-acetyl asulam	0.66	0.19	0.04	0	0.78	0.19	0.13	0.03	0.03	0.09
n-acetyl sulphanilamide	-	0.51	0.27	0	0.73	0.72	0.65	0.11	0.27	0.51
quinone	0.70	0.64	0.64	0.60	-	0	0.69	0	0.62	0
Methyl benzenesulfonyl carbamate	0.35	0.50	0.34	0.34	0.78	0.75	0.59	-	0.42	0.18
Peak I	0.20	0.14	0.08	0.05	0.80	0.24	0.27	0	0.11	0.03
Peak II	0.37	0.15 0.54	0.11 0.36	0 0.37	0.78	0.19 0.75	0.23 0.67	0 0.08	0.07 0.48	0.14
Peak III	0.73	0.68	0.48	0.22	0.77	0.78	0.68	0.40	.066	0.58

Solvent system A, single peak: asulam and sulphanilamide
Solvent system B, two peaks: asulam, sulphanilamide, 2-methoxyasulam, benzene sulphonamide, n-acetyl asulam, n-acetyl sulphanilamide.
Solvent system C, 2 peaks: 2-methoxy asulam, sulphanilamide or methyl benzene sulphonyl carbamate.
Solvent system D, two peaks: n-acetyl asulam, n-acetyl sulphanilamide, methyl benzene sulphonyl carbamate.
Solvent system E, single peak: n-acetyl asulam or methyl benzene sulphonyl carbamate.
Solvent system F, two peaks: sulphanilamide, Benzene sulphonamide, n-acetyl asulam, n-acetyl sulphanilamide, methyl benzene sulphonyl carbamate.
Solvent system G, two peaks: asulam, sulphanilamide, Benzene sulphonamide, n-acetyl sulphanilamide, quinone.
Solvent system H, two peaks: asulam, sulphanilamide, n-acetyl sulphanilamide, quinone.
Solvent system J, two peaks: asulam, methyl benzene sulphonyl carbamate.
Solvent system K, single peak: methyl benzene sulphonyl carbamate.

In addition, only a selection of the TLC chromatograms are presented in the report therefore it is not possible to verify many of the findings, whether single or multiple peaks or Rf values. The Rf values vary and therefore do not correlate well.

Overall the study was not well reported. Chromatograms in Appendix IV are not clearly labelled, i.e. it is difficult to identify which sample the chromatogram refers to and the assignments could be correlated to multiple peaks and are very uncertain. In the opinion of the RMS, the report cannot be relied upon.

B.8.1.1.2 Anaerobic degradation

This study has previously been evaluated at EU level. The conclusions are consistent with the EFSA conclusions report and no concerns were raised at EU level.

Report:

b) P. Yeomans (2003a) [^{14}C]-Asulam Sodium: Metabolism in Flooded Soil. Covance Laboratories Ltd, Harrogate, North Yorkshire, England for Bayer CropScience SA, Unpublished report No.: 1849/21-D2149

Guidelines:
EU 95/36/EC
Deviations: No

GLP: Yes

A laboratory degradation study was conducted in one anaerobic soil. The study was conducted in accordance with EC Directive 95/36/EC and SETAC guidelines (1995) and to GLP. The study was considered acceptable by the Rapporteur.

The route of degradation was investigated in a UK sandy loam soil, using ring labelled asulam sodium (specific activity 5.617 MBq/mg; radiochemical purity >97%). The soil was maintained at 45% of MWHC and incubated in the dark at 20±2°C prior to study initiation. Soil characteristics (see Table B.8.36) were similar to one of the soils used in the aerobic study.

Table B.8. 36 Characterisation of soil used to investigate the anaerobic degradation of ^{14}C-asulam sodium

Soil Code	PT102
UK Particle Size Distribution:	
Sand % (2000-63 µm)	53
Silt % (63-2 µm)	36
Clay % (<2 µm)	11
Textural Class ADAS	Sandy loam
Textural Class USDA	Sandy loam
Textural Class BBA	mittel lehmiger Sand
Organic Carbon (%)	2.2
Organic Matter (%)	3.8
pH in Water	6.7
pH in KCl (1 M)	6.6
pH in 0.01M $CaCl_2$	6.5
Cation Exchange Capacity mEq/100 g	25.0
Water holding capacity at pF 0 (0.001 bar) %	69.8
Water holding capacity at pF 2.5 (0.33 bar) %	26.4
Biomass (µg C/g)	
Study start	344.5
Study end	150.6

Distilled water was added to a level of 3 cm above separate 100g soil samples (dry weight, 2mm sieved) and samples were purged with nitrogen and allowed to equilibrate for 32 days to establish anaerobic conditions. ^{14}C-asulam in equimolar sodium hydroxide was added to the surface of the water at a rate equivalent to 4.4 kg a.s./ha. The vessels were fitted with 5 traps, an empty security trap, ethanediol and

liquid paraffin/xylene to trap polar and non-polar volatiles, and two sodium hydroxide traps to collect CO_2. Six further samples were prepared and dosed with an equivalent amount of non-labelled asulam for biomass determination at the beginning and the end of the study. The redox potential in the soil was measured before application and at each sampling interval and confirmed that anaerobic conditions were maintained throughout the study.

Sampling was performed at intervals of 0h (immediately after application), 1, 3, 7, 14, 30, 62 and 120 days after application. The surface water was aspirated from the incubation units and analysed directly by HPLC and LSC. The soil was extracted 4 x with acetone:water (1:1 v/v) which were combined for LSC quantitation. The combined extracts were evaporated to near dryness and reconstituted in acetonitrile for HPLC analysis with UV and radiochemical detection. The extracted soil was further Soxhlet extracted first with acidified water (pH2) followed by acidified acetonitrile (pH2) and extracts combined for LSC. Since this accounted for <7% of applied radioactivity, the extracts were not analysed.

The radioactivity in the trapping reagents was quantified by LSC. The traps were analysed when the units to which they were attached were removed for analysis, or were analysed and replenished with fresh reagents 30, 62 and 120 days after application.

The recovery of and distribution of applied radioactivity between surface water and soil is shown in Table B.8. 37.

Asulam sodium and its degradation products were identified by co-chromatography with reference compounds by HPLC. Levels of asulam sodium and its degradation products are presented in Table B.8. 38 to Table B.8. 40.

The total recovery of radioactivity was 94 to 103% for all samples. The radioactivity in the surface water decreased from 93% initially to 44% after 120 days.

The only degradation product detected using HPLC was sulphanilamide (at <1% AR) together with an area of diffuse radioactivity (labelled unknown 1) aggregated by a rapid change in the mobile phase gradient to allow quantification . The maximum level of unknown 1 was 9% applied radioactivity. None of the unknowns were reported by the Notifier to exceed 10% applied radioactivity. The Rapporteur agreed with this on the basis of the HPLC evidence provided.

Table B.8. 37 Recovery of applied radioactvity from a flooded soil system incubated in the dark at 20°C following a single application of ^{14}C-asulam sodium

Time (days)	Surface Water	Soil Extract	Soxhlet Extract 1	Soxhlet Extract 2	Bound Residue	Traps (Total)	Mass balance
0	93.3	1.8	nd	nd	nd	na	95.1
1	83.5	18.8	0.1	0.2	0.3	nd	102.9
3	74.4	20.2	0.3	0.3	0.7	nd	95.9
7	74.2	21.2	0.4	0.6	1.5	nd	97.9

14	63.5	28.8	1.1	1.8	1.7	0.1	97.0
30	58.3	31.1	0.8	2.6	3.4	0.2	96.4
62	51.7	33.3	2.2	2.7	5.4	0.2	95.5
120	43.5	35.4	4.6	2.2	7.9	0.4	94.0

nd = not detected na = not applicable

Table B.8. 38 Percentage of applied radioactvity present as parent and its degradationproducts in the surface water from a flooded soil system incubated in the dark at 20°C following a single application of ^{14}C-asulam sodium

Time (days)	Asulam	Sulfanil -amide	Unknown 1	Largest Unknown	Other Unknowns	Unresolved Background	Total
0	90.4	nd	2.6	nd	nd	0.3	93.3
1	82.7	nd	nd	nd	nd	0.8	83.5
3	69.0	nd	4.1	nd	0.8	0.5	74.4
7	68.4	nd	4.2	nd	0.5	1.1	74.2
14	62.5	nd	nd	nd	nd	1.0	63.5
30	57.8	nd	nd	nd	nd	0.5	58.3
62	50.9	nd	0.3	nd	nd	0.5	51.7
120	40.9	nd	2.5	nd	nd	0.2	43.5

nd = not detected

Table B.8. 39 Percentage of applied radioactivity present as parent and its degradation products in the soil extract from a flooded soil system incubated in the dark at 20°C following a single application of ^{14}C-asulam sodium

Time (days)	Asulam	Sulfanil -amide	Unknown 1	Largest Unknown*	Other Unknowns	Unresolved Background	Total
0	na	na	na	na	na	na	na
1	11.2	0.2	4.1	1.2	3.1	0.2	18.8
3	17.2	0.3	2.5	nd	nd	0.2	20.2
7	12.6	nd	4.8	1.8	3.6	0.2	21.2
14	20.3	0.2	4.2	2.0	4.0	0.1	28.8
30	20.5	0.4	3.3	2.8	6.7	0.2	31.1
62	24.8	0.4	3.4	2.3	4.6	0.2	33.3
120	26.8	0.8	3.1	2.5	4.7	nd	35.4

* included in sum of other unknowns (i.e. not unknown 1) in next column
na = not applicable nd = not detected

Table B.8. 40 Percentage of applied radioactvity present as parent and its degradation products in the total system (soil + water) from a flooded soil system incubated in the dark at 20°C following a single application of ^{14}C-asulam sodium

Time	Asulam	Sulfanil	Unknown 1	Largest	Other	Unresolved	Total

(days)		-amide		Unknown*	Unknowns	Background	
0	90.4	nd	2.6	nd	nd	0.3	93.3
1	93.9	0.2	4.1	1.2	3.1	1.0	102.3
3	86.2	0.3	6.6	nd	0.8	0.7	94.6
7	81.0	nd	9.0	1.8	4.1	1.3	95.4
14	82.8	0.2	4.2	2.0	4.0	1.1	92.3
30	78.3	0.4	3.3	2.8	6.7	0.7	89.4
62	75.7	0.4	3.7	2.3	4.5	0.7	85.0
120	67.7	0.8	5.6	2.5	4.7	0.2	78.9

* included in sum of other unknowns (i.e. not unknown 1) in next column nd = not detected

(Yeomans, 2003a)

This study has not previously been evaluated at EU level. It has been evaluated at member-state level. The conclusions are in agreement with the EFSA conclusions.

Report:

c) G. Morlock (2009a) Degradation of Asulam (^{14}C- labelled) in One Soil under Anaerobic Conditions at 20 °C in the Dark. Separate Analysis of Water and Soil. eurofins GAB GmbH, Niefern-Oschelbronn, Germany for AgriChem BV. Unpublished Report No. S08-00327.

Guidelines:
EU 95/36/EC, OECD 307
Deviations: None

GLP: Yes

The objective of this study was to determine the disappearance rate and degradation products of asulam in one soil under anaerobic conditions in the dark. The soil was the German standard soil LUFA 2.2. The applied rate of asulam was equivalent to 4.4 kg a.s. /ha. To establish and maintain anaerobic conditions, the soil was treated with the test item and incubated under aerobic conditions for 12 days then water logged (1-3 cm water layer) and the incubation system flushed with nitrogen. Samples were investigated over a period of 118 days. Water and soil were analysed separately. Duplicate samples were taken for analysis at specified intervals up to 118 days after application. Organic volatiles and carbon dioxide were trapped. The radioactivity in the soil extract and water phase (only during anaerobic phase) was quantified (separately) and characterised by reversed phase thin layer chromatography (RP-TLC) and normal phase thin layer chromatography (NP-TLC).

The soil system showed a mean recovery of 101.8 % of the applied radioactivity (AR) during the aerobic phase (day 0 to day 12) and a mean recovery of 102.8 % AR during the anaerobic phase (day 13 to day 118).

The extractable radioactivity was found to decrease during aerobic phase from 94.8% (day 0) to 50.6% AR (day 12) in soil extracts. During the anaerobic phase, the extractable radioactivity (water phase and soil extract) increased again from 51.4% one day after flooding (day 13) to 57.1% AR at the end of incubation (day 118). The extractable radioactivity was partitioned in soil extract and water phase. In the soil extract the amount of radioactivity decreased from 36.6% (day 13) to 22.5% AR (day 118) and in the water phase the amount increased from 14.8% (day 13) to 34.6% AR (day 118) at the end of incubation. The non-extractable radioactivity (bound residues) reached a maximum value of 52.7% AR during the aerobic phase and kept stable at a plateau of about 46% AR during anaerobic phase. $^{14}CO_2$ accounted for 1.2% of the applied radioactivity after 118 days. It was trapped from the gas space during the aerobic phase (0.5% AR), the gas space during the anaerobic phase (0.5% AR) and found dissolved in water (0.2% AR). No other organic volatiles were trapped.
The results of this study showed that asulam disappeared rapidly under aerobic conditions. After flooding, the total amount of asulam increased from 36.2% (day 13) to 47.0% AR at the end of incubation (day 118). While in soil extracts the amount of

asulam dissipated from 23.9% one day after flooding (day 13) to 16.1% AR at the end of incubation (day 118), the amount in the water phase increased from 12.4% to 30.9% AR at the end of incubation. This result indicated that asulam re-dissolved from non-extractable residues (bound residues) during the anaerobic phase. The extraction procedure in this study was less exhaustive than those used in the aerobic soil studies. Only one metabolite, identified by co-chromatography as sulphanilamide, was observed during the 118 days of incubation. It reached a plateau within the aerobic phase of about 18% AR. During the anaerobic phase the total amount of sulphanilamide decreased from 15.3% one day after flooding (day 13) to 10.2% AR at the end of incubation (day 118). In the water phase, the amount sulphanilamide was always below 5% AR. In the soil extract it disappeared within the anaerobic phase from 12.8% to 6.4% AR at the end of incubation.

Materials and Methods

Test Material:
[^{14}C]-Asulam sodium, radiochemical purity 98.6%, Batch no.: 2873JJN1-1

Test Design:
The study was conducted with radiolabelled asulam in a single soil (LUFA 2.2), the characteristics of which are shown overleaf. The application solution was prepared in water containing 1.6% acetone in which the radiolabelled asulam was diluted with non-radiolabelled asulam (76.1 µg labelled to 511.2 µg non-labelled).
Samples of soil (50 g dry weight equivalent) were dispensed into all-glass flasks, for treatment with the application solution. In addition samples of 100 g were dispensed for use in aerobic biomass determination and 150 g samples for use in anaerobic biomass determination. The soil samples were wetted to 45% of their water-holding capacity. They were allowed to pre-incubate in the dark at 20°C for a period prior to application. The soils were treated at a rate equivalent to 4.4 kg/ha (maximum field application rate) which corresponded to 587 µg/50 g dry soil per flask. Biomass samples were treated at the same equivalent rate.

Soil characteristics:

Soil description	LUFA 2.2 F220608
pH	5.69
Organic carbon (%)	1.82
Water capacity (%)	47.9
Cation exchange capacity (mval/100 g)	9.88
Soil density (g/L0	1188
Soil type	weak loamy sand
Particle size:	
2000 to ≥ 630 µm	0.7
630 to ≥ 200 µm	41.7
200 to ≥ 63 µm	34.6
63 to ≥ 20 µm	8.1
20 to ≥ 6.3 µm	5.2
6.3 to ≥ 2 µm	3.5
< 2 µm	6.2

Soil type (USDA)	loamy sand
Particle size:	
0.05 to 2.00 mm	79.3
0.002 to 0.050 mm	14.5
< 0.002 mm	6.2

After application the vessels were closed. The organic volatiles in the flasks were trapped by Tenax as adsorbent and the carbon dioxide in the flasks was trapped by a sodium hydroxide reservoir. The oxygen content inside the test vessels was determined by a pressure transducer system on two flasks with similar air volume. The pressure reduction by binding the carbon-dioxide to sodium hydroxide gave exact information about the oxygen consumption of the test system during the aerobic phase. The samples were aerated twice during the aerobic phase. The samples were incubated at 20 ± 2 °C, protected from light. The traps for organic volatiles and carbon dioxide were changed at least every 4 weeks.

After 12 days the system was water logged (about 3 cm water layer). The test system was connected with two traps of sodium hydroxide, one trap of ethylene glycol, a tenax air sampler tube and connected to a continuous stream of nitrogen. The samples were incubated at 20 ± 2 °C9 protected from light. At sampling time during the anaerobic phase the whole system was flushed with nitrogen to drive gaseous metabolism products from the gas phase into the traps. After opening the system, pH, redox potential and oxygen concentration were monitored.

Samples were taken for analysis at 0, 1, 3, 7 and 12 days during the aerobic phase and 13, 14, 16, 20, 28, 47, 69, 96, 118 days during the anaerobic phase. At each sampling time-point the trapping agents were radioassayed. Soil from the aerobic phase was spiked with a mixture of unlabelled asulam and sulphanilamide and extracted with 80 mL acetonitrile/water (1:1, v/v) four times and then with 80 mL acetone, twice. When anaerobic phase samples were taken for analysis the water was decanted and placed in vessels protected from light. The water was submitted to the following extraction scheme: The radioactivity in the water was determined directly by LSC of an aliquot (3 x 1 g) before it was poured out of the incubation flask. To a further 3 aliquots 100 µL acetic acid was added and the next day the radioactivity remaining in the aliquots was measured to determine the amount of carbon dioxide, which was dissolved in the water phase. The soil was subjected to a series of extractions. It was spiked with a mixture of unlabelled asulam and sulphanilamide and 80 mL of acetonitrile/water (1:1, v/v) and 1 mL of acetic acid sufficient to get a pH below 5.0 was added.

The incubation flask was closed with a carbon dioxide trap (30 mL 2 M sodium hydroxide solution) to determine the amount of carbon dioxide which was bound to soil. The assembly was shaken overnight to allow evolution of the carbon dioxide and the extraction of the soil. The amount of radioactivity in the carbon dioxide trap was then determined by liquid scintillation counting. The dispersed soil was then transferred to a 200 mL glass centrifuge tube and the extract was separated from the soil by centrifugation. The extraction was repeated three times with 60 mL acetonitrile/ water (1/1; v/v). Subsequently, the soil was extracted two times with 60 mL of pure acetone under the previously described conditions. The radioactivity in the extracts

was determined by LSC using an aliquot. The acetone and acetonitrile/water extracts were combined and radioassayed by LSC of aliquots.
After the final extraction, the soil was dried prior to combustion. The total amount of non-extractable radioactive residues in soil after extraction was determined by combustion and LSC of sub-samples.

The radioactivity in the extracts was characterised reversed-phased TLC and by normal-phase TLC (i.e. two systems based on different principles). The fractions were co-chromatographed with the available reference compounds. For reversed-phase TLC the extracts (water phase and soil extracts) were applied directly to thin layer plates (stationary phase: Macherey-Nagel RP 18W/UV 254). The mobile phase was acetonitrile/water (30/70 v/v). Normal -phase TLC was used as confirmatory method to the reversed phase TLC. The extracts were applied directly to thin layer plates (stationary phase: Merck Silica gel 60 F254). The mobile phase was ethyl acetate/ hexane/ acetic acid (50:50:20 v/v/v). The radioactivity on the TLC plates was determined optically by a Fluorescence Radioluminography Scanner. The non-labelled reference compounds were identified visually by UV treatment at 254 nm as dark spots against a fluorescent background.

Results and Discussion:
The mean recovery during the aerobic phase was 101.8 % AR (99.0% -105.8 %). The CC>2 increased to 0.6 % AR after 12 days. No organic volatiles were observed. The non-extractable radioactivity (bound residues) increased to a maximum value of 52.7% AR 12 days after application. There was a corresponding reduction in extractable radioactivity from 94.8 % AR directly after application to 50.6 % AR at the end of the aerobic phase. Asulam decreased from 77.6% directly after application to 32.4% AR after 12 days during aerobic incubation. The major and only metabolite, identified as sulphanilamide by co-chromatography, reached a plateau of about 18 % AR after 3 days.

Within the anaerobic phase, the pH of the water phase ranged between 6.1 and 8.3. The oxygen content was low, not exceeding 2.6 mg/L. The redox potential decreased from values between 253-280 mV directly after flooding (day 13) to values between 22-30 mV at the end of incubation (day 118). In the soil the redox potential decreased from values between 243 and 247 mV directly after flooding (day 13) to values below -262 mV at the end of incubation. The determination of the biomass at the start, during and at the end of anaerobic phase shows the viability throughout the incubation period.
The mean recovery from the anaerobic soil system was 102.8 % AR. The distribution of recovered radioactivity is summarized in Table B.8.41 below.

Table B.8. 41 **Recovery and distribution of applied radioactivity from flooded LUFA 2.2 soil following treatment with [^{14}C]-asulam sodium at 4.4 mg/kg**

Time (days)	% applied radioactivity in:						
	Aerobic Phase CO2	Anaerobic phase CO2	Water Phase	Soil Extracts	Total Extracted	Bound Residue	Total
13	0.6	0.4	14.8	36.6	51.4	47.4	99.7
14	0.6	0.2	13.2	37.5	48.4	48.5	95.9
16	0.4	0.4	24.3	28.6	52.9	48.3	101.8

Time (days)	% applied radioactivity in:						
	Aerobic Phase CO2	Anaerobic phase CO2	Water Phase	Soil Extracts	Total Extracted	Bound Residue	Total
20	0.7	0.9	27.8	27.7	55.6	46.3	103.3
28	0.6	0.5	33.2	25.9	59.2	46.2	106.4
47	0.8	0.6	33.7	21.6	55.2	49.2	105.7
69	0.5	0.6	35.1	23.9	59.0	45.4	105.4
96	0.6	0.6	33.5	21.5	54.9	47.4	103.4
118	0.5	0.7	34.6	22.5	57.1	45.9	104.0

The table above shows that the total extractable radioactivity (sum of water phase and soil), increased from 51.4 % one day after flooding (day 13) to 57.1 % AR at the end of the test (day 118). In soil extracts, the amount of radioactivity decreased from 36.6 % one day after flooding (day 13) to 22.5% AR at the end of incubation (day 118) while in the water phase the amount of applied radioactivity increased from initial 14.8 % to a plateau of about 34 % AR two weeks after flooding (day 28). The non-extractable residues (bound residues) in soil fluctuated between 45.4% and 49.2% of applied radioactivity throughout the complete anaerobic phase. Within the 118 days of incubation no organic volatiles were detected throughout the incubation period. Total mineralisation to carbon dioxide was 1.2% AR after 118 days taking into account the amount of carbon dioxide evolved during aerobic and anaerobic phase.
The composition of extracted radioactivity at each sampling time-point is shown in Table B.8.42 below.

Table B.8. 42 **Recovery and distribution of applied radioactivity from flooded LUFA 2.2 soil following treatment with [^{14}C]-asulam sodium at 4.4 mg/kg**

Time (days)	% applied radioactivity as:			
	asulam	Sulphanil-amide	Total Identified	Total Extractable
13	36.2	15.3	51.5	51.4
14	34.1	14.5	48.6	48.4
16	38.2	14.9	53.1	52.9
20	42.5	13.1	55.6	55.6
28	47.4	11.9	59.3	59.2
47	44.6	10.6	55.2	55.2
69	46.2	12.9	59.1	59.0
96	44.3	10.7	55.0	54.9
118	47.0	10.2	57.2	57.1

The amount of asulam increased within the anaerobic phase from 36.2% (day 13) to 47.0% AR (day 118) with a plateau of 44.3% to 47.4% AR reached after 28 days. The increase of asulam corresponded to the increase in total extractable radioactivity in the water phase. The amount of the metabolite sulphanilamide decreased slightly from 15.3 % to about 10 % AR during the anaerobic phase.

Conclusions:
Asulam disappeared rapidly under aerobic conditions but under anaerobic conditions no disappearance of asulam was observed. The only metabolite, sulphanilamide

increased rapidly in the aerobic phase and decreased slowly during the anaerobic phase.

(Morlock, 2009a)

RMS opinion on study

The overall recoveries were acceptable with overall mean material balance ranging from 95.9 to 106.4% AR. The recovery at day 28 is in excess of 105% but still considered acceptable by the RMS The amount of asulam increased during the anaerobic phase although this corresponded to the recovery in the water phase. From this it can be concluded that overall, asulam was not degrading during the anaerobic phase. The major metabolite was sulphanilamide which decreased from 15.3% at the start of the anaerobic phase to 10.2% AR at the study end. No other minor metabolites were detected.

Overall the study of Morlock, (2009a) was well reported and considered acceptable by the RMS.

B.8.1.1.3 Soil rate of degradation studies - laboratory

B.8.1.1.3.1 Photolysis in soil

This study has previously been evaluated at EU level. The conclusions are consistent with the EFSA conclusions report and no concerns were raised at EU level.

Report:
P. Yeomans, S. Swales (2000a) [^{14}C]-Asulam Sodium: Photodegradation on a Soil Surface. Covance Laboratories Ltd, Harrogate, North Yorkshire, England for Aventis CropScience, Unpublished report No.: 68/172-D2142

Guidelines:
EU 95/36/EC, US EPA 161-3
Deviations: None

GLP: Yes

c) A laboratory soil photolysis study was conducted in one soil. The study was conducted in accordance with EPA guideline N, 161-3 (1982), EC Directive 95/36/EC and SETAC guidelines (1995) and to GLP and the study was considered acceptable by the Rapporteur.

The photodegradation in soil of ring labelled asulam (specific activity 5.617 MBq/mg; radiochemical purity >98%) was studied in a clay loam soil. A sandy silt loam soil was used in the preliminary test but both soils were taken from the same location in the UK and had similar characteristics and therefore the Rapporteur did not consider that this affected the validity of the study. (see Table B.8. 43 for soil characteristics used in the definitive study).

Table B.8. 43 Characterisation of soil used to investigate the photolytic degradation of ^{14}C-asulam sodium on soil

UK Particle size distribution:	
Sand % (2000 – 63 µm)	24
Silt % (63 - 2 µm)	55
Clay % (<2 µm)	21
Textural Class ADAS	Clay loam
Textural Class BBA	schluffiger Lehm
Textural Class USDA	Silt loam
Organic carbon (%)	3.5
Organic matter (%)	6.0
pH (water)	7.1
pH (1M KCl)	6.2
pH ($CaCl_2$)	6.4
Cation exchange capacity (g/kg)	13.7
Moisture holding capacity (% at 0.01 bar)	96.6
Moisture holding capacity (% at 0.33 bar)	33.5
Biomass (µg C/g soil)	490.6

Soil samples (15g dry weight equivalent) were dispensed into glass vials equipped with quartz glass lids (for the irradiated samples) and air inlet/outlet ports. Radiolabelled asulam in an equimolar solution of sodium hydroxide was applied as the sodium salt to the soil surface, equivalent to an application rate of 4.4 kg a.s./ha. Irradiated samples were exposed to simulated sunlight from a Heraeus Suntest CPS accelerated exposure machine equivalent to UK summer sunlight at 54°N (nominally 445 watts/m^2) and their temperature maintained at 25±1°C. The sunlight exposure times were then converted to exposure times in Florida, USA (30°N). The light was filtered to exclude wavelengths below 290 nm to simulate natural sunlight. The dark controls were placed in an incubator at 25±1°C. Humidified air was drawn over the surface of the irradiated and dark control soil samples through a series of traps. In the preliminary study, six traps were employed, a polyurethane foam trap being inserted between the units and the first trap (an empty vessel as a security trap). The third and fourth traps were ethanediol and liquid paraffin in xylene to collect polar and non-polar volatiles respectively followed by 2 sodium hydroxide traps to collect liberated CO_2. No radioactivity was recovered in the polyurethane foam or the ethanediol or liquid paraffin xylene therefore the number of traps used in the definitive study was reduced to three. The first being the empty vessel as a security trap. The second and third containing sodium hydroxide to trap liberated CO_2.

The moisture content was adjusted daily to 75±10% of its moisture holding capacity at 33 kPa. Dupilcate samples were removed for analysis immediately after application and at 0.5, 1, 2, 3, 5, 10, 14, and 24 days (equivalent to 0.5, 1.07, 2.04, 2.90, 4.81, 10.59, 15.33 and 29.08 days Florida summer sunlight). Duplicate dark control samples were removed at the same time as irradiated units.

Samples were extracted three times with acetone:water (1:1 v/v) and extracts combined and evaporated to near dryness and reconstituted in acetonitrile prior to HPLC analysis.

The extracted soil samples were air dried prior to combustion in oxygen to determine bound residue levels. Recoveries of radioactivity from samples are displayed in Tables Table B.8.44 and Table B.8.45. The overall recovery of AR was 95 to 105% for irradiated and dark controls.

Levels of asulam and degradation products are shown in Tables B.8.46 and B.8.47. Products were quantified by HPLC, and the presence of asulam and sulphanilamide were confirmed by mass spectroscopy.

In the presence of light, degradation of asulam was accelerated without producing any unique photodegradates in comparison with the dark controls.

The single first order DT50's for asulam sodium in the irradiated and dark control samples was 3.71 and 6.69 d respectively. The single first order DT_{50} for asulam sodium due to photolysis only (corrected for degradation in the dark controls) was 8.3d. Overall the Rapporteur considered that photolysis was unlikely to be a significant rate of dissipation in the field compared with biotic degradation in the absence of light.

Table B.8. 44 Recovery and distribution of applied radioactivity from irradiated soil samples following application of ^{14}C-asulam sodium to the surface (means of replicate samples)

Time (days)	Soil Extract	Soil Residue	Sodium Hydroxide Traps	Mass balance
0	96.7	4.1	na	100.8
0.5	80.6	18.5	0.1	99.1
1	73.4	24.8	0.2	98.4
2	61.5	35.4	0.6	97.4
3	53.8	48.4	0.7	102.9
5	38.2	52.1	0.6	90.9
10	31.6	63.5	1.1	96.1
14	24.3	71.5	0.7	96.5
24	15.7	77.6	1.6	94.8
Overall mean recovery				97.4

Table B.8. 45 Recovery and distribution of applied radioactivity from non-irradiated soil samples following application of ^{14}C-asulam sodium to the surface (means of replicate samples)

Time (days)	Soil Extract	Soil Residue	Sodium Hydroxide Traps	Mass balance
0	96.7	4.1	na	100.8
0.5	88.5	10.8	0.0	99.3
1	83.8	14.8	0.1	98.6
2	75.0	22.7	0.2	97.9
3	69.8	24.9	0.4	95.0
5	64.2	40.4	0.3	104.8
10	42.9	54.1	0.6	97.5
14	27.7	68.2	1.3	97.2
24	16.9	78.9	2.1	97.8
Overall mean recovery				98.8

Table B.8. 46 Percentage of applied radioactivity present as asulam and its degradation products in extracts of irradiated soil maintained at 25°C following a single application of ^{14}C asulam

Time (days)	Asulam	Sulfanilic Acid	Sulfanil -amide	Acetyl Sulfanil -amide	Acetyl Asulam	Largest Unknown*	Total Unknowns	Un-resolved Bkg	Total allocated
0	92.7	nd	0.3	0.1	nd	0.6	1.4	0.1	94.6
0.5	77.8	nd	0.7	nd	nd	0.9	1.6	0.5	80.6
1	66.3	0.2	0.1	nd	0.2	3.7	5.3	0.6	72.7
2	55.1	nd	1.7	nd	0.1	1.8	3.1	0.4	60.4
3	43.7	0.1	1.0	nd	0.3	6.1	8.1	0.6	53.7
5	29.7	0.0	1.4	nd	0.4	4.5	5.8	0.4	37.7
10	23.0	nd	3.6	nd	0.3	3.2	4.2	0.3	31.4
14	13.1	0.0	1.7	nd	0.9	3.5	7.5	0.3	23.4
24	5.8	0.1	1.7	nd	1.3	3.0	6.3	0.2	15.4

* included in total unknowns in next column

Table B.8. 47 Percentage of applied radioactivity present as asulam and its degradation products in extracts of dark control soil maintained at 25°C following a single application of ^{14}C asulam

Time (days)	Asulam	Sulfanilic Acid	Sulfanil -amide	Acetyl Sulfanil -amide	Acetyl Asulam	Largest Unknown*	Total Unknowns	Un-resolved Bkg	Total allocated
0	92.7	nd	0.3	0.1	nd	0.6	1.4	0.1	94.6
0.5	86.9	0.1	0.6	nd	0.1	0.3	0.6	0.3	88.5

1	79.1	0.1	0.2	nd	0.3	0.7	1.3	0.9	81.9
2	72.9	nd	1.4	nd	0.2	0.5	0.5	0.5	75.5
3	64.8	nd	2.1	nd	0.2	1.7	1.7	0.4	69.1
5	56.9	nd	3.3	nd	0.2	2.7	2.9	0.6	63.8
10	30.2	nd	10.7	nd	nd	1.4	1.7	0.2	42.8
14	20.8	nd	3.5	nd	nd	1.9	2.6	0.2	27.1
24	10.8	nd	4.2	nd	nd	1.2	1.3	0.1	16.4

* included in total unknowns in next column

(Yeomans and Swales, 2000a)

This study has not previously been evaluated at EU level. It has been evaluated at member-state level. The conclusions are consistent with those of the previously submitted study and the EFSA conclusions

Report:
M. Doble and A. Oddy (2007a) [^{14}C]-Asulam: Soil Photolysis. Battelle UK Ltd, Ongar, Essex, England for AgriChem BV. Unpublished report No.: OU/07/007

Guidelines:
EU 95/36/EC, US EPA 161-3
Deviations: None

GLP: Yes

RMS opinion on study: fully acceptable. Please note the summary below is the applicant's. It is an accurate account of the report and considered acceptable by the RMS. The RMS has provided comments at the end of the study summary.

Executive Summary:
The photolysis of [^{14}C]- asulam on soil was investigated under aerobic conditions, at 20 ± 2°C, with a soil moisture content of 75% of ⅓ bar water holding capacity and with continuous irradiation by artificial sunlight for a period of 18 days. The artificial sunlight was provided by a xenon arc lamp with filters to cut off any radiation below 290 nm (Heraeus Suntest).

The nominal treatment rate per photolysis vessel was 271 µg which was based on a single maximum application rate in the field of 4400 g/ha and a surface area of the soil sample of 6.16 cm^2. Due to the number of samples required being greater than could be fitted under the Suntest the dosing was split into two series. The concentration of [^{14}C]-asulam in the first dosing solution was 5.19 mg mL which required dosing with 52 µl (277 µg) onto the soil in each photolysis vessel. The concentration of [^{14}C]-asulam in the second dosing solution was 2.23 mg/mL which then required dosing with 113 µl (255 µg) onto the soil in the photolysis vessel.

The test vessels were connected to traps for the collection of carbon dioxide and organic volatiles. Irradiation was continued for 18 days (equivalent to ca 30.7 days natural sunlight at Phoenix, Arizona). Duplicate samples from the irradiated regime were taken at 1, 2, 3, 4, 7, 11 and 18 days. Duplicate samples from the non irradiated

regime (dark controls) were taken at 0, 1, 2, 3, 4, 7, 11, and 18 days. The control samples were incubated under the same conditions except kept in the dark. The soil samples were extracted three times with 50:50 v/v acetone/water and the extracts were then analysed by HPLC using ^{14}C-flow-through detection techniques. Identification and confirmation of asulam and metabolites was performed by HPLC with comparison to certified analytical standards as the primary analytical method and, for selected samples, by LC/MS as the secondary method.

The overall mean recovery of applied radioactivity was 95.7% (range 88.4% to 101.7%) in the irradiated samples and 96% (range 90.3% to 106.2%) in the non-irradiated samples.

In the irradiated samples, the amount of extractable radioactivity decreased from 95.2% (mean of duplicates) at time zero to 26.6% (mean of duplicates) by the end of the study period. At the same time, the total unextractable radioactivity increased from 1.2% at time zero to 66.2% (mean of duplicates) by day 18. At the end of the experimental phase, evolved $^{14}CO_2$ and volatile organics accounted for up to 1.7% (in one sample at day 18) of the applied radioactivity.

In the irradiated samples, parent asulam was found to be the major component in the extracts throughout the study. HPLC analysis showed that the amount of asulam in the soil extracts declined from 93.3% to 10.1% by the end of the study. Some minor transformation products were also detected; sulphanilic acid, sulphanilamide and acetyl asulam plus eight minor, unknown degradation products none exceeding 3% of the applied radioactivity individually, with the exception of two of the unknowns (RRT 1.36 and RRT 1.53) which reached *ca* 4% of the applied dose at day 18.

In the non-irradiated samples, the amount of extractable radioactivity decreased from 95.2% (mean of duplicates) at time zero to approximately 51.8% by the end of the study period at day 18 (mean of duplicates). At the same time, the total unextractable radioactivity increased from 1.2% at time zero to 40.5% (mean of duplicates) by day 18. At the end of the experimental phase, evolved $^{14}CO_2$ and volatile organics accounted for up to 0.3 % of the applied radioactivity.

In the non-irradiated system, parent asulam was found to be the major component in the extracts throughout the study. HPLC analysis showed that the amount of asulam in the soil extracts declined from 93.3% at time zero to 47% by the end of the study. Some minor transformation products were also detected; sulphanilic acid, sulphanilamide and acetyl asulam plus six minor unknown degradation products none of which exceeded 2% of the applied radioactivity.

The half lives/DT_{50}s were estimated using the KinGUI kinetic model (single first order) and were found to be 5.6 days for irradiated samples and 18.4 days for dark controls.

The results showed that the degradation of asulam on a soil surface is accelerated by light without the production of unique photoproducts. In the soil environment photolysis will have no affect on the route of degradation of asulam but will increase its rate of dissipation.

Materials and Methods

Test Material:
[^{14}C]-Asulam sodium, radiochemical purity 98.1%, Batch no.: 2414MFO004-2

Test Design:
The photodegradation of asulam (as the sodium salt) on the surface of soil was investigated. The soil was a clay loam (USDA) or clay (ADAS) soil. Its characteristics are shown in the table below.

Soil was placed into uniquely labelled photolysis dishes which were positioned onto an inverted scintillation vial inside a glass incubation unit. Eleven out of twelve labelled glass incubation units were used and each sealed with a threaded cap containing a quartz window and PTFE washers providing an airtight seal. The twelfth vessel was used to monitor the temperature of the system (see below). The photolysis vessels were placed on a twelve position frame and raised into position under the Suntest apparatus with a lab-jack.

Characteristics of soil used for soil photolysis study:

Soil Reference	07/012
Source Geographic Location	UK Rutland Empingham Chapel Hill Farm
Soil Description	Clay/Clay Loam
Soil mapping reference	SK 961089
Textural classification (USDA) Sand (63-2000 µm) Silt (2-63 µm) Clay (<2 µm)	Clay Loam 22 38 40
Textural classification (ADAS) Sand (50-2000 µm) Silt (2-50 µm) Clay (<2 µm)	Clay 26 34 40
% Moisture Content	33.7
Water Holding Capacity at 0.1bar (% w/w) Water Holding Capacity at 0.33 bar (% w/w)	46.0 33.3
Organic Carbon (%)	5.6
Organic Matter	9.6
Cation Exchange Capacity meq/100g	34.7
Soil pH after 24 hr equilibration (1:1 in water) (1:1 in 0.01M CaCl2) (1:1 in 1M KCl)	 7.6 7.3 6.3
Microbial biomass: mg C/100g % organic matter carbon	 87.46 1.58

Approximately 2.5 g of moist soil, equivalent to *ca* 2.0 g of oven dried soil, at a soil moisture content of 75% of the moisture content at ⅓ bar was weighed into

individually labelled vessels and the exact weight was recorded. Sufficient samples were prepared to allow for duplicate samples at each sampling interval. The photolysis dishes numbered as 1 and 2 were used for time zero and the soil was weighed out into extraction vessels rather than photolysis dishes. To allow for the number of samplings required, two separate treatments occurred during the study. The first treatment was for the 0, 1, 2, 3, 7 and 18 day samplings and the second treatment for the 4, 11 and one irradiated vessel at day 7.

Each photolysis incubation unit had an inlet and an outlet to allow moist, sterile, carbon dioxide free air to be pumped across the soil surface. This was produced by bubbling air through two scrubbers, one containing aqueous KOH solution (2 M) and the other containing de-ionised water. From the scrubbers the air was split into twelve streams, pumped through tubing containing capillary flow restrictors (to regulate flow), across the sample surface, and then into a series of traps. One ethylene glycol and two KOH (2 M) traps were used to capture any volatile degradates evolved. The [^{14}C]-asulam (10.766 mg, 52.9 MBq for first sample series and 4.777 mg, 23.22 MBq for second sample series) was dissolved in 2 mL of water containing an equimolar portion of sodium hydroxide. The stock solutions were diluted by removing an aliquot of 0.05 mL and diluting to 50 mL with acetone/water 50:50 v/v mix. Aliquots of this dilution were removed and counted by LSC to determine the exact concentration of the stock solutions. The concentration of the stock solutions were determined to be 5.19 mg mL^{-1} (first treatment) and 2.23 mg mL^{-1} (second treatment). An aliquot of 52 µL was therefore required to achieve the target application rate of 271 µg per test vessel in the first treatment series. For the second series an aliquot of 113 µL was required to achieve the target application rate. Treatment checks were taken at the time of application. Following treatment, each vessel was incubated at a constant temperature of 20 ± 2°C. The whole system of non-irradiated samples was placed in the dark, in a temperature-controlled room at 20°C with vessels additionally covered with black plastic sheets to ensure continuous darkness. The temperature was automatically recorded using a 'squirrel' data logging system.

The series of samples for irradiation were placed in the photolysis vessels under the Suntest apparatus. A water/ethylene glycol mixture, circulating through the water jacket around the vessels, dissipated the heat generated by the xenon light source. The coolant passed through a flow heater/cooler for temperature control to keep samples at 20°C as monitored by a temperature probe in the twelfth photolysis vessel which contained 2 g of soil. The irradiation was continuous.

For non-irradiated samples the incubation time was recorded as the time interval from treatment to the test vessel being taken from the controlled temperature room. For the irradiated samples the time of sampling was the total irradiation time. At each sampling point, the time was recorded when the lamp was turned off and switched on again in order to determine the total irradiation time. The moisture content of all samples was checked each day and was adjusted by addition of de-ionized water. Duplicate of irradiated and non-irradiated samples were taken for analysis at 0, 1, 2, 3, 4, 7, 11 and 18 days.

Soil samples were extracted three times with acetone/water (1:1, v/v) and the extracts were combined. Following extraction, the soil samples were air-dried, weighed and ground to a fine powder. They were radioassayed by combustion and LSC of sub-

samples. The trapping solutions were removed for analysis at each sampling time except for time zero samples. The volume of each solution was measured and the radioactivity determined by removing 1 mL aliquots for LSC.

Extracts were examined by HPLC. The system comprised a Phenomex Synergy Hydro 4µm 250 x 4.6 mm i.d. column connected to a UV detector and a radiodetector fitted with a liquid cell. The identity of the [^{14}C]-asulam wand its presence in selected extracts was confirmed by LC/MS (Liquid Chromatography-Electrospray Mass Spectrometry coupled with [^{14}C] analysis) in comparison with equivalent data for the certified reference standard. Analyses were carried out on a Micromass QUATTRO-LC triple quadrapole mass spectrometer in positive ion Electrospray Ionisation (ESP+).

The limit of quantification for LSC was 0.0011% of applied radioactivity and that for HPLC was 0.08% of applied radioactivity.

Results and Discussion:
The recoveries of applied radioactivity were good being in the range of 90-110% of applied radioactivity for all samples, except for one individual sample (day 3, irradiated, vessel 10) where the radioactivity recovered was only 88.44%. For individual samples, the material balance was in the range of 88.4% to 101.7% (mean value 95.7%) for irradiated samples and 90.3% to 106.2% (mean 96%) for the non-irradiated samples.

The extractability of asulam from the soil declined with time with the majority of the radioactivity being accounted for as unextractable residues by the end of the study (95% of radioactivity recovered in the soil extract at time zero, declining to 26% by day 18). The unextracted residues increased steadily from 1.2% at time zero to 66% of the applied radioactivity (mean of duplicates) by day 18. The distribution of radioactivity in the non-irradiated system showed a similar pattern. The extractability declined with time and the majority of the radioactivity was found to be unextractable residues by day 18. The total extracted radioactivity declined from 95.2% to 51.8% by day 18. The unextracted residues increased from 1.2% at the beginning of the study to 40.5% by the end of the study.

Parent asulam was found to be the major component of the extractable residues throughout the study. HPLC analysis showed that the amount of asulam in the irradiated soil extracts declined from 93.3% at time zero to 10.1% by day 18. Some minor transformation products were also detected. These comprised sulphanilic acid, sulphanilamide and acetyl asulam plus eight, unknown degradation products none exceeding 3% of the applied radioactivity individually, except for two unknowns (RRT 1.36 and RRT 1.53) which reached approximately 4% of the applied dose by day 18. In non-irradiated samples the amount of parent asulam decreased steadily from 93.3% at time zero to approximately 47% by the end of the study period (mean of duplicates). A number of minor transformation products were also detected, and were characterised as sulphanilic acid, sulphanilamide, acetyl asulam plus five very minor unknown degradation products none exceeding 2% of the applied radioactivity.

Conclusions:
The results show that the degradation of asulam on a soil surface is accelerated by light without the production of unique photoproducts. In the soil environment photolysis will have no effect on the route of degradation of asulam but will increase the rate of its dissipation.

(Doble and Oddy, 2007a)

RMS opinion on study

The total recovery in one replicate of the irradiated samples on day 3 was a little low (88.44 % AR). No explanation was provided although taking the mean of the replicates, the recovery was 90.3% and considered acceptable by the RMS. The overall recoveries were acceptable with overall mean material balance ranging between 95.7 and 95.99 % AR in irradiated and non-irradiated samples respectively. Unextracted radioactivity increased with time in both irradiated and non-irradiated samples, both showing a similar pattern.

The major component in the irradiated samples from the extractable residues was asulam declining from 93% at time zero to 10% after 18 days irradiation compared to 47% in the dark controls. Transformation products comprised sulphanilic acid, sulphanilamide and acetyl asulam plus eight unknown degradation products none exceeding 4% AR. A number of minor degradates were detected in the dark controls, including sulphanilic acid, sulphanilamide, acetyl asulam plus five unknown products none exceeding 2% AR.

The RMS considers the study of Doble and Oddy (2007a) acceptable. Overall, in the presence of light, degradation of asulam was accelerated in comparison with the dark controls.

B.8.1.3 Field studies

Field dissipation

Since the DT50 of asulam, determined in laboratory soil metabolism studies is less than 60 days, field studies are not required.

Field accumulation

Since the DT90 of asulam, determined in laboratory soil metabolism studies is less than 1 year, field accumulation studies are not required.

This report has not previously been evaluated at EU level. It is a new report with the calculations conducted according to the latest guidance.

Report:
I.A.J. Hardy. (2013a) Kinetic Modelling Analysis of Asulam and Sulphanilamide Rate of Degradation Studies to Derive Modelling Endpoints. Battelle UK Ltd, Ongar, Essex, England for United Phosphorus Limited, Unpublished report No.: NZ/10/003C

I.A.J. Hardy. (2011a) Kinetic Modelling Analysis of Asulam and Sulphanilamide Rate of Degradation and Asulam Water / Sediment Studies to Derive Modelling Endpoints Battelle UK Ltd, Ongar, Essex, England for Agrichem BV,
Unpublished report No.: OU/10/001

Guidelines:
EU 91/414/EEC, Section 5, Point CA 7.1.1.
Deviations: None

GLP:
No, conducted according to Good Modelling Practice recommendations

The kinetics of the degradation of asulam in the results studies Yeomans (2000a), Yeomans and Swales 2003a, Roohi and Mackenzie 2009 and Völkel, 2000 have been re-evaluated in a new study (Hardy 2013a and Hardy 2011a) in accordance with current FOCUS kinetics Guidance (June 2006) for derivation of modelling parameters.

RMS opinion on study: fully acceptable

The soil properties for the above studies are listed below.

Study reference	Soil properties
Yeomans (2000a)	Table B.8. 1
Yeomans & Swales (2003a)	Table B.8. 2
Roohi & Mackenzie (2009)	
Völkel, 2000	

Only the kinetics of the soils incubated at 20°C are being re-evaluated. The results of the %AR present as asulam in the soils studied (reproduced from the original DAR).are presented in tables B.8.48 to B.8.49 below.

(PT102 soil at 10°C SFO DT50 = 9.3 d, normalised DT50 3.6 d at 20°C)

Table B.8. 48 Study 1, Yeomans 2000a, (%AR)

Time (days)	Soil PT102	Time (days)	Soil PT103	Soil SK15556090	Soil SK961089
0	94.1	0	97.8	93.7	99.2
0	91.4	1	66.3	54.9	68.8
1	68.6	3	44.3	35	51.4
1	69	7	27.6	15.6	29.1
3	51.9	14	11.5	1.8	14.7
3	45.2	28	3.0	0.5	4.2
7	26.8	59	ND (0.05*)	ND (0.05*)	ND (0.05*)
7	26.3	120	-	-	-
14	11.3				
14	11				

28	4.1
28	3.2
59	0.3
59	0.05*
120	0.05*
120	-

* ½ LOQ of 0.05% used in the kinetic evaluation

Table B.8. 49 Study 2, Yeomans and Swales (2003), %AR using different scintillant

Time (days)	Soil PT103	Soil SK15556090	Soil SK960087
0	94.4	95.7	97.0
1	61.2	36.5	65.8
3	36.4	35.8	41.1
7	17.2	13.7	18.3
14	8.9	4.6	3.1
21	2.7	3.5	1.6
28	1.6	1.1	1.7
42	1	0.5	1.3
62	0.5	0.4	0.5
90	0.3	0.1	0.3
120	0.4	ND (0.05*)	0.2

* ½ LOQ of 0.05% used in the kinetic evaluation

The laboratory soil metabolism study of Yeomans 2000a was performed on four soils, PT102, PT103, SK15556090 and SK961089. Due to formation of an artefact peak considered to be produced by the scintillant, a follow up study was performed using a different scintillant on three soils to demonstrate that the artefact peak was not present. The kinetics of degradation data from these studies has been re-evaluated in the study of Hardy (2013a) in accordance with FOCUS kinetics guidance (FOCUS, 2006) to derive modelling and persistence endpoints for use in the exposure assessment.

All datasets were initially evaluated using SFO kinetics with free optimisation of M0 and Kp. Where datasets were statistically and or visually unacceptable, further evaluation with FOMC, and DFOP kinetics were applied. A comparison between the models was made and the best-fit kinetic model was selected.

The kinetic evaluation was performed by the applicant using the KinGUI (v2.0). The acceptability of the kinetic fit was determined by visual inspection of the residual plot and the χ^2 error. The RMS has validated the kinetic fits using KinGUI v 2.1 using non-linear least square settings.

It is noted that the initial concentrations derived experimentally (see Tables B.8.2 to B.8.5 were low particularly for the 'SK' soils: 76.3 and 88.2% AR). In the original DAR, the initial concentrations were used in the derivation of the DT50s. In the current re-evaluations, the time zero values have been set to the total recovered

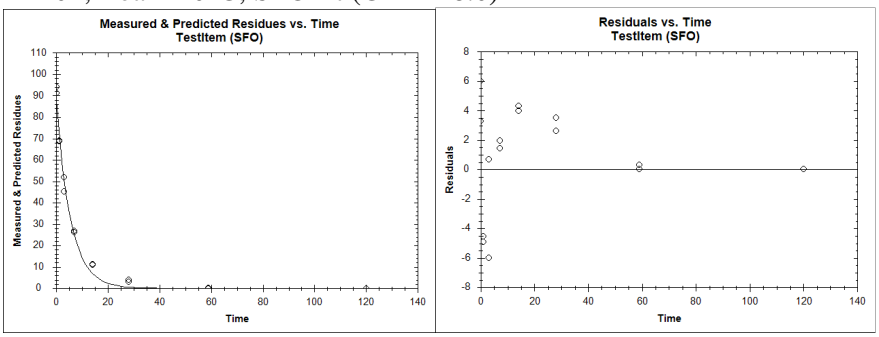
radioactivity (mass balance at time zero) for use in the kinetic evaluation in line with the current FOCUS kinetics guidance.

The results of the optimisations for the four soils at 20°C are presented in Tables B.8.50 to B.8.53.

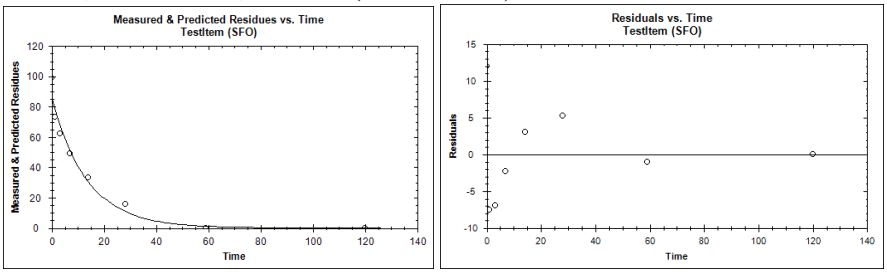
Table B.8. 50 Yeomans (2000a) study data: Asulam – normalised (20°C and pF2) aerobic soil SFO DT50 values for use as model input

Soil system	DT_{50} (days)	DT_{90} (days)	Min Chi^2 error (%)	t-test (-)	Visual assessment
PT102 Loam	3.8	12.7	8.0	2.14E-09	Acceptable
PT103 Sandy loam	3.4	11.5	12.3	0.00152	Acceptable
SK15556090 Silt loam	2.1	7.1	13.0	0.00103	Acceptable
SK961089 Clay loam	4.0	13.4	11.0	0.00105	Acceptable
PT102 Loam at 10°C	9.3	31.4	11.6	0.000835	Acceptable

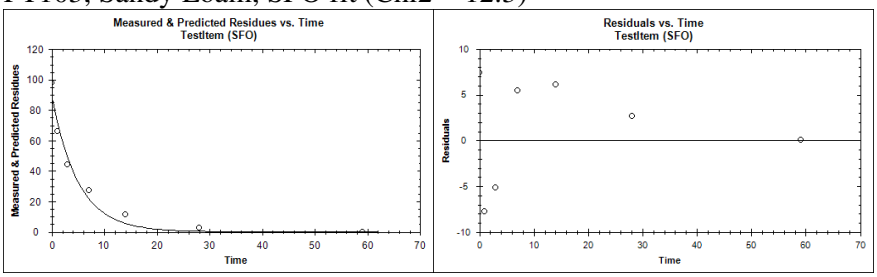
PT102, Loam 20°C, SFO fit (Chi2 = 8.0)



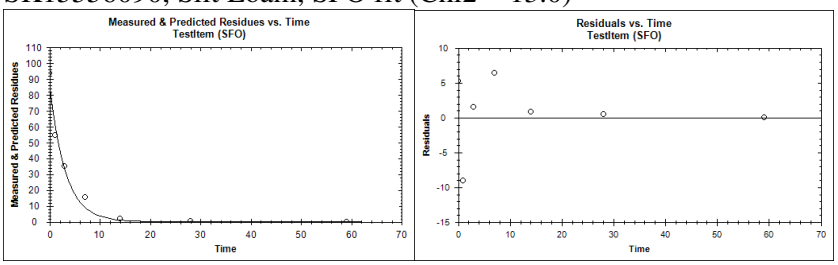
PT102, Loam 10°C, SFO fit (Chi2 = 11.6)



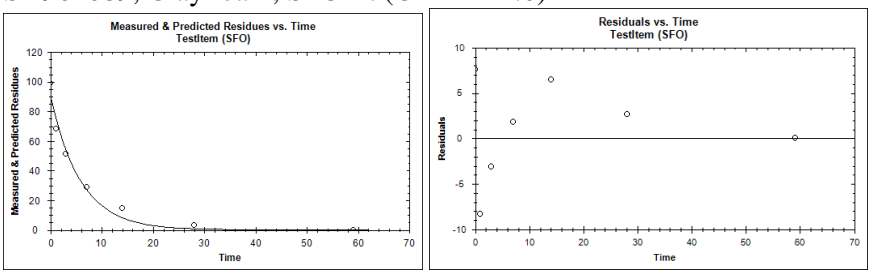
PT103, Sandy Loam, SFO fit (Chi2 = 12.3)



SK15556090, Silt Loam, SFO fit (Chi2 = 13.0)



SK961089, Clay loam, SFO fit (Chi2 = 11.0)



In the opinion of the RMS the results of the optimisations for the four soils in the study of Yeomans (2000). The results were validated by the RMS using KinGUII v2.1 and Microsoft Excel software. All show acceptable SFO model fits, where the residual plot showing random scatter of data and the chi^2 values are less than 15%. The significance level for all estimated parameters was >99%.

The asulam degradation data for the three datasets from Yeomans & Swales, 2003, were entered into KinGUI .

Table B.8. 51 Yeomans & Swales (2003) study data: Asulam – SFO Parameter optimisation results

Soil Type (USDA)	Kinetics	DT_{50} (days)	DT_{90} (days)	Min Chi^2 error (%)	t-test (-)	Visual assessment
PT103, Sandy loam	SFO	2.4	8.0	14.7	9.41E-06	Acceptable
SK15556090, Silt loam	SFO	1.7	5.5	37.9	0.0105	Poor
SK960087, Clay loam	SFO	2.6	8.6	9.6	1.16E-07	Good

Table B.8. 52 Yeomans & Swales study data: Asulam – FOMC Parameter optimisation results

Soil Type (USDA)	Kinetics	DT_{50} (days)	DT_{90} (days)	Min Chi^2 error (%)	t-test (-)	Visual assessment
SK15556090, Silt loam	FOMC	0.8	10.4	23.4	n/a	Poor

alpha: 0.7833, lower CI: 0.3338, Upper CI: 1.233
beta: 0.5829, lower CI: -0.2048, upper CI: 1.371 – confidence interval includes zero, not acceptable

Table B.8. 53 Yeomans & Swales study data: Asulam – DFOP Parameter optimisation results

Soil Type (USDA)	Kinetics	DT_{50} (days)	DT_{90} (days)	Min Chi^2 error (%)	t-test (-)	Visual assessment
SK15556090, Silt loam	DFOP	0.2	10.9	11.9	k1: 15.89404 (0.48139) k2: 0.14365 (0.00018) g: 0.55205 (1.80e-06)	Very good

The visual and kinetic fits for soils PT103 and SK960087 from SFO kinetics was acceptable and no further kinetics were investigated for these soils.

Figure B.8.15 PT103 soil, SFO kinetics, Chi2 = 14.7%

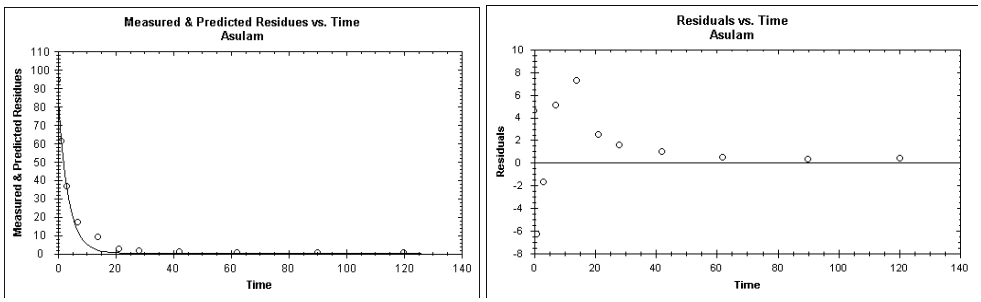
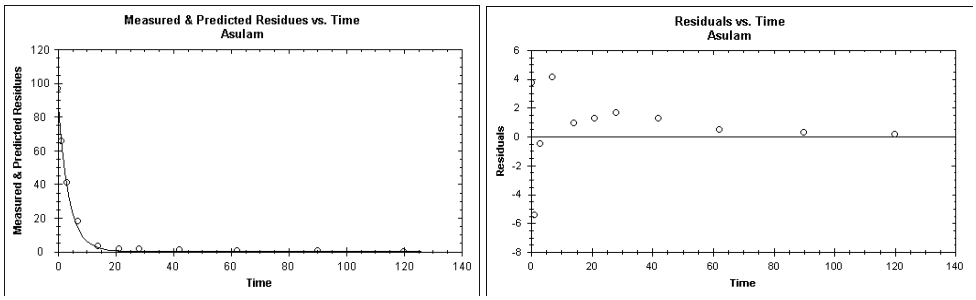


Figure B.8. 16 SK960087 soil, SFO kinetics, Chi2 = 9.6%



For the SK15556090 soil, neither SFO nor FOMC kinetics provided an acceptable fit. The visual and kinetic fit from DFOP kinetics provided a very good fit both visually and statistically. The fits are presented in Figures B.8.1.??? below.

Figure B.8. 17 SK15556090 soil, SFO kinetics, Chi2 = 37.9%

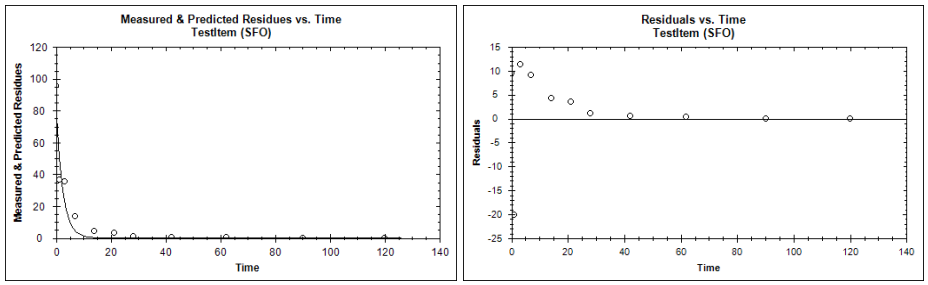


Figure B.8. 18 SK15556090 soil, FOMC kinetics, Chi2 = 23.4%

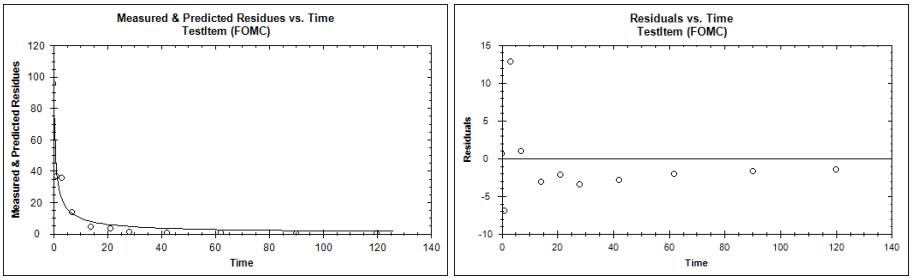
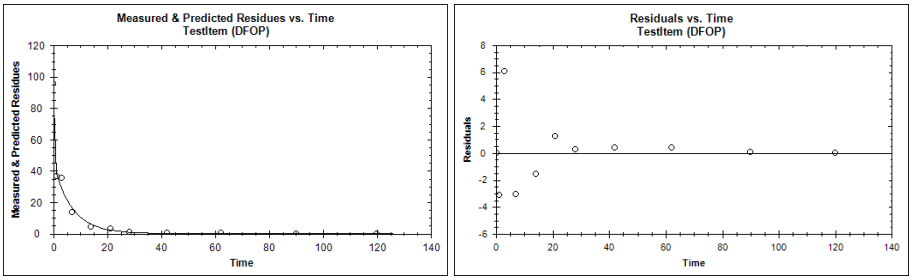


Figure B.8. 19 SK15556090 soil, DFOP kinetics, Chi2 = 11.9%



DFOP kinetics (Figure B.8. 19) provides an improved fit both visually and statistically over SFO and FOMC kinetics for the SK15556090 soil. The applicant calculated a pseudo SFO DT50 by dividing the DFOP DT90/3.322 which afforded a DT50 of 3.3 days. This is acceptable in accordance with current FOCUS kinetics guidance.

The results from the two studies (Yeomans 2000a and Yeomans & Swales 2003a) normalised to 20°C and pF2 are presented in Table B.8. 54 below. The normalised values are considered acceptable by the RMS.

Table B.8. 54**Asulam – normalised (20°C and pF2) aerobic soil SFO DT_{50} values for use as model input from two soil studies**

Soil Type (USDA)	Kinetic	DT_{50} (days)	Temperature (°C)	Study moisture content (%w/w)	Reference moisture content (%w/w)	DT_{50REF} (days)
PT102, Loam, 20°C	SFO	3.8	20	26.5	25 [a]	3.8
PT102, Loam, 10°C	SFO	9.3	10	26.5	25 [a]	3.6[e]
PT103, Sandy loam	SFO	3.4	20	16.9	14 [b]	3.4
SK961089, Silt loam	SFO	2.1	20	49.7	38.8 [c]	2.1
SK15556090, Clay loam	SFO	4.0	20	38.7	31.2 [c]	4.0
PT103, Sandy loam	SFO	2.4	20	19.2	14 [b]	2.4
SK15556090, Silt loam	SFO	3.3 [d]	20	39.1	31.8 [c]	3.3
SK960087, Clay loam	SFO	2.6	20	28.0	28 [a]	2.6

[a] FOCUS default pF 2 [b] default pF 2 for loamy sand, measured pF 2.5 closer to loamy sand than sandy loam
[c] measured study pF 2.5 data > default pF 2, so pF 2.5 data used [d] 3.3 days calculated from DFOP DT90/3.322
e not used in mean calculation.

The asulam degradation data taken from the study report and used for the kinetic evaluations are shown below.

Table B.8. 55 Summary of asulam study data used for kinetic evaluation: Roohi and Mackenzie, 2009

Time (days)	% Applied radioactivity			
	Speyer 2.2 07/022	Further field, 07/024	Flint Hall, 07/028	Warsop A1, 07/030
0	101.63	102.96	102.66	100.78
0	103.20	103.06	104.28	101.51
1	77.12	81.63	82.92	84.15
1	84.43	78.82	83.86	85.17
3	77.76	71.54	71.06	73.17
3	77.53	67.50	75.40	74.20
7	63.90	47.72	55.25	65.01
7	64.04	52.56	66.48	61.95
14	43.85	23.64	46.44	38.48
14	39.39	26.70	41.62	39.42
21	25.30	11.37	25.12	21.13
21	22.17	11.89	25.48	24.39
28	16.34	9.16	11.21	17.88
28	15.68	8.01	13.83	15.53
57	7.13	4.22	4.34	14.65
57	5.71	3.85	3.87	15.68
119	2.71	2.42	0.97	5.50
119	4.07	2.11	1.47	6.03

The rate of degradation of asulam has also been investigated under aerobic conditions at 20 ± 2°C in one soil in the dark [Völkel, 2000]. The asulam degradation data taken from the study report and used for the kinetic evaluations are shown below.

Table B.8. 56 Summary of asulam study data used for kinetic evaluation: Völkel, Sandy Loam Soil (Borstel 98)

Time (days)	% Applied radioactivity
0	96.2
1	37.6
4	26.0
7	16.3
14	8.9
35	7.1
56	3.5
118	3.6

For both studies the time zero data were set to the total recovered radioactivity (mass balance at time zero) for use in the kinetic evaluation in line with the current FOCUS kinetics guidance.

The applicant performed the kinetic analysis on the data from the studies of Roohi and Mackenzie, 2009 and Völkel, 2000 with KinGUI (v1.0 and 1.1). The RMS has validated the evaluation of the applicant using KinGUI v 2.1 using non-linear least squares optimisation.

Roohi and Mackenzie, 2009

Results of the optimisations for the four soils from Roohi and Mackenzie, 2009, at 20°C are presented in Table B.8.57

Table B.8. 57 Results for optimisations of the four soils from Roohi and Mackenzie (2009)

Soil Type (USDA)	Kinetics	DT_{50} (days)	DT_{90} (days)	Min Chi^2 error (%)	t-test (-)	Visual assessment
Speyer 2.2, 07/022	SFO	11.2	37.2	7.5	1.60E-11	Very good
Further field, 07/024	SFO	7.1	23.6	7.7	4.9E-12	Very good
Flint Hall, 07/028	SFO	10.8	36	7.8	4.8E-11	Very good
Warsop A1, 07/030	SFO	11.1	36.8	9.6	4.4E-10	Good

The visual fits are presented in Figure B.8. 20 to Figure B.8. 23 below.

Figure B.8. 20 **Speyer 2.2 07/022, SFO fit, Chi2 7.5**

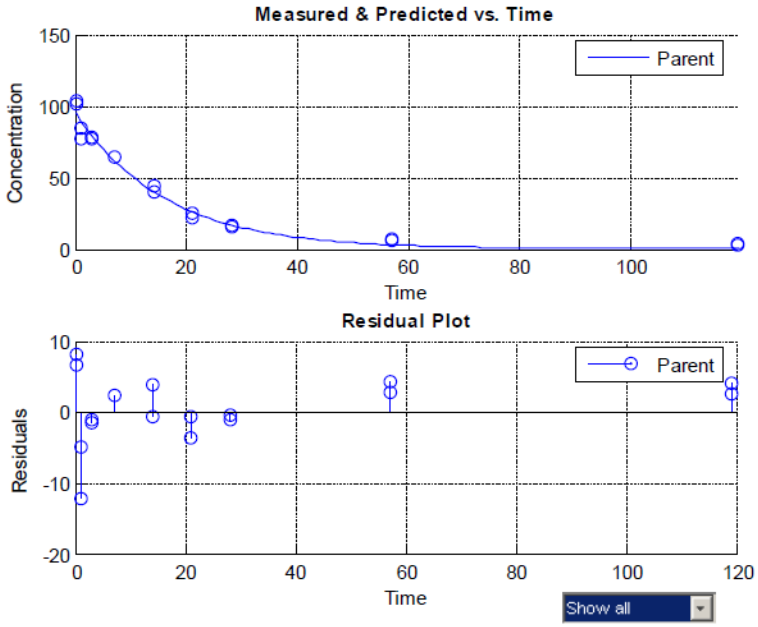


Figure B.8. 21 **Further field, 07/024, SFO fit, Chi2 7.7**

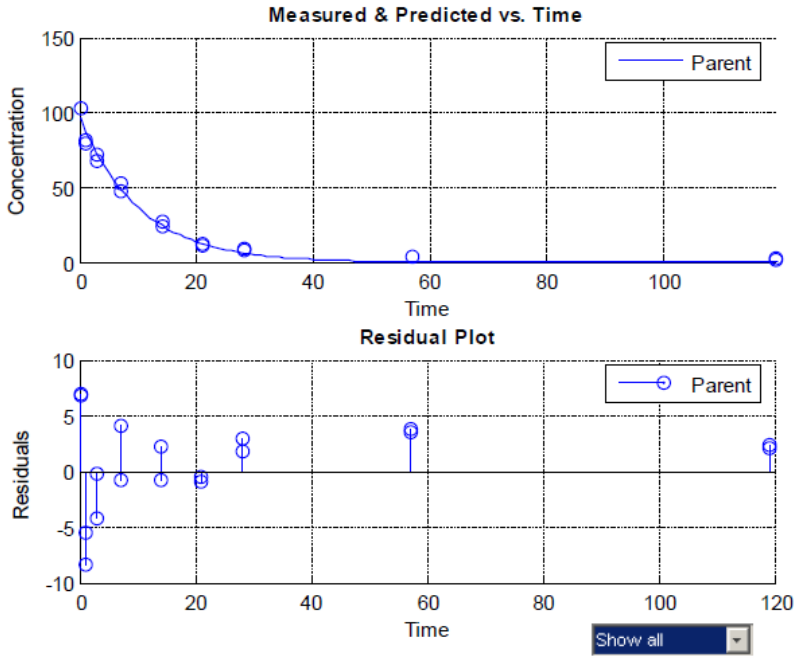


Figure B.8. 22 **Flint Hall, 07/028, SFO fit, Chi2 7.8**

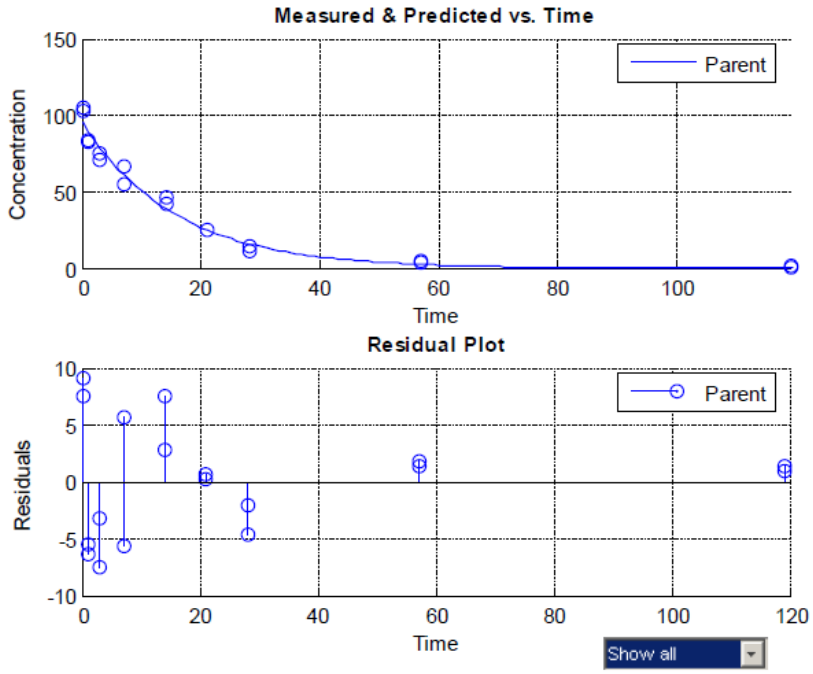
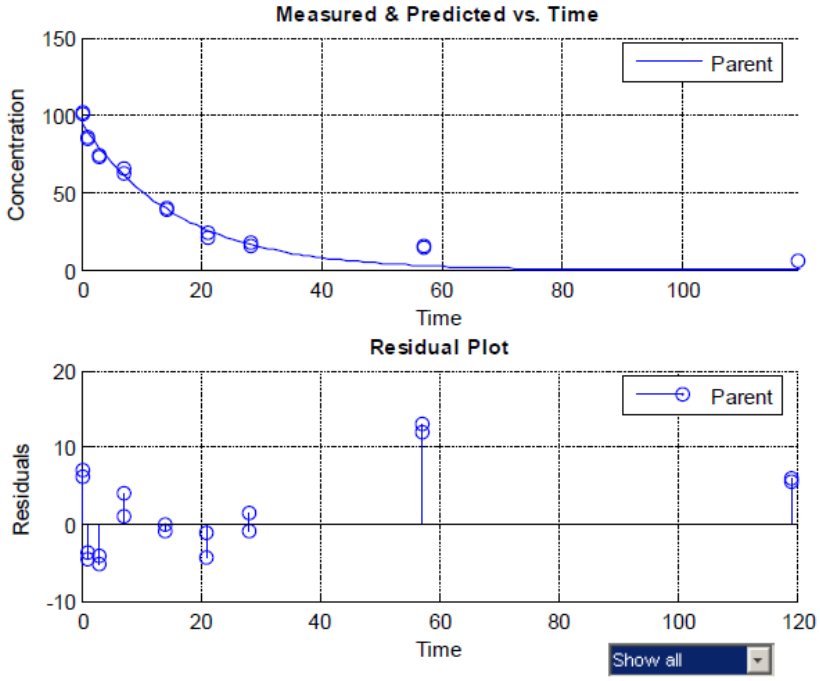


Figure B.8. 23 **Warsop A1, 07/030, SFO fit, Chi2 9.6**



The visual and statistical fits for the 07/022, 07/024 and 07/028 are considered very good using SFO kinetics. The SFO fit of the Warsop A1 soil (07/030) is very good up to 57 day timepoint which is less well described but will have no effect on the overall DT50 and describes the overall degradation pattern well and considered visually and statistically acceptable.

Völkel, 2000

The data from the single soil investigated in the Völkel 2000 study (Borstel 98) did not provide an acceptable fit using SFO kinetics. FOMC kinetics provided improved fit both visually and statistically (Chi2 7.9% and confidence intervals did not include zero). In addition the DT90 was reached (i.e. <10% remained) before the study end therefore FOMC fit was considered acceptable. The optimisation results are summarised in Table B.8.58 and the visual fit is presented in Figure B.8. 24 and Figure B.8. 25 below.

The pseudo SFO DT50 calculated from the FOMC DT90/3.322 was calculated as 5.9 days.

Table B.8. 58 Völkel 2000 study asulam aerobic soil (Borstel 98) – parameter optimisation results (SFO and FOMC) – free optimisation

Soil Type (USDA)	Kinetics	DT_{50} (days)	DT_{90} (days)	Min Chi^2 error (%)	t-test (-)	Visual assessment
Borstel 98, Sandy Loam	SFO	1.6	5.3	33	0.0146 (>98%)	Poor
Borstel 98, Sandy Loam	FOMC	0.6	19.6	7.6	lower CI upper CI α: 0.372 0.638 β: 0.041 0.374	Very good

Figure B.8. 24 Asulam, Borstel 98 SFO fit (Chi2 33%)

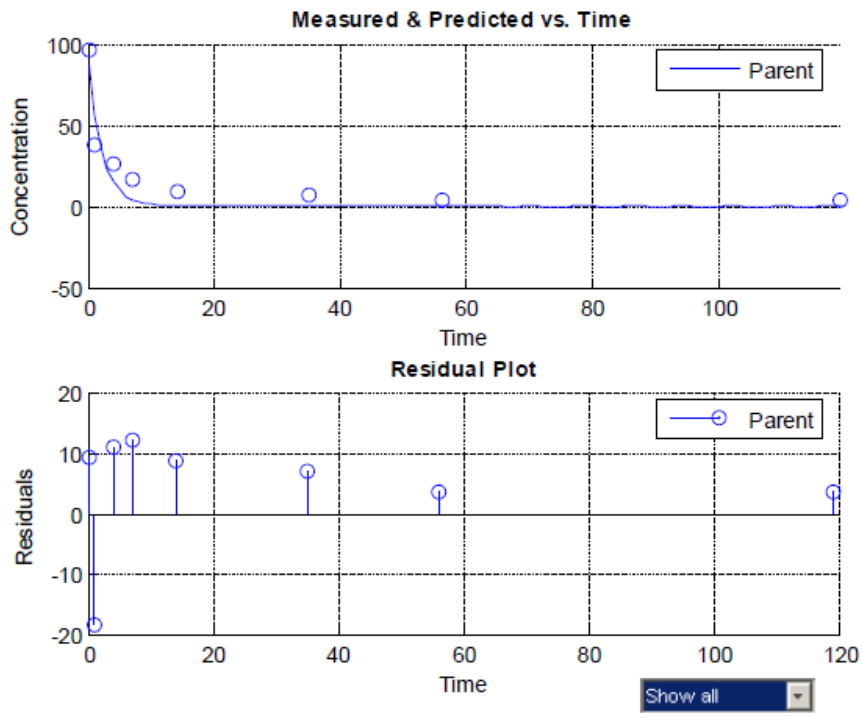
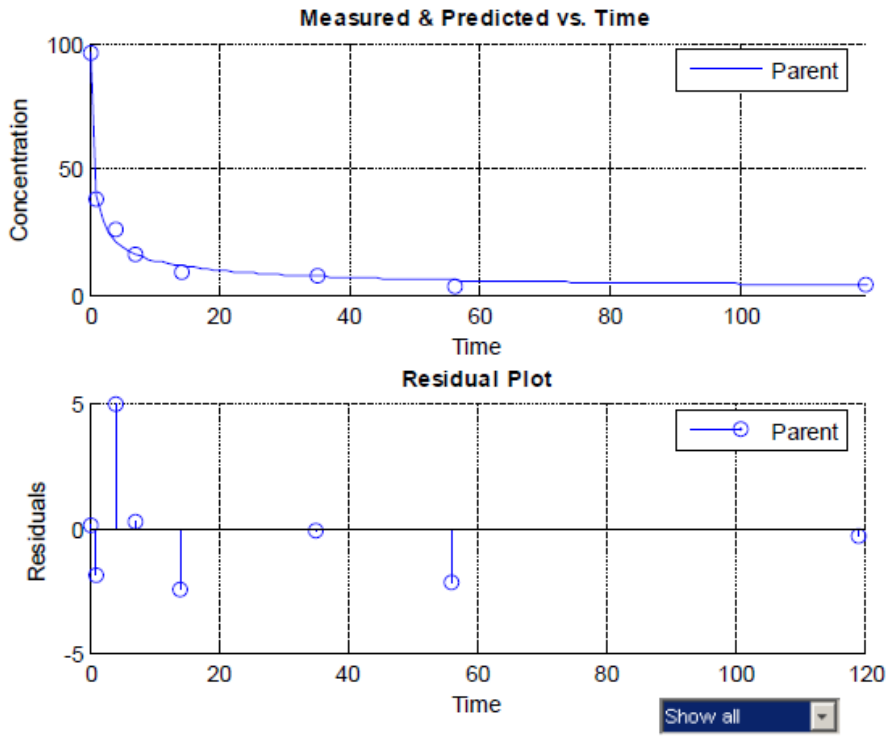


Figure B.8. 25 Asulam, Borstel 98 soil, FOMC fit, Chi2 7.6



The results of the kinetic evaluations of data from the studies of Mackenzie, 2009 and Völkel, 2000 normalised to 20°C and pF2 are presented in Table B.8. 59 below. Overall the normalised DT50 values calculated by the applicant are considered acceptable by the RMS.

Table B.8. 59 Laboratory DT50 values for asulam normalised to FOCUS temperature and moisture conditions of 20°C and pF2

Soil	Kinetic	DT_{50} (days)	Temperature (°C)	Study moisture content (%w/w)	Reference moisture content (%w/w)	DT_{50REF} (days)
Speyer 2.2, Loamy sand	SFO	11.2	20	15.1	19.7 [a]	9.3
Further field, Clay loam	SFO	7.1	20	28.2	28.5 [a]	7.0
Filnt Hall, Clay loam	SFO	10.8	20	29.6	31.3 [a]	10.4
Warsop A1, loamy sand	SFO	11.1	20	6.6	6.5 [a]	11.1
Borstel 98, Sandy loam	FOMC	5.9 [c]	20	14.53	19 [b]	4.9

[a] FOCUS default pF 2 [b] default pF 2 for loamy sand, measured pF 2.5 closer to loamy sand than sandy loam
[c] FOMC DT90/3.322

(Hardy, 2011a)

The geometric mean DT50 normalised to 20°C and filed capacity (pF2) for asulam has been calculated from the results of the kinetic evaluation of laboratory soil data from the studies of Yeomans 2000a, Yeomans and Swales 2003a, Mackenzie and Roohi 2009a and Völkel 2000a. The results of the kinetic evaluation from the studies of

Hardy 2013a and 2011a for asulam are summarised in Table B.8.60 below, the RMS agrees with the quoted values.

Table B.8. 60 Summary of kinetic evaluations for asulam from Hardy 2011a and 2013a

Soil (texture)	Kinetic Model	Best-fit DT_{50} (days)	Best-fit DT_{90} (days)	Calculated DT_{50} (days)	Actual Temp (˚C)	MC study (%)	MC ref (%)	DT_{50} ref (days)
PT102, Loam	SFO	3.8	12.7	3.8	20	26.5	25 [a]	3.8
PT103, Sandy loam	SFO	3.4	11.5	3.4	20	16.9	14 [b]	3.4
SK15556090, Silt loam	SFO	2.1	7.1	2.1	20	49.7	38.8 [c]	2.1
SK961089, Clay loam	SFO	4.0	13.4	4.0	20	38.7	31.2 [c]	4.0
PT103, Sandy loam	SFO	2.4	8.0	2.4	20	19.2	14 [b]	2.4
SK15556090, Silt loam	DFOP	0.2	10.9	3.3 [d]	20	39.1	31.8 [c]	3.3
SK960087, Clay loam	SFO	2.6	8.6	2.6	20	28.0	28 [a]	2.6
07/022,	SFO	11.2	37.2	11.2	20	15.1	19.7 [a]	9.3
07/024,	SFO	7.1	23.6	7.1	20	28.2	28.5 [a]	7.0
07/028,	SFO	10.8	36	10.8	20	29.6	31.3 [a]	10.4
07/030,	SFO	11.1	36.8	11.1	20	6.6	6.5 [a]	11.1
Borstel 98, Sandy Loam	FOMC	0.6	19.6	5.9 [e]	20	14.53	19 [b]	4.9
Geometric Mean								4.6

[d] DFOP DT90/3.322
[e] FOMC DT90/3.322

Metabolites

This study has previously been evaluated at EU level. The conclusions presented are consistent with the EFSA conclusion report and no concerns were raised at EU level.

Report:
A.M. Oddy (2003b) [^{14}C]-Sulphanilamide: Rate of Degradation in Three Soils at 20°C. Battelle AgriFood Ltd., Unpublished report No.: CX/02/023

Guidelines: EU 96/36/EC

Deviations: None

GLP: Yes

RMS opinion on study: fully acceptable. Please note the summary below is the applicant's. It is an accurate account of the report and considered acceptable by the RMS. The RMS has provided comments at the end of the study summary.

The rate of degradation of ^{14}C-sulphanilamide was investigated in accordance with EC Directive 95/36/EC (1995) and SETAC guidelines (1995) and to GLP and was considered acceptable by the Rapporteur.

The study was conducted in three aerobic soils at 20±2°C and 40 to 50% MWHC. Soil characteristics are presented in Table B.8.60. ^{14}C-sulphanilamide (radiochemical purity >99.0%, specific activity 1274.3 MBq/mmol) in acetonitrile was added to separate acclimatised samples of soil (100g oven dried equivalent) at a rate equivalent to 1.1 kg/ha. Sample flasks were fitted with an ethylene glycol trap and 2 potassium hydroxide traps to collect CO_2.

The soils were sampled at intervals of 0h, 1, 3, 7, 15, 29, 59, 99 and 120 days after application. Radioactivity in the traps was quantified by LSC. The traps were analysed when the respective units were removed for analysis with an additional trap change at 76 days. Soils were extracted with acetonitrile:water (1:1 v/v), 0.1M sodium hydrogen carbonate solution, acetone:0.1 M sodium carbonate (1:1 v/v) and then Soxhlet extraction using acetonitrile:water (80:20 v/v) with duplicate aliquots taken for LSC analysis at each stage.

Table B.8. 61 Characteristics of soils used to investigate the aerobic degradation of ^{14}C-sulphanilamide (Oddy 2003b)

Soil Reference	**02/03**	**02/05**	**02/06**
	%	%	%
Textural classification, ADAS:	Sandy Loam	Clay Loam	Clay Loam
Particle size distribution (µm)			
Sand (63-2000 µm)	73.93	27.98	20.94
Silt (2-63 µm)	15.93	48.60	48.63
Clay (<2 µm)	10.13	23.42	30.42
Organic Matter (%)	6.03	3.28	3.97
Maximum water holding capacity (%)	53.8	48.3	59.4
Moisture content in study (%)	22.6	24.2	29.7
pH in water	5.2	7.3	8.1
pH in 1M KCl	3.9	7.0	7.9
pH in 0.01M $CaCl_2$	4.1	6.9	7.6
Microbial Biomass initial	880	1826	1028
(µg C/g soil) final	402	923	506

Overall mean recoveries of radioactivity were generally in the range 89 to 93% (see Table B.8.27) although some individual flasks fell outside the range 90-110% AR. Procedural recoveries were greater than 90% AR. Recoveries from HPLC were >90% and no significant quantities of radioactivity remained on the column.

The distribution, recovery and characterisation of radioactivity are presented in Table B.8. 62. The distribution of radioactivity between extractable, unextractable and volatile radioactivity was not found to be soil dependant. In all cases the extractable radioactivity decreased with time with a corresponding increase in unextractable and volatile radioactivity.

All extracts were analysed by reverse phase HPLC. Selected samples were analysed by LC-MS for confirmation of sulphanilamide by comparison with an unlabelled sulphanilamide reference standard. Quantification and identification of sulphanilamide (the only substance identified in the soil extracts) was performed by duplicate analysis of extracts at each timepoint.

The DT_{50} and DT_{90} values were calculated by the notifier for each soil type assuming single first order and First Order Multi-Compartment (FOMC) kinetics using the Model Manager modelling program but the initial values used by the Notifier were constrained to 100%. The Rapporteur did not consider that this treatment gave the best fit and DT_{50} values quoted were shorter than if unconstrained values had been used. The DT50 and DT90 values have been recalculated by the Rapporteur assuming single first order and FOMC kinetics using Microsoft Excel software and non-linear regression from the values shown in Table B.8. 62Table B.8.61 using unconstrained values for initial concentration and the results are summarised in Table B.8. 63.

Table B.8. 62 Distribution and recovery of radioactivity in % of Applied radioactivity in 3 aerobic soils after application of 1.1kg/ha of ^{14}C-sulphanilamide at 20°C (Oddy 2003b)

Incubation Time (days)	Extract 1	Extract 2	Extract 3	Extract 4	Total* extracted	Volatiles	Unextracted	TOTAL
Sandy loam 02/03								
0	72.6	6.9	2.8	4.4	86.7	0.0	6.0	92.8
1	56.8	4.9	na	15.7	77.3	0.0	18.2	95.5
3	35.7	na	na	26.4	62.1	0.1	31.5	93.6
7	27.5	na	na	24.2	51.7	0.3	38.6	90.7
15	11.4	na	na	19.7	31.1	1.0	60.2	92.3
29	9.6	na	na	19.0	28.6	1.4	59.8	89.8
59	na	na	na	16.7	16.7	1.4	74.0	92.0
99	na	na	na	17.2	17.2	2.5	72.9	92.6
120	na	na	na	11.1	11.1	2.7	79.9	93.6
Clay loam 02/05								
0	74.6	6.0	1.7	2.3	84.7	0.0	6.3	90.9
1	47.7	4.9	na	16.7	69.2	0.0	25.6	94.8
3	33.3	na	na	20.2	53.5	0.2	34.1	87.7
7	9.9	na	na	20.2	30.1	0.3	56.1	86.4
15	8.4	na	na	14.8	23.2	0.7	62.5	86.4
29	4.6	na	na	14.7	19.3	0.8	68.7	88.8
59	na	na	na	12.6	12.6	1.6	76.8	90.9
99	na	na	na	11.9	11.9	2.1	73.1	87.0
120	na	na	na	7.4	7.4	1.8	80.6	89.8
Clay loam 02/06								
0	78.9	5.5	2.1	2.4	89.0	0.0	4.7	93.6
1	54.3	4.0	na	14.6	73.0	0.0	21.4	94.4
3	32.2	na	na	19.2	51.4	0.5	40.6	92.5
7	8.5	na	na	18.4	26.9	1.0	60.0	87.8
15	6.3	na	na	11.8	18.0	1.5	66.8	86.4
29	3.3	na	na	12.7	16.0	2.1	68.9	86.9
59	na	na	na	10.5	10.5	3.3	77.5	91.3
99	na	na	na	9.6	9.6	3.7	76.5	89.9
120	na	na	na	7.5	7.5	3.7	83.5	94.6

Extract 1 = acetone/water
Extract 2 = sodium hydrogen carbonate solution
Extract 3 = acetone/sodium carbonate solution
Extract 4 = soxhlet (acetonitrile/water)

* all sulphanilamide

Table B.8. 63 DT50 and DT90 values for sulphanilamide in 3 soils at 20°C

Soil Type	Analysis Model	DT_{50} (days)	DT_{90} (days)	Fit (r^2)
Sandy Loam (02/03)	Single first order kinetics	15.2	50.6	0.866
	First order multi compartment	7.8	238.7	0.992
Clay Loam (02/05)	Single first order kinetics	6.4	21.2	0.876
	First order multi compartment	3.8	99.7	0.992
Clay Loam (02/06)	Single first order kinetics	4.5	15.1	0.920
	First order multi compartment	3.2	54.1	0.990

(Oddy 2003b)

This study has not previously been evaluated at EU level. It has been evaluated at member-state level. The conclusions presented are consistent with those in the EFSA conclusion report.

Report:
E. Mackenzie and A. Roohi (2008a) [^{14}C]-Sulfanilamide: Rate of Degradation in Three Soils at 20°C. Battelle AgriFood Ltd, Ongar, Essex, England for AgriChem BV. Unpublished report No.: OU/07/011.
Guidelines: EU 96/36/EC
Deviations: None
GLP: Yes

Executive Summary:
The rate of degradation of [^{14}C]-sulphanilamide has been investigated under aerobic conditions in three contrasting soils incubated at 20°C in the dark. The soils were LUFA Speyer 2.2 loamy sand (Soil 07/022), Further Field clay loam (Soil 07/024) and Warsop A1, a second, more acidic loamy sand (Soil 07/030). The soils were incubated at a soil moisture content of between pF 2.0 and pF 2.5. Sulphanilamide was applied to the soil surface to give an application rate of 120 µg/100g of ode soil, equivalent to 0.45 kg/ha, and the soil samples were incubated for 118 days.

Following treatment, the soil flasks were connected to a series of trapping solutions to collect any volatile products evolved. At intervals (0, 1, 3, 7, 14, 29, 58 and 118 days) throughout the study duplicate flasks were removed from the incubation system for analysis. The soil samples were extracted first with acetonitrile / water (1:1, v/v) at room temperature and then by soxhlet apparatus with acetonitrile / water (1:4, v/v). Extracted soil samples were air-dried, ground to a fine powder and the residual radioactivity quantified by combustion.

Solvent extracts from each soil sample were concentrated and analysed by reverse phase high performance liquid chromatography (HPLC). In addition, selected soil extracts were analysed by Liquid Chromatography-Mass Spectrometry-Mass Spectrometry (LC-MS/MS) to provide confirmation of structural identity. At each sampling interval, the radioactivity in the trap solutions associated with each sample was quantified by liquid scintillation counting (LSC). Traps were also changed between sampling intervals and the radioactivity quantified by LSC.

The overall recoveries for the soils were within 90 to 110% of applied radioactivity, with mean values of 98% for Soil 07/022, 94% for Soil 07/024 and 96% for Soil 07/030.

The levels of non-extractable soil bound residues increased rapidly at time-points up to 14 days in the clay loam (Soil 07/024) or up to 60 days in the loamy sands (Soil 07/022 and Soil 07/030). Thereafter the levels of non-extractable residues increased slowly to a maximum of 84-93% by the end of the study at 118 days. Small quantities of volatile compounds were evolved, reaching a maximum of 1 to 2% by 118 days. Sulphanilamide was very rapidly degraded in soil *via* a large number of transient intermediate metabolites (maximum 5% of applied radioactivity) and, despite repeated extraction of soil samples, rapidly formed significant quantities of unextractable soil bound residues. DT50 values of between 1.3 and 3.4 days were calculated for soil incubated at 20ºC.

Materials and Methods

Test Material:
[^{14}C]-Sulphanilamide, radiochemical purity 99.7%, Batch no.: 2415MFO001-1

Test Design:
The rate of degradation of sulphanilamide, the major soil metabolite of asulam, was investigated in three agricultural soils the characteristics of which are shown in Table B.8. 64 below.

Table B.8. 64 Soil characteristics

Soil Reference Batch ID	LUFA Speyer 2.2 07/022	Further Field 07/024	Warsop A1 07/030
Source Geographic Location	LUFA, Grober Striet Hanhofen Rheinland-Pfalz Germany	Boarded Barns Farm Ongar Essex UK	Site A1 Warsop Nottinghamshire UK
OS Map Reference	not available	TL557047	SK566696
Textural classification (USDA) Sand % (50-2000 µm) Silt % (2-50 µm) Clay (< 2 µm)	Loamy sand 83 8 9	Clay loam 22 47 31	Loamy sand 87 6 7
Textural classification (ADAS) Sand % (63-2000 µm) Silt % (2-63 µm) Clay (< 2 µm)	Loamy sand 84 8 9	Silty clay loam 19 50 31	Loamy sand 86 6 8
pH Deionised water	5.8	6.7	5.1

0.01 M $CaCl_2$ 1 M KCl	5.2 5.2	6.1 5.8	4.2 3.9
Organic carbon (%)	1.9	1.6	0.9
Organic matter (%)	3.3	2.7	1.5
Cation exchange capacity (meq/100 g)	7.7	17.1	5.3
Soil moisture during incubation	15.1	28.2	6.6
Water holding capacity (%) pF 2.0 – WHC 0.1 bar pF 2.5 – WHC 0.33 bar	 19.7 10.4	 28.5 27.8	 6.5 6.7
Bulk density (fresh g/L)	980	854	1176
Microbial Biomass initial (µg C/g soil) final	233.4 232.6	466.4 323.1	155.8 93.4

The soils were dispensed (100 g oven-dried equivalent) into the glass sample flasks, the water content of the soil was adjusted to pF 2 to pF 2.5 by addition of deionised water and the samples allowed to acclimatise under study conditions for approximately one week prior to treatment. Twenty flasks for each soil were prepared and treated with [^{14}C]-sulphanilamide, allowing duplicate samples to be collected at eight specified time-points and leaving four spare flasks. Eight untreated flasks for biomass determination (four for initial biomass and four for final biomass) were also prepared.

Moist carbon dioxide-free air, produced by bubbling the air through two scrubber systems (one containing 4M potassium hydroxide solution to remove carbon dioxide and the other containing de-ionised water to moisten the air), was pumped through tubing, containing in-line capillary tube flow restrictors, across the soil surface and out through the trapping solutions. The use of flow restrictors before each flask regulated the airflow and allowed flasks to be removed without disruption of the airflow to the remaining flasks.

Following treatment, each flask was placed in a labelled lightproof plastic bag and returned to an incubation room where the temperature was maintained at 20 ± 2oC throughout the course of the study.

The trapping solutions, consisting of three calibrated 50 mL tubes, the first containing ethylene glycol followed by two containing 2M potassium hydroxide, were connected in series to the incubation flasks. Moist carbon dioxide free air was bubbled constantly, at a consistent rate, through the flasks and trap vessels during the course of the study, with interruption to the flow only occurring during flask maintenance. The traps were removed and replaced at appropriate intervals to ensure efficient trapping of evolved volatile compounds.

[^{14}C]-Sulphanilamide (10.397 mg, batch 2415MFO001-1, specific activity 6.52 MBq/mg) was dissolved in acetonitrile (10 mL) to give a treatment solution with a nominal concentration of 1.04 mg/mL. The treatment solution was analysed by high performance liquid chromatography (HPLC) prior to use. The identity of the test item was confirmed by LC/MS/MS.

The soil samples were each treated with the [^{14}C]-treatment solution using a positive displacement Microman pipette, adding the solution drop-wise onto the soil surface, ensuring even distribution. Treatment checks for each soil were made before, during

and after treatment by dispensing an equal volume of treatment solution directly into a 100 mL volumetric flask. The treatment checks were diluted to volume with acetonitrile and the radioactivity determined by LSC to obtain the treatment rate achieved. The soil flasks were each treated with 120 µL of the [^{14}C]-treatment solution containing 120 µg (46,873,000 dpm) of sulphanilamide, equivalent to an actual treatment rate of 1.20 mg/kg, 97.4% of target.

All sample and biomass flasks (other than the zero-time samples) were incubated in the dark at 20 ± 2°C. Aerobic conditions were maintained by the constant passage of moist carbon dioxide free air through the sample flasks and out through the trap solutions. At selected times during the course of the study, the sample flasks were removed from the system and weighed. In order to maintain the flasks at the selected soil moisture content any loss in weight was compensated for by the addition of de-ionised water. Duplicate flasks, and their associated traps, were removed at each sampling interval. Sampling was conducted at zero time and following 1, 3, 7, 14, 29, 58 and 118 days incubation.

Soil samples were extracted on the day that they were collected for analysis. Two ambient solvent extractions using acetonitrile/water (1:1, v/v) were employed. Further extraction of the soil residues by soxhlet apparatus with acetonitrile/water (4:1, v/v) was conducted for all time-points. The soil residues were then air dried and the radioactive content of the extracted soil quantified by the combustion of multiple aliquots. The non-extractable soil residue was also subjected to organic matter fractionation.

Selected ambient and all soxhlet extracts were concentrated prior to analysis. A known volume of the extract (100 mL) was concentrated to a small volume using a turbovap evaporator set at 35-40°C. The concentrated extract was re-dissolved in water/acetonitrile/ formic acid (95:5:0.5, v/v/v). Aliquots of the concentrated extracts were removed for LSC to quantify procedural recoveries.

With the exception of the zero time samples, trap solutions were removed for analysis at each sampling time. The volume of each trap solution was measured, recorded and the radioactivity present was determined by LSC. The total radioactivity trapped as volatile material was then calculated as the sum of the radioactivity measured at the sampling time and the total radioactivity found from any previous trap changes. Samples were analysed by high performance liquid chromatography (HPLC). The system comprised a Synergy Hydro-RP 4µm 80A, 250 x 4.6mm i.d. column connected to a UV detector set at 260 nm and a radiodetector fitted with a liquid cell.

The test item solution and selected samples were also analysed by liquid chromatography /tandem mass spectroscopic analysis (LC/MS/MS) to confirm the identity of sulphanilamide. The mass spectrometer was a MICROMASS QUATTRO-LC Triple Quadrupole, run in Positive Ion Electrospray Ionisation (ESP+) mode.
The limits of quantification for both LSC and HPLC were <<1% of applied radioactivity.

Results and Discussion:
Prior to use the purity of the test compound was determined to be 99.7% (mean value) by HPLC. The results of the soil biomass determinations confirmed that all of the soils

were microbiologically active throughout the course of the study. The extraction efficiency, using both ambient temperature solvent extraction and soxhlet extraction with heat, ranged from 94 to 104% of applied radioactivity for the zero time samples, indicating the method was suitable for the extraction of [^{14}C]-sulphanilamide from the test soil matrices. The recovery of radioactivity on concentration was generally quantitative, with average recoveries of 91% and 96% on concentration of ambient extracts and soxhlet extracts, respectively.

The overall recoveries were good for all soils with mean values for each soil ranging from 94% to 98%. Material balances are shown in Table B.8. 65 to Table B.8. 67. Individual recoveries all fell within the range 90 to 110% with the exception of the occasional sample flasks, which fell just below 90% (Flasks 105, 109, 124, 125, and 145). Observed trends for mass balance did not suggest loss during a specific analytical technique or indeed the loss of any specific metabolite.

The distribution of radioactivity was similar in all three soils. The levels of radioactivity extractable ranged from 97 to 102% at time zero and rapidly decreased with time, with a corresponding increase in the levels of unextractable radioactivity. Initially the majority of the radioactivity was extractable at ambient temperature with further extraction by soxhlet removing only 10% of the extractable soil residue. With time, an increasing proportion of the extractable radioactivity was extracted by soxhlet, indicating the residue was more difficult to extract with time. By the end of the incubation period at 118 days, the total amount of extractable radioactivity was 13% in Soil 07/030 and 5-8% in Soils 07/022 and 07/024.

Unextractable radioactivity reached maximum values ranging from 84 to 93% after 118 days. Levels of volatile radioactivity reached 1 to 2% by the end of the incubation period. The distribution of residual radioactivity amongst the soil organic matter fractions showed that in the two loamy sands (LUFA Speyer 2.2 and Warsop A1) the unextracted radioactivity was principally associated with the fulvic and humic acid fractions (ca 30% – 53% in each fraction) with < 10% in the humin fraction. The situation in the clay loam (Further Field) was somewhat different with a more equal distribution between all three fractions, although predominantly in the fulvic acid and humin fractions.

Table B.8. 65 Distribution of Radioactivity in LUFA Speyer 2.2 Loamy Sand (07/022) Incubated at 20ºC

Incubation Time (Days)	% of Applied Radioactivity					
	Ambient Extract	Soxhlet Extract	Total Extracted	Total Volatiles	NER *	Total
Zero	89.32	9.44	98.76	na	4.76	103.52
1	68.43	13.84	82.36	0.03	16.02	98.31
3	37.36	20.21	57.57	0.13	32.26	89.96
7	35.77	14.24	50.01	0.34	46.79	97.14
14	20.38	8.68	29.07	0.31	63.92	93.30
29	9.21	6.81	16.02	0.88	81.14	98.04
58	5.70	4.05	9.75	1.19	93.16	104.10
118	4.27	3.47	7.74	0.92	93.44	102.09
Overall mean recovery						98.32
Standard deviation						5.15

Table B.8. 66 **Distribution of Radioactivity in Further Field Clay Loam (07/024) Incubated at 20ºC**

Incubation Time (Days)	% of Applied Radioactivity					
	Ambient Extract	Soxhlet Extract	Total Extracted	Total Volatiles	NER *	Total
Zero	87.72	9.54	97.26	na	8.81	106.08
1	61.52	11.20	72.72	0.00	23.17	95.89
3	25.73	14.39	40.12	0.21	46.67	87.00
7	13.66	11.96	25.62	0.77	66.77	93.15
14	6.92	5.19	12.12	0.34	78.34	90.79
29	4.69	3.73	8.42	1.12	81.41	90.95
58	4.27	2.92	7.20	1.48	86.98	95.65
118	2.79	2.05	4.84	2.21	85.49	92.53
Overall mean recovery						94.01
Standard deviation						5.58

Table B.8. 67 **Distribution of Radioactivity in Warsop A1 Loamy Sand (07/030) Incubated at 20ºC**

Incubation Time (Days)	% of Applied Radioactivity					
	Ambient Extract	Soxhlet Extract	Total Extracted	Total Volatiles	NER *	Total
Zero	95.12	7.31	102.43	na	3.84	106.27
1	68.10	12.19	80.28	0.00	16.81	97.09
3	49.71	15.83	65.54	0.08	24.22	89.84
7	38.02	12.18	50.21	0.20	41.41	91.82
14	26.05	12.41	38.46	0.54	53.67	92.67
29	17.09	8.19	25.29	1.16	66.53	92.97
58	11.75	6.16	17.91	2.00	78.87	98.78
118	8.85	4.63	13.48	2.19	83.76	99.43
Overall mean recovery						96.11
Standard deviation						5.42

Mackenzie and Roohi (2008a)

This report has not previously been evaluated at EU level.

Report:
H. Willems (1997a), Determination Of The Degradation Rate Of Methyl-Benzene-Sulfonyl-Carbamate In Soil, NOTOX B.V. Hambakenwetering 3, 5231 DD 's-Hertogenbosch, The Netherlands for AgriChem BV.
Unpublished report No.: 187728.
Guidelines: EU 96/36/EC
Deviations: None
GLP: Yes

A laboratory soil degradation study was conducted on asulam metabolite [^{14}C] methyl benzene sulphonyl carbamate (MBSC) also referred to in the DAR as des-amino asulam. The study, which was conducted in accordance with EC Directive 95/36/EC and SETAC guidelines (1995) and to GLP, was considered acceptable by the Rapporteur. This study was requested by the Rapporteur since MBSC is a potential major metabolite in surface water.

The rate of degradation was investigated in 3 soils at 20°C using radiolabelled asulam sodium (specific activity 740 MBq/mmol; radiochemical purity 98.5%). The test soils (2mm sieved) were characterised as shown in Table B.8. 68.

Table B.8. 68 Characterisation of soils used to investigate the aerobic degradation of ^{14}C-MBSC.

1. Speyer soil 2.1

Location	Rheinland-Pfalz Rheinzabern Teufelskanzel	
Horizon	0-20 cm	
Texture	Sand (USDA)	
Charge number	F12095	
Organic carbon content	0.62	%
Particles <20µm	6.5	%
Clay (<2µm)	1.9	%
Silt (2-63 µm)	9.8	%
Sand (63-2000 µm)	88.4	%
pH (0.01 M CaCl2)	5.9	
Maximum water capacity	31	%
Cation Exchange Capacity	5.0	meq/100g

Soil history:

Crop:	1996-97	None
	1995	Onions
	1994	no crop
	1993	Grass
	1992	no crop
	1991	no crop
Manuring:	1991-97	None
Plant protection products		
	1991-97	None

2. Speyer soil 2.2

Location	Rheinland-Pfalz Hanhofen	
	Grosser Striet, Nr. 585	
Horizon	0-20 cm	
Texture	Loamy sand (USDA)	
Charge number	F21995	
Organic carbon content	2.32	%
Particles <20µm	12.1	%
Clay (<2µm)	5.5	%
Silt (2-63 µm)	13.4	%
Sand (63-2000 µm)	81.2	%
pH (0.01 M CaCl2)	5.6	
Maximum water capacity	48	%
Cation Exchange Capacity	10.9	meq/100g

Soil history:

Crop:	1996-97	None
	1995	Meadow-grass
	1994	Meadow-grass
	1993	Meadow-grass
	1992	Meadow-grass
	1991	Meadow-grass
Manuring:	1993-97	None
	1992	NPK
	1991	NPK and Ca
Plant protection products		
	1991-97	None

3. Westmaas soil

Location	ROC Westmaas, Parcel 16	
Horizon	0-30 cm	
Texture	Silt loam (USDA)	
Charge number	950427	
Organic matter	2.4	%
Particles <20ym	33.3	%
Clay (<2ym)	17.5	%
Silt (2-63 ym)	50.6	%
Sand (63-2000 ym)	31.9	%
pH (0.01 M CaClo)	7.4	
Maximum water capacity	39	%
Cation exchange capacity	12.9	meq/100g

Soil history:

Crop:	1994-97	no crop
	1993	Sugar beets
	1992	Wheat
	1991	Potatoes
Manuring:	1995-97	None
	1994	N and K20
	1993	NP
	1992	N
	1991	NP
Plant protection products:		
	1995-97	None
	1994	Glyphosate
	1993	Chloridazon, Desmedipham, Ethofumesate, Phenmedipham, Deltamethrin and Pirimicarb
	1992	Methabenzthiazuron, Chlormequat, MCPA, Fluroxypyr, Fenpropimorph, Prochloraz and Pirimicarb

After receipt at the testing facility the soils were stored outdoors in open, well drained containers.

The soil samples (100 g dry weight equivalent) were pre-incubated at 20±2°C and 40 to 45% of the maximum water holding capacity for 28 days prior to dosing. The soil samples were purged with moist air. A series of traps were fitted, the first and second containing sodium hydroxide to collect CO_2 and the third containing 2-methoxyethanol to collect organic volatiles. Soils were maintained at 40 -45% of the maximum water holding capacity in the dark.

^{14}C-MBSC was dosed at a rate of 0.0508 mg MBSC/100g dry soil equivalent to an application rate of 4.4 kg/ha asulam correcting for 11.6% metabolite formation and assuming that 1 kg/ha = 1 mg/kg.

The Speyer 2.1 and 2.2 soils were sampled at intervals of 0, 1, 2, 7, 14, 42 and 63 days after application. The Westmaas soil was sampled at intervals of 0, 1, 2, 7, 14, 42, 69,

and 82 days of incubation. The soils removed from the incubation system were stored in the freezer at -20°C until extraction. Storage was for a maximum of 15 days.

All soils were extracted four times with 100 mL of MeOH for 30 minutes on a shaker. The separated extracts were combined and concentrated on a rotary evaporator. The remaining aqueous phase was acidified and partitioned four times with dichloromethane. The combined dichloromethane extracts were evaporated and redissolved in 5 mL MeOH. Radioactivity was quantified by LSC. Radioactivity in the remaining aqueous phase was also determined by LSC. All extracts were stored in the freezer until further analysis.

In some cases (Speyer 2.1 from day 14 and Speyer 2.2 from day 7 onwards) an additional partitioning was performed with dichloromethane (4 x 20 mL) after addition of tetrabutyl ammonium bromide to the aqueous phase. The dichloromethane extracts were evaporated and the residues redissolved in 5 mL MeOH. Radioactivity remaining in the aqueous and organic phase were determined by LSC. All extracts were stored in the freezer until further analysis.

The soil was further Soxhlet extracted overnight with MeOH. The extracts were extracts were evaporated and redissolved in 5 mL MeOH. Radioactivity was determined by LSC and extracts stored in the freezer until further analysis.

Additional Soxhlet extractions in acetone were performed on soil samples collected after day 2. The acetone extract was evaporated to dryness and redissolved in MeOH. Radioactivity was determined by LSC. The Westmaas soil sampled after Day1 was acidified and further Soxhlet extracted firstly with dichloromethane followed by acetone and finally ethyl acetate. Each extract was evaporated and redissolved in MeOH. Radioactivity was determined by LSC.

Radioactivity in the NaOH traps to determine CO_2 and 2-methoxy-ethanol traps to determine organic volatiles was quantified by LSC.

An aliquot of moist soil residues remaining after the final extraction step were combusted on a Harvey OX-500 oxidizer. The released CO_2 was trapped and quantified by LSC.

Extracts were then analysed by TLC. Location and distribution of radioactivity on the plates was determined using a Bioscan System 200 Imaging Scanner and compared with the location of MBSC reference material.

Microbial biomass at the end of the pre-incubation period was 29, 48, and 42 mg/100 g for the Speyer 2.1, Speyer 2.2 and Westmaas soils respectively. At the end of the study, microbial biomass measured 21, 38, and 39 mg/100 g respectively.

Recoveries of radioactivity from samples is presented in Tables B.8.69 to B.8.71. Extractable radioactivity is presented in Tables B.8.72 to B.8.74. Distribution and characterisation of radioactivity from TLC analysis is presented in Tables B.8.75 to B.8.77. It is noted by the Rapporteur that the recovery of %AR is a little low particularly in the Westmaas soil (79.8% at day 1) see Table B.8. 71. It is noted that at

day 2, a further extraction step was included in the procedure for day 2 which the RMS notes improved the recovery to 90.5% AR.

Table B.8. 69 Recovery of radioactivity from Speyer 2.1. soil (% of applied)

Days	CO2	Volatiles	Extractable	Unextractable	Total
0	-	-	87.6	0.4	88
1	0.1	< 0.01	93.4	0.4	93.9
2	0.3	< 0.01	86.2	0.9	87.4
7	1.8	< 0.01	86	1.7	89.5
14	2.8	< 0.01	94.6	4.7	102.1
28	12.3	< 0.01	77.7	9.8	99.8
56	11.3	< 0.01	61.8	25.5	98.6
63	19.7	< 0.01	53.1	17.9	90.7

Table B.8. 70 Recovery of radioactivity from Speyer 2.2. soil (% of applied)

Days	CO2	Volatiles	Extractable	Unextractable	Total
0			89	0.4	89.4
1	0.1	< 0.01	95.7	0.5	96.3
2	0.5	< 0.01	82.3	1.3	84.1
7	0.9	< 0.01	91	1.3	93.2
14	2.7	< 0.01	100.3	3.6	106.6
28	5.8	< 0.01	78.9	10.5	95.2
56	20.4	< 0.01	51.1	22.4	93.9
63	27.9	< 0.01	55.6	18.6	102.1

Table B.8. 71 Recovery of radioactivity from Westmaas soil (% of applied)

Days	CO2	Volatiles	Extractable	Unextractable	Total
0			82.6	7.6	90.2
1	0.1	< 0.01	66.1	13.6	79.8
2	0.2	< 0.01	78.1	12.2	90.5
7	1.4	< 0.01	88.3	8.8	98.5
14	4.9	< 0.01	92.1	8	105.0
42	28.7	< 0.01	50.7	18.1	97.5
69	41.9	< 0.01	25.8	25.1	92.8
82	52.9	< 0.01	12.8	26.3	92

Table B.8. 72 Extractable radioactivity in Speyer 2.1 soil (% of applied)

Day	MeOH		MeOH soxhlet	Total extractable
	Organic	Aqueous		
0	85.4	0.5	1.7	87.6
1	91.9	0.3	1.2	93.4
2	82.1	1.2	2.9	86.2
7	80.7	1.7	3.6	86
14	84.7	0.5	9.4	94.6
28	67.4	1	9.3	77.7
56	44.7	2.2	14.9	61.8
63	36	2.1	15	53.1

Table B.8. 73 Extractable radioactivity in Speyer 2.2 soil (% of applied)

Day	MeOH		MeOH soxhlet	Total extractable
	Organic	Aqueous		
0	83.9	0.5	4.6	89
1	91.9	1.1	2.7	95.7
2	77.4	2	2.9	82.3
7	87.3	1	2.7	91
14	94.2	1.4	4.7	100.3
28	70.5	1.9	6.5	78.9
56	43.2	2.1	5.8	51.1
63	46.7	1.8	7.1	55.6

Table B.8. 74 Extractable radioactivity in Westmaas soil (% of applied)

Day	MeOH		MeOH soxhlet	Total extractable
	Organic	Aqueous		
0	71.8	0.2	10.6	82.6
1	62.1	0.1	3.9	66.1
2	70.3	0.1	7.7	78.1
7	83.2	0.2	4.9	88.3
14	83.3	0.7	8.1	92.1
42	47.2	0.5	3	50.7
69	23.2	0.5	2.1	25.8
82	10.6	0.8	1.4	12.8

Table B.8. 75 Distribution of extractable radioactivity in Speyer 2.1 (% of applied)

Days	MBSC Rf 0.56-0.7	Met A Rf 0.03-0.07	Met B Rf 0.81-0.86	Aqueous phase	Not identified
0	80.2	3.4	1.8	0.5	1.7
1	84.4	3.4	4.2	0.3	1.2

2	74.9	1.3	8.8	1.2	-
7	68.6	1.5	14.2	1.7	-
14	52.8	2.3	37.6	0.5	1.4
28	15.6	1.7	59.4	1	-
56	1.6	2	55	2.2	1
63	ND	2.2	47.4	2.1	1.4

Table B.8. 76 Distribution of extractable radioactivity in Speyer 2.2 (% of applied)

Days	MBSC Rf 0.56-0.7	Met A Rf 0.03-0.07	Met B Rf 0.81-0.86	Aqueous phase	Not identified
0	79	3.6	5.7	0.5	0.1
1	84.5	2.1	8	1.1	-
2	64.3	1.4	14.6	2	-
7	48.1	2.2	39.8	1	-
14	29	2.5	67.4	1.4	-
28	9.6	0.5	67	1.9	-
56	0.2	1.5	47.4	2.1	-
63	0.1	1.7	52	1.8	-

Table B.8. 77 Distribution of extractable radioactivity in Westmaas (% of applied)

Days	MBSC Rf 0.56-0.7	Met A Rf 0.03-0.07	Met B Rf 0.81-0.86	Aqueous phase	Not identified
0	79.3	2.7	0	0.2	0.4
1	62.8	2.9	0.4	0.1	-
2	74.7	1.8	1.5	0.1	-
7	83.7	2.3	2	0.2	-
14	85.5	2.6	3.4	0.7	-
42	44.3	2.9	3.1	0.5	-
69	22.7	0.5	2.1	0.5	-
82	8.6	ND	2	0.8	1.4

The Applicant provided DT_{50}/DT_{90} calculations however these were based on Timm and Frehse. The RMS has re-evaluated the kinetics based in accordance with FOCUS (2006).

All time zero levels in soil are taken from the mass balance.

The Speyer 2.1 and 2.2 soils both provided an acceptable visual fit and statistical fit with SFO kinetics (Chi2 7.56 and 7.54 respectively. The SFO fits are presented in Table B.8. 78 below.

Table B.8. 78 MBSC Speyer 2.1 and 2.2 soils – parameter optimisation results (SFO)

Soil system	DT50(d)	DT90 (d)	Chi2	t-test	Visual assessment
Speyer 2.1	14.4	48.0	7.56	4.23E-04	Acceptable
Speyer 2.2	8.3	27.6	7.54	4.15E-05	Good

The visual fits are presented in Figure B.8. 26 and Figure B.8. 27.

Figure B.8. 26 Visual fit (SFO) for Speyer 2.1 soil (Chi2 7.56)

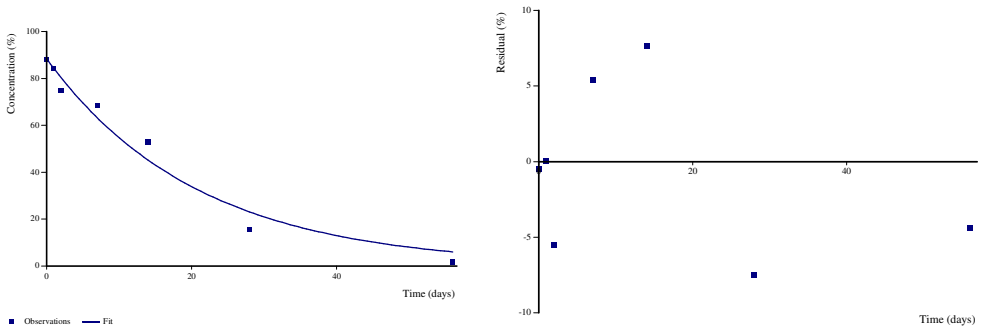
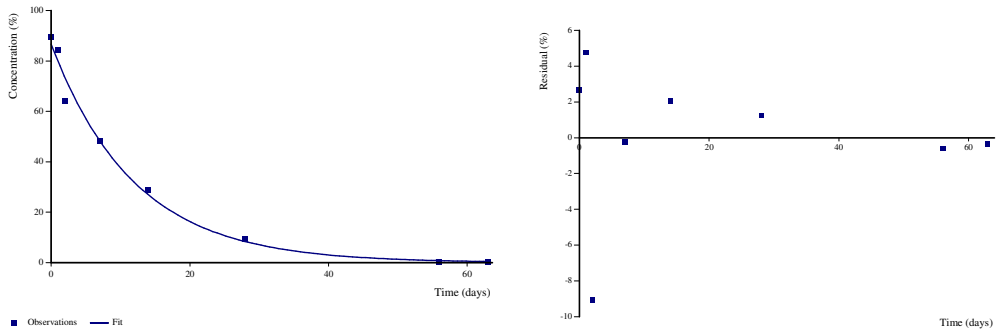


Figure B.8. 27 Visual fit (SFO) for Speyer 2.2 soil (Chi2 7.54)



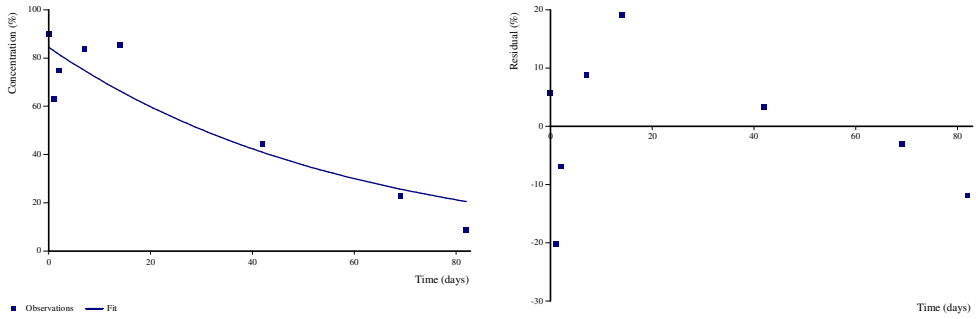
The data for the Westmaas soil shows significant scatter for the initial timepoints showing an initial decrease in concentration followed by an increase up to day 14, potentially a lag phase. This may be due to the microbial population acclimatising although evidence for this is inconclusive. In addition the recovery at day 1 is low (79.8%) which is considerably below the regulatory requirement of 90 – 110%. The fit initially was determined using SFO kinetics using the whole dataset. The timepoints up to 14 days were not described well. The Chi2 error was 15.8%, although the t-test was 0.0058.

Table B.8. 79 MBSC Westmaas soil – parameter optimisation results (SFO) – free optimisation, whole dataset

Soil system	DT50(d)	DT90 (d)	Chi2	t-test	Visual assessment
Westmaas	40.2	134	15.8	0.005796	poor

The SFO visual fit is presented in Figure B.8. 28 below.

Figure B.8. 28 Visual fit (SFO) for Westmaas soil, full dataset (Chi2 15.8)



FOMC and DFOP did not provide improvement either visually or statistically, acceptable statistical fits could not be achieved. The RMS investigated the kinetics using the data from the highest value at day 14. An acceptable SFO fit was provided both visually and statistically. The Chi2 was 7.11% and t-test (0.006172) were acceptable, the DT50/DT90 were 26.4 d and 87.8 days respectively.
However the fit does not include the first four timepoints and it is based only on four timepoints from day 14 onwards therefore this was not considered acceptable by the RMS.

Since the recovery was very low at day 1, the RMS considers that it is appropriate to exclude this point from the dataset. The kinetic fit was rerun with the day 1 data point removed. Improved fit was provided both visually and statistically, Chi2 12.1, t-test 0.0024.

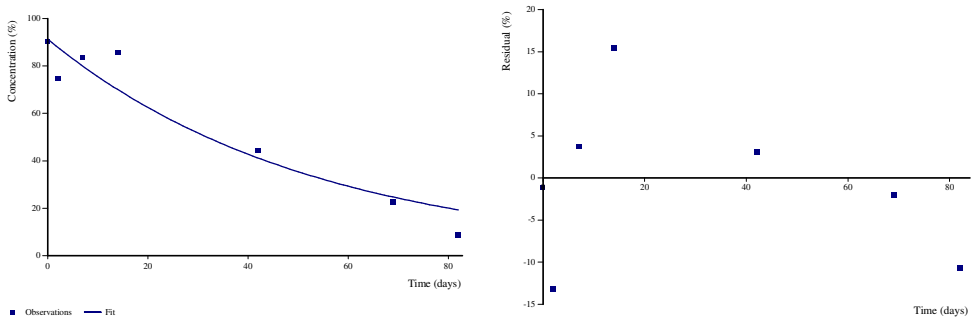
The kinetic fit using SFO kinetics is summarised in

Table B.8. 80. The visual fit is presented in Figure B.8. 29. An acceptable fit could not be achieved with either FOMC or DFOP kinetics.

Table B.8. 80 MBSC Westmaas soil – parameter optimisation results (SFO) – day 1 datapoint removed

Soil system	DT50(d)	DT90 (d)	Chi2	t-test	Visual assessment
Westmaas	36.6	122	12.1	0.002387	acceptable

Figure B.8. 29 Visual fit (SFO) for Westmaas soil, day 1 removed (Chi2 12.1)



The RMS considers that the SFO fit with the day 1 datapoint removed acceptable.

The SFO DegT50/DegT90 values for the three soils investigated for the rate of degradation of MBSC are summarised in Table B.8. 81 below. The geometric mean DT50 for modelling calculated from the three soils is 14.9 days.

Table B.8. 81 DT50 and DT90 values for MBSC in 3 soils at 20°C

Soil (texture)	Kinetic Model	Chi2 error	DT_{50} (days)	DT_{90} (days)	Actual Temp (˚C)	MWHC (%)	MC study (%)	MC ref (%)	DT_{50} ref (days)
Speyer 2.1 (sand)	SFO	7.56	14.4	48.0	20	31	13.95	12	14.4
Speyer 2.2 (loamy sand)	SFO	7.54	8.3	27.6	20	48	21.6	14	8.3
Westmaas (silt loam)	SFO	12.1	36.6	122	20	39	17.55	26	27.9

(Willems 1997a)

This report has not previously been evaluated at EU level. It is a new report with calculations conducted according to the latest guidance.

Report:
I.A.J. Hardy. (2013a) Kinetic Modelling Analysis of Asulam and Sulphanilamide Rate of Degradation Studies to Derive Modelling Endpoints. Battelle UK Ltd. For United Phosphorus Limited, Unpublished report No.: NZ/10/003C.

Guidelines:
EU 91/414/EEC, Section 5, Point 7.1.1.
Deviations: None

GLP:
No, conducted according to Good Modelling Practice recommendations

The kinetics of the degradation of sulphanilamide in the 3 soils from the study of Oddy, (2003b) has been re-evaluated in a new study (Hardy 2013a) in accordance with current FOCUS kinetics Guidance (June 2006) for derivation of modelling parameters.

RMS opinion on study: fully acceptable. It should be noted that the analysis was not conducted on the parent soils for sulphanilamide (no suitable decline phase to derive DT50).

The three soils studied (ADAS classification) were sandy loam (02/03), clay loam (02/05), and clay loam (02/06).

The characterisation of the soils is presented in Table B.8.26.

Summary of sulphanilamide study data used for kinetic evaluation: Oddy, 2003b is presented in Table B.8. 62 above. For all soils, Day 0 values were set to the total recovered amount of radioactivity (%) (mass balance) and used in the kinetic evaluation.

The applicant has re-evaluated the kinetics of the 3 soils using KinGUI (v 2.0) in accordance with FOCUS kinetics guidance (2006) using free optimisation of parameters.

The results of the optimisations of the data from the Oddy 2003b study are summarised below.

SFO kinetics did not provide acceptable fits for any of the three soils, visually or statistically, the chi2 values ranged between 18.7 and 22%. The SFO fits are summarised in Table B.8. 82 below.

Table B.8. 82 Oddy 2003b (sulphanilamide aerobic soil) – parameter optimisation results (SFO) – free optimisation

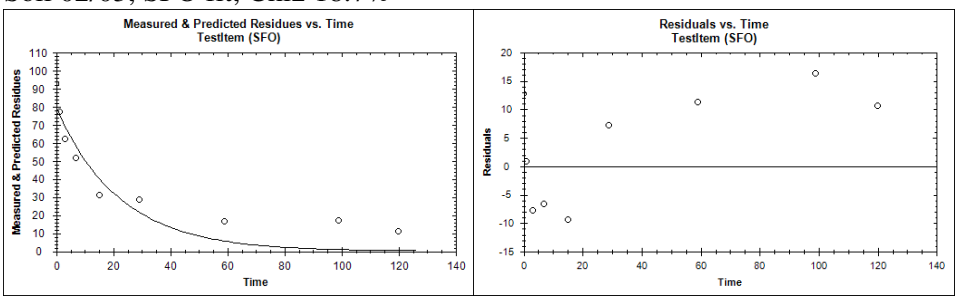
Soil system	DT50(d)	DT90 (d)	Chi2	t-test	Visual assessment
Soil 02/03	15.3	50.7	18.7	2.57E-02	Poor
Soil 02/05	6.4	21.1	22	0.0213	Poor
Soil 02/06	4.5	15.1	19.7	4.98E-03	Poor

FOMC kinetics provided excellent fits for the 02/03 and 02/05 soils both visually and statistically, the Chi2 being 4.8 and 5.8% respectively. The FOMC fit for the 02/06 soil was good (Chi2 7.3).

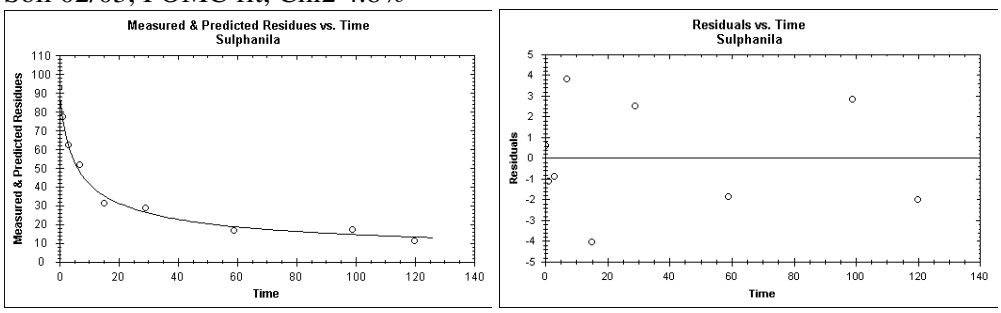
Table B.8. 83 Oddy 2003b (sulphanilamide aerobic soil) – parameter optimisation results (FOMC) – free optimisation

Soil system	DT50(d)	DT90 (d)	Chi2	Parameter	Lower CI	Upper CI	Visual assessment
Soil 02/03	7.8	238.4	4.8	α: 0.5130 β: 2.7105	α:0.3907 β:1.1248	α:0.635 β:0.8090	Excellent
Soil 02/05	3.8	99	5.8	α: 0.5457 β:1.47834	α:0.4227 β:0.66595	α:0.669 β:2.291	Excellent
Soil 02/06	3.2	54.2	7.3	α: 0.6662 β: 1.7658	α:0.4843 β:0.7412	α:0.848 beta:2.790	Good

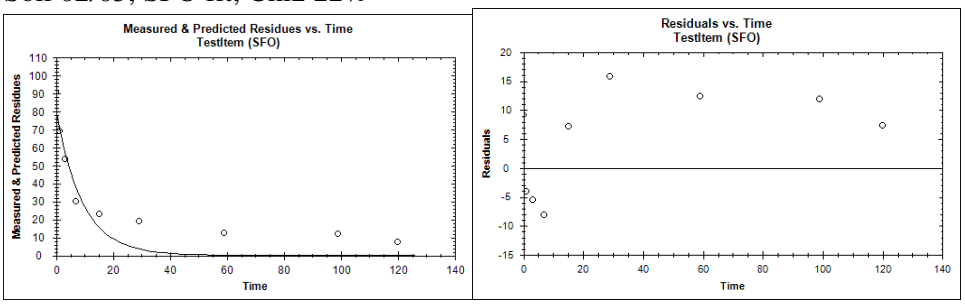
Soil 02/03, SFO fit, Chi2 18.7%



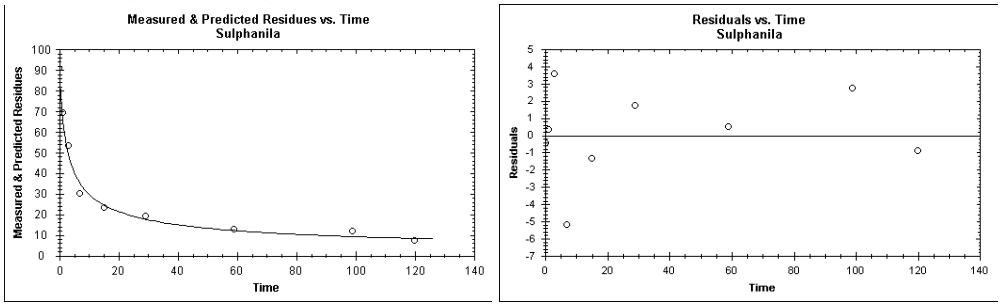
Soil 02/03, FOMC fit, Chi2 4.8%



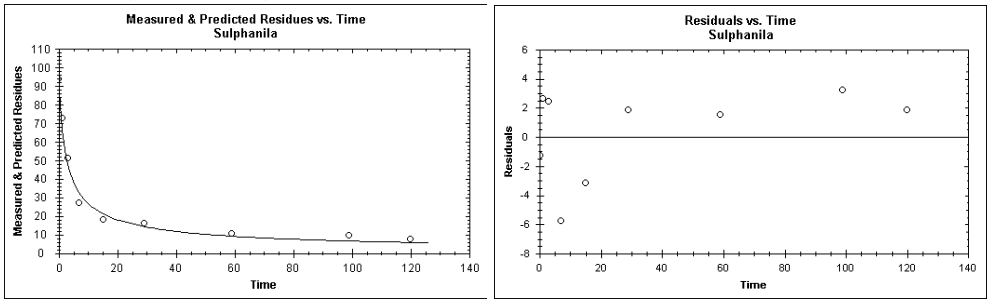
Soil 02/05, SFO fit, Chi2 22%



Soil 02/05, FOMC fit, Chi2 5.8%



Soil 02/06, FOMC fit, Chi2 7.3%



For soil 02/03, the FOMC DT90 (234.4 days) was outside the experimental period and not acceptable in accordance with FOCUS (2006). In addition, 10% of the initial concentration was not reached at the study end. The DFOP fit was investigated by the applicant which provided an excellent visual and statistical fit. DFOP kinetics also provided improved fit for soil 02/06 both visually and statistically (Chi2 2.7) and although the FOMC was good, the DFOP fit was better. DFOP was therefore chosen as the best fit for soil 02/03.

The model fits and calculated DT50s were independently validated by the RMS using KinGUI (v 2.1) and Microsoft XL software. DFOP was correctly chosen by the applicant for soil 02/03 since the 10% of the initially measured concentration is not reached within the experimental period of 120 d. DFOP was found to be visually and statistically acceptable. The optimised model fit, optimisation results and residual plot for soil 02/03 (sandy loam) are presented in Table B.8.13 and Figure B.8. 30 respectively.

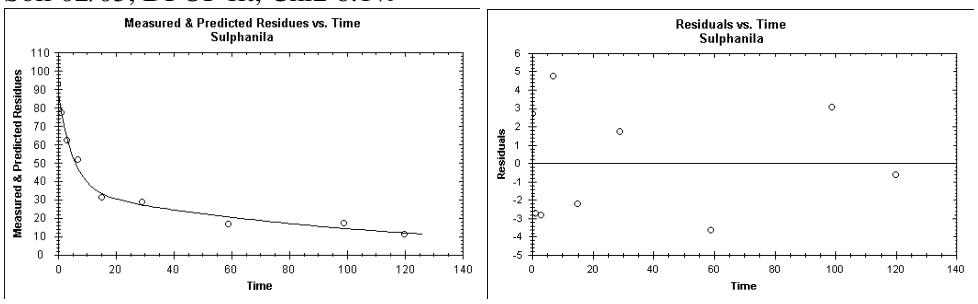
Table B.8. 84 Oddy 2003b (sulphanilamide aerobic soil) soil 20/03 – parameter optimisation results (DFOP) – free optimisation

Soil system	DT50(d)*	DT90 (d)*	Chi2	Estimate	t-test	Visual assessment
Soil 02/03	7.7	148.9	6.1	k1: 0.192707 k2: 0.009061 g: 0.614756	0.009333 0.017285 0.000160	Excellent
Soil 02/06	3.4	92.1	2.7	k1: 0.286701 k2: 0.007676 g:0.797196	7.46E-06 0.00196 4.09E-08	Excellent

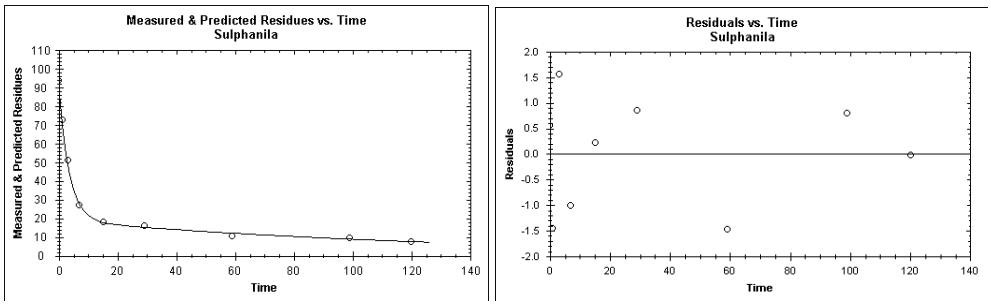
* overall DFOP DT50/DT90

Figure B.8. 30 Optimised model fit for Soil 02/03 (DFOP)

Soil 02/03, DFOP fit, Chi2 6.1%



Soil 02/06, DFOP fit, Chi2 2.7%



For soil 02/05 FOMC kinetics was considered by the RMS to provide the best visual and statistical fit. FOMC kinetics were acceptable for soil 02/05 since 10% of the initial concentration was reached within the experimental period. Overall the model fits were good as demonstrated by the r^2 value (see Table B.8. 63), minimum Chi^2 value and residual plots. The parameters alpha and beta and calculated DT50 and DT90 values were considered acceptable by the RMS.

The calculated DT50 values were normalised to 20°C and soil moisture at field capacity`. The results are presented in Table B.8. 85 below.

Table B.8. 85 DT50 and DT90 values for sulphanilamide in 3 soils at 20°C

Soil (texture)	Kinetic Model	Chi2 error	Best-fit DT_{50} (days)	Best-fit DT_{90} (days)	Calculated DT_{50} (days)	Actual Temp (˚C)	MC study (%)	MC ref (%)	DT_{50} ref (days)
02/03 (Sandy loam)	DFOP	6.1	7.7	148.7	76.2[a]	20	22.6	22.7	75.9
02/05 (Clay loam)	FOMC	5.8	3.8	100.9	30.4[b]	20	24.2	36.4	22.8
02/06 (Clay loam)	DFOP	2.7	3.4	92.1	21.9[c]	20	29.7	41.6	21.9

[a] DT50 from slow phase DFOP model (ln[2]/0.0091) which is the method recommended by FOCUS for modelling endpoints since 10% of the initial concentration is not reached.
[b] Best-fit FOMC DT_{90}/3.32
[c] DFOP DT90/3.32

Hardy 2013a

This report has not previously been evaluated at EU level. It is a new report with calculations conducted according to the latest guidance.

Report:

I.A.J. Hardy. (2011a) Kinetic Modelling Analysis of Asulam and Sulphanilamide Rate of Degradation and Asulam Water / Sediment Studies to Derive Modelling Endpoints Battelle UK Ltd. For Agrichem BV, Unpublished report No.: OU/10/001

Guidelines: EU 91/414/EEC, Section 5, Point 7.1.1.

Deviations: None

GLP: No, conducted according to Good Modelling Practice recommendations

The kinetics of the degradation of sulphanilamide in the 3 soils from the study of Mackenzie & Roohi (2008a) has been re-evaluated in a new study (Hardy 2011a) in accordance with current FOCUS kinetics Guidance (June 2006) for derivation of modelling parameters.

RMS opinion on study: fully acceptable

The characterisation of the soils is presented in Table B.8.1.29.

Summary of sulphanilamide study data used for kinetic evaluation Mackenzie & Roohi (2008a) is presented in Table B.8. 65 to Table B.8. 67 above. For all soils, Day 0 values were set to the total recovered amount of radioactivity (%) (mass balance) and used in the kinetic evaluation.

The applicant has re-evaluated the kinetics of the 3 soils using KinGUI (v 1.0 and 1.1) in accordance with FOCUS kinetics guidance (2006) using free optimisation of parameters.

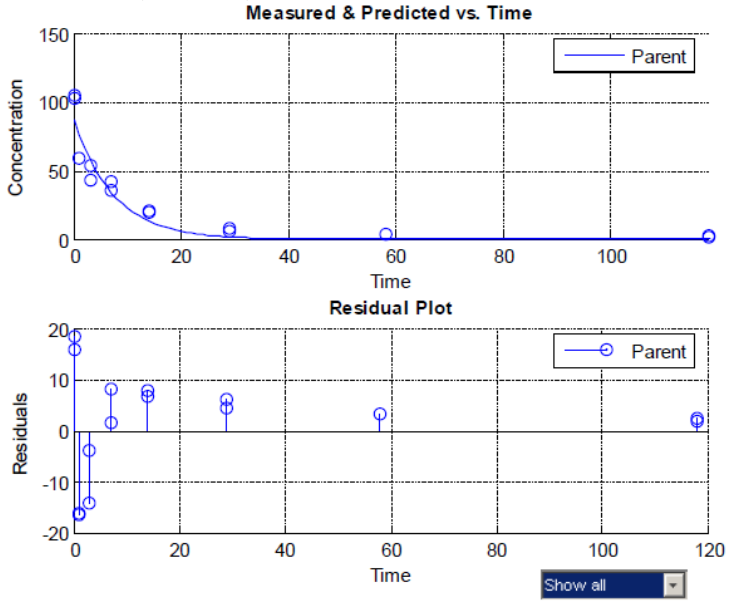
The results of the optimisations of the data from the evaluation Mackenzie & Roohi (2008a) study are summarised below.

SFO kinetics did not provide acceptable fits for any of the three soils, all produced poor visual fits and chi2 value greater than 15%. The SFO fits are summarised in Table B.8. 86.

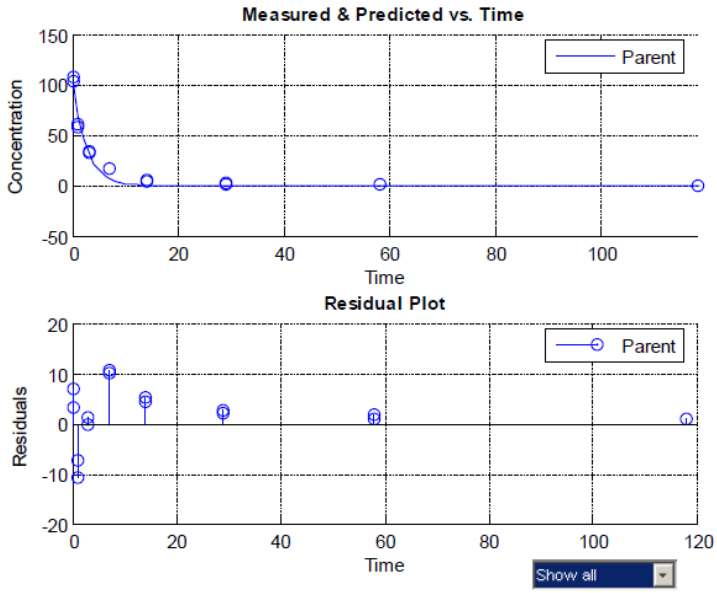
Table B.8. 86 Mackenzie & Roohi 2008a (sulphanilamide aerobic soil) – parameter optimisation results (SFO) – free optimisation

Soil system	DT50(d)	DT90 (d)	Chi2	t-test	Visual assessment
07/022	5.2	17.2	21.9	0.000054, >99%	Poor
07/024	1.8	6.1	15.6	5.0E-8, >99%	Poor
07/030	5.9	19.7	16.5	4.3E-6, >99%	Poor

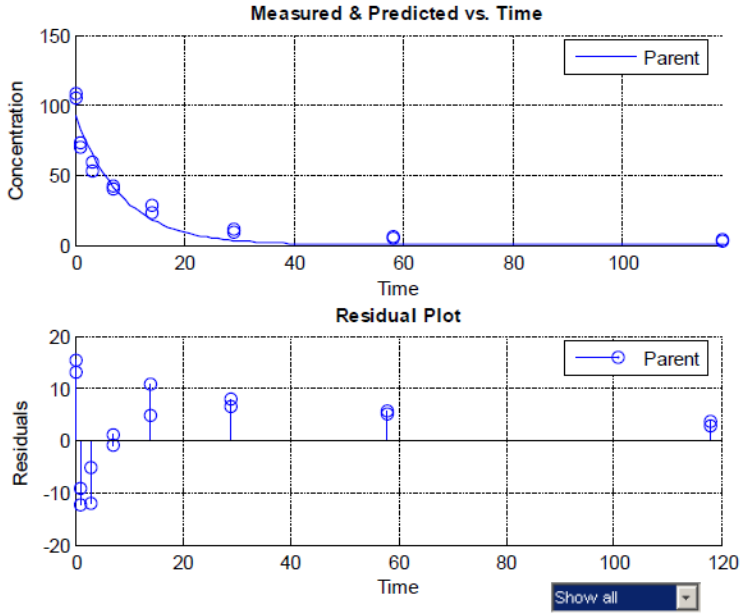
Soil 07/022, SFO fit, Chi2 = 21.9



Soil 07/024, SFO fit, Chi2 = 15.6%



Soil 07/030, SFO fit, Chi2 = 16.5%

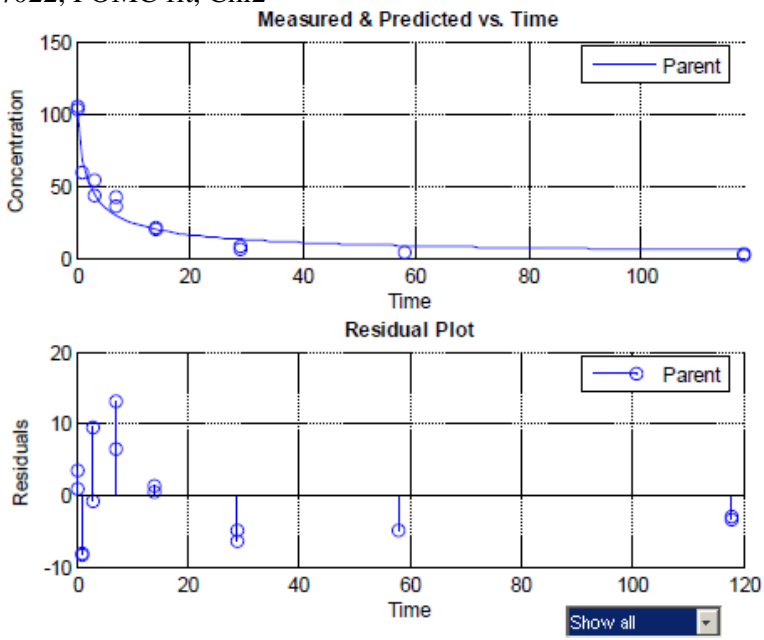


Since SFO did not provide acceptable fits FOMC kinetics were investigated by the applicant. FOMC provided improved fit for soil 07/024 only. The FOMC fits are summarised below in
Table B.8. 87.

Table B.8. 87 Mackenzie & Roohi 2008a (sulphanilamide aerobic soil) – parameter optimisation results (FOMC) – free optimisation

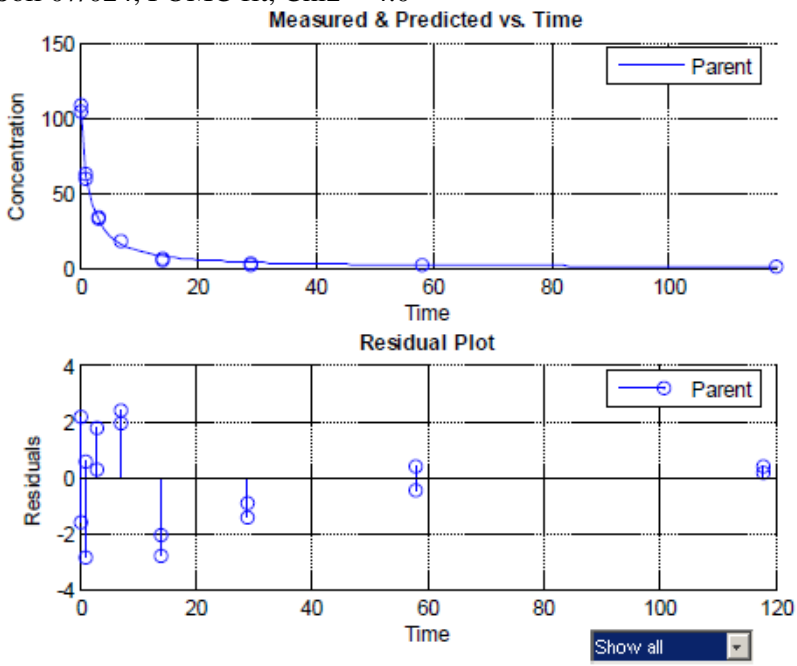
Soil system	DT50(d)	DT90 (d)	Chi2	t-test	Visual assessment
07/022	2.2	42.4	13.5	-	Poor
07/024	1.4	10.3	4	-	Very good
07/030	3.3	45.1	8.4	-	Poor

Soil 07/022, FOMC fit, Chi2 =

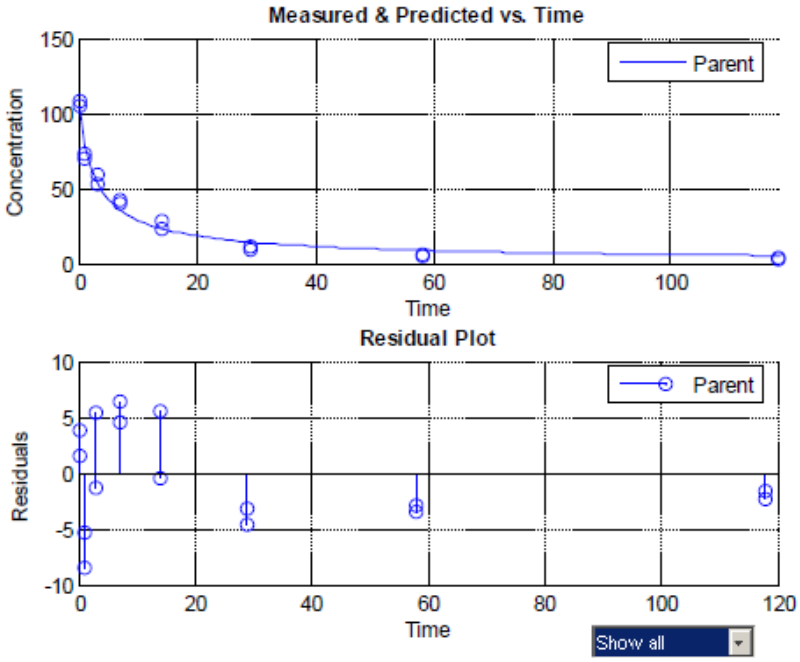


13.5%

Soil 07/024, FOMC fit, Chi2 = 4.0



Soil 07/030, FOMC fit, Chi2 = 8.4%



FOMC kinetics were considered by the applicant to be inappropriate for Soils 07/022 and 07/030 due to poor fitting around the measured DT90. DFOP kinetics provided improved fit both visually and statistically for 07/022 and 07/030 soils. The fits which are considered appropriate by the RMS are presented in Table B.8. 88 and Figures B.8. 31 and B.8. 32 below.

Table B.8. 88 Mackenzie & Roohi 2008a (sulphanilamide aerobic soil) soils 07/022 and 07/030 – parameter optimisation results (DFOP) – free optimisation

Soil system	DT50(d)	DT90 (d)	Chi2	Value	t-test	Visual assessment
07/022	2.4	24.2	3.9	k1: 3.068 k2: 0.0739 g: 0.4028	0.0579, >94% 2.60E-07, >99%	Excellent
07/030	3.4	28	4.4	k1: 1.4492 k2: 0.0653 g: 0.3779	6.90E-04, >99% 1.20E-07, >99%	Excellent

Figure B.8. 31 Soil 07/022, DFOP fit, Chi2 = 3.9

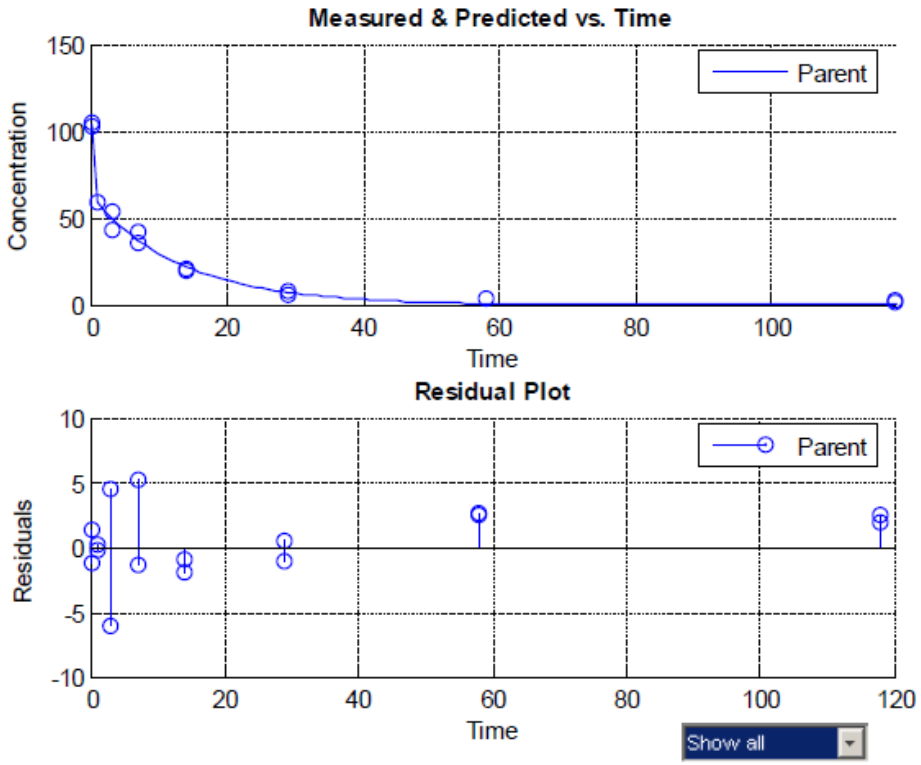
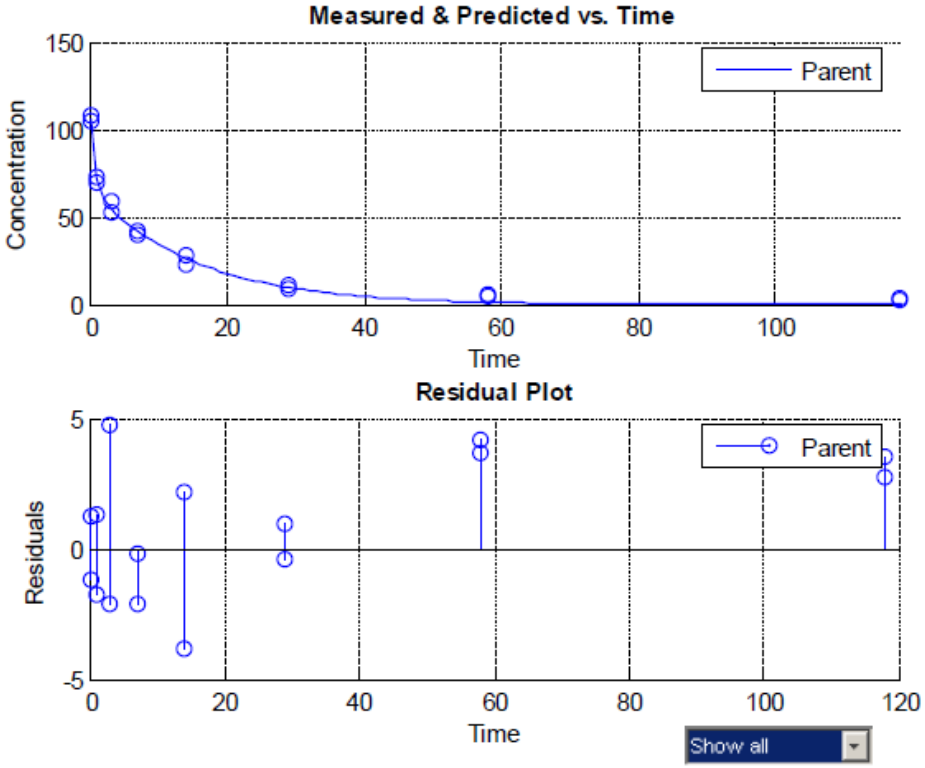


Figure B.8. 32 Soil 07/030, DFOP fit, Chi2 = 4.4



DFOP kinetics were evaluated for these two soils resulting in significantly improved fits to the data around the DT90. As DT90 was reached within the experimental period, conservative SFO DT50 values for modelling endpoints were derived by the applicant from the FOMC or DFOP DT90/3.32, resulting in values of 7.3 days for soil 07/022 (24.2/3.32), 3.1 days for soil 07/024 (10.3/3.32) and 8.4 days for soil 07/030 (28.0/3.32). The RMS agrees with the DT50s calculated by the applicant, the values of which are presented in Table B.8. 89 below.

Table B.8. 89 Laboratory DT50 values for sulphanilamide normalised to FOCUS temperature and moisture conditions of 20°C and pF2

Soil	Kinetic	Chi2	Best-fit DT_{50} (days)	Best-fit DT_{90} (days)	Calculated DT50 (d)
07/022	DFOP	3.9	2.4	24.2	7.3 [a]
07/024	FOMC	4.0	1.4	10.3	3.1 [b]
07/030	DFOP	4.4	3.4	28	8.4 [a]

[a] DFOP DT90/3.322
[b] FOMC DT90/3.322

Hardy, (2011a)

The geometric mean DT50 at 20°C and field capacity (pF2) has been calculated below from the results of the kinetic evaluation for all six soils from the studies of Oddy (2003b) and Mackenzie and Roohi (2008a) and are presented in Table B.8. 90.

Table B.8. 90 Summary of DT50s for sulphanilamide normalised to 20°C and pF2 from studies of Oddy (2003b) and Mackenzie and Roohi (2008a)

Soil (texture)	Kinetic Model	Best-fit DT_{50} (days)	Best-fit DT_{90} (days)	Calculated DT_{50} (days)	Actual Temp (˚C)	MC study (%)	MC ref (%)	DT_{50} ref (days)
02/03 (Sandy loam)	DFOP	7.7	148.7	76.5 [1]	20	22.6	22.7	76.3
02/05 (Clay loam)	FOMC	3.8	100.9	30.4 [2]	20	24.2	36.4	22.4
02/06 (Clay loam)	DFOP	3.4	92.1	21.9 [3]	20	29.7	41.6	21.9
07/022 (Loamy sand)	DFOP	2.4	24.2	7.3 [3]	20	15.1	19.7	6.0
07/024 (Clay loam)	FOMC	1.4	10.3	3.1 [2]	20	28.2	28.5	3.1
07/030 (Loamy sand)	DFOP	3.4	28	8.4 [3]	20	6.6	6.5	8.4
Geometric Mean								13.4

[1] DFOP slow phase DT50 [ln(2)/k2]
[2] FOMC DT90/3.322
[3] DFOP DT90/3.322

B.8.1.1.4 Supplementary studies

Anaerobic studies (IIA 7.1.1; IIIA 9.1.1)

This study has previously been evaluated at EU level. The conclusions are consistent with the EFSA conclusions report and no concerns were raised at EU level.

Report:
P. Yeomans (2003a) [^{14}C]-Asulam Sodium: Metabolism in Flooded Soil. Covance Laboratories Ltd. for Bayer CropScience SA, Unpublished report No.: 1849/21-D2149, 31099, C032795

Guidelines: EU 95/36/EC
Deviations: None

GLP: Yes

c) The route and rate of degradation of ^{14}C-asulam sodium was investigated in one anaerobic soil at 20°C in the dark. The details of this study are reported in section B.8.1.1.2 (b).

The levels of ^{14}C-asulam sodium over the period of the study are presented in Tables B.8.38 to B.8.40. The DT50 for asulam sodium in the water phase and the total system was calculated by the Notifier using Double First Order in Parallel (DFOP) kinetics using Microsoft Excel software. The Rapporteur independently calculated the DT_{50} values. Using Single First Order (SFO) kinetics, although the Chi^2 error were <15%, the visual fits were not acceptable. DFOP provided improved visual fits and the Chi2 values were <2%.

The DT50 and DT90 values (DFOP) were calculated by the Notifier from the results in Table B.8. 38 and Table B.8. 40. The half life, DT50 and DT90 are shown in Table B.8. 91 and were considered acceptable by the Rapporteur. The Notifier did not report the DT90s as they were not considered to be representative. The Rapporteur agrees since the DT90 values were far in excess of the experimental period of 120 days.

Table B.8. 91 DT50 and DT90 values for asulam in anaerobic soil at 20°C

System	Analysis Model	DT_{50} (days)	DT_{90} (days)	Chi2 Error (%)	Fit (r^2)
Surface water	Double first order in parallel	91.1	471.6	1.95	0.990
Total system	Double first order in parallel	356.35	1343.4	1.67	0.942

(Yeomans, 2003a)

This study has not previously been evaluated at EU level. It has been evaluated at member-state level. The conclusions presented are consistent with those in the EFSA conclusions report.

Report:
G. Morlock (2009a) Degradation of Asulam (^{14}C- labelled) in One Soil under Anaerobic Conditions at 20 °C in the Dark. Separate Analysis of Water and Soil. eurofins GAB GmbH for AgriChem BV. Unpublished Report No. S08-00327.
Guidelines:

EU 95/36/EC, OECD 307
Deviations: None
GLP:
Yes

Details of the study of Morlock (2009a) have been reported in section B.8.1.1.2 (c).

The applicant only calculated the rate of decline for asulam during the aerobic phase using first order kinetics (DT50 = 9.2 d, DT90 = 30.6 d, r^2 = 0.9949). Asulam did not degrade in the anaerobic phase (see Table B.8. 42). It is considered by the applicant that during the anaerobic phase, asulam is re-dissolved from the bound residues in the soil which would account for the increase in concentration. A single metabolite (sulphanilamide) was formed under the anaerobic degradation of asulam. Sulphanilamide reached a maximum of 18.4% during the aerobic phase. During the anaerobic phase a maximum of 15.3% after 13 days was reached. For sulphanilamide the DT50 was calculated by the applicant using FOMC kinetics, for total system of 305 days (total system, water + soil). The Rapporteur could not obtain an acceptable fit using FOMC kinetics. An SFO DT50 of 195 days (Chi2 = 7%). The DT90 was beyond the duration of the study.

(Morlock, 2009a)

d) A laboratory soil photolysis study was conducted on ^{14}C-asulam sodium in one soil. The details of this study are reported in section B.8.1.1.3 (c).

In the presence of light degradation of asulam was accelerated without producing any unique photodegradates in comparison with the dark controls. The levels of asulam in the irradiated and dark control samples are presented in Table B.8. 44 and

Table B.8. 45 respectively (see section B.8.1.1.3 (c)). The DT50 values (SFO and DFOP) and DT90 values (DFOP) have been calculated by the Rapporteur and found to be in close agreement to those reported by the Notifier and are summarised below. The single first order DT_{50} for asulam sodium due to photolysis only was 8.3d (corrected for degradation in the dark controls). Overall the Rapporteur considered that photolysis was unlikely to be a significant rate of dissipation in the field compared with biotic degradation in the absence of light.

	half-life (days) (SFO)	r^2	DT50 (days) (DFOP)	DT90 (days) (DFOP)	r^2
Irradiated	3.71	0.959	2.66	19.4	0.996
Dark controls	6.69	0.995	6.42	25.8	0.996
Photolysis only	8.3	-	-	-	-

Soil residue studies

B.8.1.4 Summary & assessment – Soil studies

The environmental fate of asulam sodium was investigated in a number of laboratory studies using ring labelled material. Results of the laboratory aerobic route and rate of degradation carried out at 10 or 20°C are available for twelve different soils.

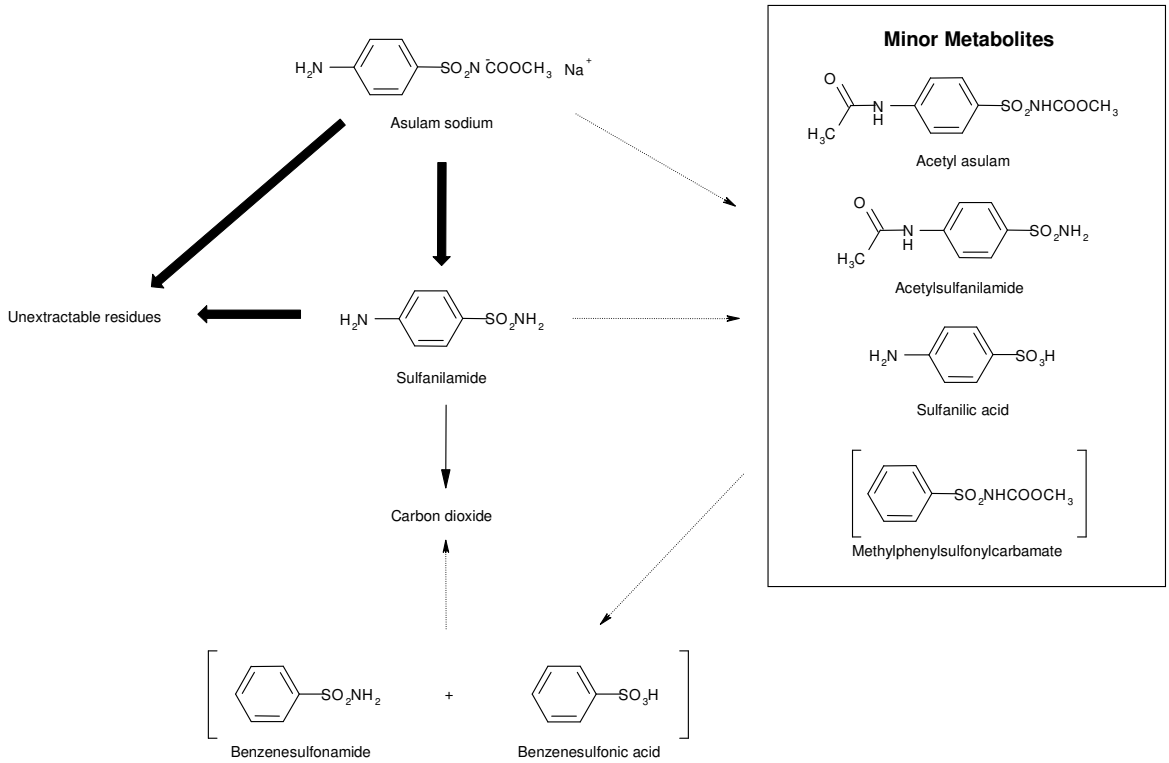
The major metabolite was identified as sulphanilamide which peaked at 14% AR after 14 days at 20°C in clay loam soil. An unidentified metabolite showed levels of up to ca. 20% AR but was found in a supplementary study to be an artefact caused by the scintillant. Bound residues were found associated with all three soil fractions (fulvic, humic and humin) the least radioactivity being associated with the fulvic acids (19 to 32%) and the most associated with the humic acids (22 to 53%) and humin (29 to 67%). The total unextracted residues accounted for 46 to 66% AR. Mineralisation to CO_2 ranged from 4 to 6% AR by 59 days and accounted for between 6 to 8% AR after 120 days (study end) in soils incubated at 20°C.

One study tentatively assigned a further metabolite as methyl benzenesulfonyl carbamate (MBSC) however the study was not considered reliable for a number of reasons including non-GLP, non-guideline, time zero samples not determined, no mass balance. Characterisation of radioactivity was by TLC alone without further confirmation and not considered reliable. Overall it was considered that the study was not reliable.

The major aerobic degradation path of asulam results in production of sulphanilamide. The major route of dissipation is the formation of unextractable bound residues either of asulam itself but more likely of sulphanilamide. A number of minor metabolites are formed and some of these may be further degraded to carbon dioxide along with a small portion of sulphanilamide. These mechanisms are accelerated by light, without the formation of unique photoproducts, but are slowed by anaerobic conditions.

The degradation pathway proposed by the notifier and considered acceptable by the Rapporteur is presented in Figure B.8. 33. The bold arrows represent the major pathways.

Figure B.8. 33 Proposed metabolic pathway of asulam sodium in soil



A summary of the laboratory DT50 values, normalised to 20°C and pF2, is presented in Table B.8. 92.

Laboratory single first order DT50 values for asulam normalised to 20°C and moisture at field capacity (pF2) ranged from 2.1 to 11.1 days (geometric mean 4.6 days, results from a four studies on 12 soils). Corresponding DT_{90} values were in the range of 7.1 to 37.2 days.

For metabolite sulphanilamide, laboratory DT_{50} values were available from six soils. Acceptable fits were achieved with either FOMC or DFOP kinetics, the normalised degradation rates (20°C/pF2) ranged from 3.1 to 76.3 days (geometric mean 13.4 days). Using FOMC or DFOP kinetics giving the best fit, the DT90 values were in the range 10.3 to 148.7 days

The anaerobic route of degradation has been investigated in two studies (2 soils). In the first study, asulam degraded more slowly than under aerobic conditions. DT50 values of 91 days in the water phase and 356 days for the total system were calculated using double first order in parallel kinetics (DFOP). DT90 values were not calculated. Metabolite sulphanilamide formed at peak levels of 0.8% AR in soil after 120 d but was not detected in the water. Additionally a major unidentified metabolite labelled "unknown 1" reached a maximum of 9% AR after 7 days and the largest unknown reached a maximum of 2.8% after 30 days. Bound residues reached a maximum of

7.9% AR after 120 days. In the second study, the rate of decline was only calculated by the applicant for the aerobic phase, asulam did not degrade in the anaerobic phase but actually displayed an increase. It is considered that in the anaerobic phase, asulam was re-dissolving from the bound residues in the soil. Sulphanilamide was the only metabolite reaching a maximum of 15.3% AR in the anaerobic phase.

Table B.8. 92 Summary of the single first order (non-linear regression) rates of asulam and its metabolite sulphanilamide in aerobic laboratory soils normalised to 20°C and moisture at field capacity (pF 2)

Soil / texture	Kinetic Model	Best-fit DT_{50} (days)	Best-fit DT_{90} (days)	Calc[d] DT_{50} (days)	Actual Temp (˚C)	MC study (%)	MC ref (%)	DT_{50} ref (days)	Study reference
Asulam									
PT102, Loam	SFO	3.8	12.7	3.8	20	26.5	25 [a]	3.8	Yeomans 2000a
PT103, Sandy loam	SFO	3.4	11.5	3.4	20	16.9	14 [b]	3.4	
SK15556090, Silt loam	SFO	2.1	7.1	2.1	20	49.7	38.8 [c]	2.1	
SK961089, Clay loam	SFO	4.0	13.4	4.0	20	38.7	31.2 [c]	4.0	
PT103, Sandy loam	SFO	2.4	8.0	2.4	20	19.2	14 [b]	2.4	Yeomans and Swales, 2003a
SK15556090, Silt loam	DFOP	0.2	10.9	3.3 [f]	20	39.1	31.8 [c]	3.3	
SK960087, Clay loam	SFO	2.6	8.6	2.6	20	28.0	28 [a]	2.6	
07/022, Loamy sand	SFO	11.2	37.2	11.2	20	15.1	19.7 [a]	9.3	Roohi and Mackenzie 2009a
07/024,Clay loam	SFO	7.1	23.6	7.1	20	28.2	28.5 [a]	7.0	
07/028, Clay loam	SFO	10.8	36	10.8	20	29.6	31.3 [a]	10.4	
07/030, Loamy sand	SFO	11.1	36.8	11.1	20	6.6	6.5 [a]	11.1	
Borstel 98, Sandy Loam	FOMC	0.6	19.6	5.9 [e]	20	14.53	19 [b]	4.9	Völkel, 2000a
							Geometric Mean	4.6	
Sulphanilamide									
02/03 (Sandy loam)	DFOP	7.7	148.7	76.5 [d]	20	22.6	22.7	76.3	Oddy, 2003b
02/05 (Clay loam)	FOMC	3.8	100.9	30.4 [e]	20	24.2	36.4	22.4	
02/06 (Clay loam)	DFOP	3.4	92.1	21.9 [f]	20	29.7	41.6	21.9	
07/022 (Loamy sand)	DFOP	2.4	24.2	7.3 [f]	20	15.1	19.7	6.0	Roohi and Mackenzie, 2008a
07/024 (Clay loam)	FOMC	1.4	10.3	3.1 [e]	20	28.2	28.5	3.1	
07/030 (Loamy sand)	DFOP	3.4	28	8.4 [f]	20	6.6	6.5	8.4	
							Geometric Mean	13.4	

a FOCUS default pF 2,
b default pF 2 for loamy sand measured pF 2.5 closer to loamy sand than sandy loam,
c measured study pF 2.5 data > default pF 2, so pF 2.5 data used
d DFOP slow phase DT50 [ln(2)/k2]
e FOMC DT90/3.322
f DFOP DT90/3.322

Photodegradation in soil was investigated in two studies using artificial illumination. In both studies the degradation of asulam was accelerated the presence of light compared to the dark controls without producing any unique photodegradates. Asulam was shown to have a short half-life (3.71 and 5.6 days for the irradiated samples and

6.69 and 18.4 days for the dark controls). The DT50 corrected for photolysis only was 8.3 days. The principal identified metabolite was sulphanilamide, which accounted for up to 4% in the irradiated samples and 11% AR in the dark controls. A number of minor metabolites including acetyl asulam were quantified but none of the metabolites accounted for >5% AR at the end of the study. Overall the Rapporteur considers that photolysis is unlikely to be a significant route of dissipation in the field compared with biotic degradation in the absence of light.

It is concluded that the studies whose results are summarised above were of appropriate design with appropriately positioned radiolabels. They were considered by the Rapporteur to provide sufficient information of an appropriate quality to elucidate and propose the route of degradation of asulam in soil under aerobic and anaerobic conditions and in the presence of light.

For calculating soil, surface water and groundwater PEC values, the pertinent residues in soil are therefore considered by the Rapporteur to be asulam and metabolite sulphanilamide.

Route of degradation (aerobic) in soil (Annex IIA, point 7.1.1.1.1)

Mineralization after 100 days ‡	6.1-7.5 % after 120 d, [^{14}C-Ph]-label (n[1]= 4) 31.5-6.17 % after 119 d, [^{14}C-Ph]-label (n= 4)
Non-extractable residues after 100 days ‡	63.9-76 % after 120 d, [^{14}C-Ph]-label (n= 4) 69.08-82.49 % after 119 d, [^{14}C-Ph]-label (n= 4)
Metabolites requiring further consideration ‡ - name and/or code, % of applied (range and maximum)	Sulphanilamide, Peak *13.9* % at *14* d (n= 3) [^{14}C-PH] label

Route of degradation in soil - Supplemental studies (Annex IIA, point 7.1.1.1.2)

Anaerobic degradation ‡

Mineralization after 100 days	0.4 % after 120 d, [^{14}C-Ph]-label (n= 1) 0.7 % after 118 d, [^{14}C-Ph]-label (n= 1)
Non-extractable residues after 100 days	7.9 % after 120 d, [^{14}C-Ph]-label (n= 1) 48.5 % after 14 d, [^{14}C-Ph]-label (n= 1)
Metabolites that may require further consideration for risk assessment - name and/or code, % of applied (range and maximum)	None

Soil photolysis ‡

Metabolites that may require further	Parent DT50: 3.71d irradiated; 6.69d dark controls Sulphanilamide:

[1] n corresponds to the number of soils.

consideration for risk assessment - name and/or code, % of applied (range and maximum)	Irradiated: 3.6% after 10 d, 1.7% after 24 d, Dark controls: 10.7% after 10 d, 4.2% after 24d presumed to be photo-degrading in soil

Rate of degradation in soil (Annex IIA, point 7.1.1.2, Annex IIIA, point 9.1.1)

Laboratory studies ‡

Parent	Aerobic conditions						
Soil type	X[1]	pH (KCl)	t. °C / % MWHC	DT_{50} /DT_{90} (d)	DT_{50} (d) 20°C pF2/10kPa	Chi^2 error	Method of calculation
Loam		6.7	20°C / 45 %	3.8 / 12.7	3.8	8.0	SFO
Sandy loam		5.1	20°C / 45 %	3.4 / 11.5	3.4	12.3	SFO
Silt loam		6.1	20°C / 45 %	2.1 / 7.1	2.1	13.0	SFO
Clay loam		7.3	20°C / 45 %	4.0 / 13.4	4.0	11.0	SFO
Sandy loam		4.9	20°C / 45 %	2.4 / 8.0	2.4	14.7	SFO
Silt loam		6.1	20°C / 45 %	4.8 / -	3.3	11.7	DFOP DT90/3.32
Clay loam		7.3	20°C / 45 %	2.6 / 8.6	2.6	11.9	SFO
Loamy sand		5.2	20°C / 63	11.2 / 37.2	9.3	7.5	SFO
Clay loam		6.1	20°C / 90	7.1 / 23.6	7.0	7.7	SFO
Clay loam		7.3	20°C / 93	10.8 / 36	10.4	7.8	SFO
Loamy sand		3.9	20°C / 28	11.1 / 36.8	11.1	9.6	SFO
Sandy loam		5.01	20°C / 40	0.6 / 19.6	4.9	7.6	FOMC DT90/3.322
Geometric mean/median					4.6*		
					4.7**		

* Overall geometric mean; ** geomean of sandy loam and silt loam calculated first

Sulphanilamide	Aerobic conditions							
Soil type	X[1]	pH (KCl)	t. °C / % MWHC	DT_{50} (d)	f. f. k_{dp}/k_f	DT_{50} (d) 20°C pF2/10kPa	Chi^2 error	Method of calculation
Sandy loam		3.9	20°C / 42 %	76.5 [a]	-	76.3	6.1	DFOP
Clay Loam		7.0	20°C / 50 %	30.4 [b]	-	22.4	5.8	FOMC
Clay Loam		7.9	20°C / 50 %	16.4 [b]	-	21.9	7.3	FOMC
Loamy sand		5.2	20°C / 63 %	7.3 [a]	-	6.0	3.9	DFOP
Clay loam		5.8	20°C / 90 %	3.1 [b]	-	3.1	4.0	FOMC

[1] X This column is reserved for any other property that is considered to have a particular impact on the degradation rate.

Loamy sand		3.9	20°C / 28 %	8.4 [a]	-	8.4	4.4	DFOP
Geometric mean/median			-	-	-	13.4	-	-

[a] ln(2)/DFOP slow phase rate constant [b] FOMC DT90/3.322 [c] DFOP DT90/3.322

Field studies ‡ No data, not required

~~Parent~~	~~Aerobic conditions~~								
~~Soil type (indicate if bare or cropped soil was used).~~	~~Location (country or USA state).~~	~~X[1]~~	~~pH~~	~~Depth (cm)~~	~~DT_{50} (d) actual~~	~~DT_{90} (d) actual~~	~~St. (r^2)~~	~~DT_{50} (d) Norm.~~	~~Method of calculation~~
~~Geometric mean/median~~									
~~Met 1~~	~~Aerobic conditions~~								
~~Soil type~~	~~Location~~		~~pH~~	~~Depth (cm)~~	~~DT_{50} (d) actual~~	~~DT_{90} (d) actual~~	~~St. (r2)~~	~~DT_{50} (d) Norm.~~	~~Method of calculation~~
~~Geometric mean/median~~									

Repeat for as many metabolites as necessary

pH dependence ‡ (yes / no) (if yes type of dependence)	None
Soil accumulation and plateau concentration ‡	Not studied. Not required

Laboratory studies ‡

Parent	Anaerobic conditions						
Soil type	X[2]	pH	t. °C / % MWHC	DT_{50} /DT_{90} (d)	DT_{50} (d) 20°C pF2/10kPa	St. (r^2)	Method of calculation

[2] X This column is reserved for any other property that is considered to have a particular impact on the degradation rate.

Sandy loam		6.7	20°C	356 / 1343		356	0.942	DFOP
Loamy sand		5.69	20°C	stable			n/a	Not calculated
Geometric mean/median								
Met 1	Anaerobic conditions							
Soil type	X^1	pH	t. °C / % MWHC	DT_{50}/ ~~DT_{90}~~ (d)	f. f. k_{dp}/ k_f	DT_{50} (d) 20°C pF2/10kPa	Chi^2 error (%)	Method of calculation
Loamy sand		5.69	20°C	195	-	195	7	SFO
Geometric mean/median								

Repeat for as many metabolites as necessary

B.8.2 Adsorption, desorption and mobility in soil (IIA 7.1.2, 7.1.3, IIIA 9.1.2)

B.8.2.1 Adsorption and desorption

This study has previously been evaluated at EU level. The conclusions are consistent with the EFSA conclusions report and no concerns were raised at EU level.

Report:

C.J. Lewis (1999a) [14C]-Asulam Sodium: Adsorption/Desorption in Five Soils and One Sediment. Covance Laboratories, Unpublished report No.: 68/169-D2142, C015417

Guidelines:
EU 95/36/EC, OECD 106, EPA N 163-1
Deviations: None

GLP: Yes

a) A batch equilibrium adsorption/desorption study was conducted with asulam in accordance with OECD guideline 106 (1981), EC Directive 95/36/EC and EPA guidelines N, 163-1, (1982) and to GLP and was considered acceptable by the Rapporteur. The definitive study was conducted in 3 UK soils, 2 US soils and a UK sediment, the characteristics of the which are shown in Table B.8. 93

Stock solutions of ring labelled ^{14}C-asulam (radiochemical purity >97%, specific activity 5.617 MBq/mg) prepared in aqueous solution in equimolar sodium hydroxide and 0.01M $CaCl_2$, were further diluted to concentrations of 5, 1, 0.2 and 0.04 µg/mL with 0.01M $CaCl_2$ solution prior to addition to the soils and sediment. The 5 µg/mL solution was prepared using ^{14}C-asulam and non-radiolabelled asulam in the approximate ratio 1:4. The other solutions were prepared using ^{14}C-asulam only.

Preliminary studies were performed to establish adsorption and desorption equilibrium times, soil:solution ratio and obtain preliminary stability data. Preliminary studies were also performed to demonstrate that asulam was soluble in aqueous solution at the highest concentration and that it did not adsorb to the containers. (The preliminary studies were performed on 6 soils and 1 sediment whereas the definitive study was performed on 5 soils and 1 sediment as shown in Table B.8. 93).The preliminary tests showed that a soil:solution ratio of 1:1 (w/v) and that an equilibrium time for adsorption and desorption of 24 h would be required. Equilibrium time and the adsorption and desorption studies were conducted in the dark at 20±2°C. The stability of asulam in the 24 h adsorption was verified by HPLC analysis. Degradation products were <5% of radioactivity in solution.

The determination of isotherms was performed by addition of 10 mL portions of ^{14}C-asulam solutions to duplicate 10g dry weight equivalent samples of soil which had been air dried and equilibrated for 24 h by shaking with twice its weight in water. One adsorption and 2 desorption steps were performed. After each step, samples were centrifuged and the supernatants removed. After the adsorption and first desorption phases the removed supernatants were replaced with fresh 0.01 M calcium chloride solution and the samples shaken vigorously to break up compacted material. The soils and sediment treated with the highest concentration (5 µg/mL) were extracted with acetonitrile after the second desorption stage.

Although the Notifier reported that the desorption stages were conducted with fresh $CaCl_2$ solution, it was noted by the Rapporteur that in the first desorption stage for soil types PT102 and LA99-3, and the first and second desorption stages for soil SK961089, that the quantity of asulam adsorbed per gram of soil, increased. This was not considered to significantly affect the validity of the study since the calculations of the adsorption coefficients (Kfoc) were validated by the Rapporteur.

The extracted soils and sediment were allowed to air dry. The radioactivity was determined in the adsorption and desorption solutions (and extracts) by liquid scintillation counting (LSC). The mean mass balance data was acceptable (96.0 to 99.3%).

Freundlich adsorption coefficients (K_f, K_{foc} and K_{fom}) and values of 1/n which have been validated by the Rapporteur, are shown in Table B.8. 94. Mean values for K_{foc} and 1/n were calculated to be 20 and 0.75 respectively. Only the four soils were included in the mean calculation. The sand (low organic carbon) and the sediment (not a soil) were excluded.

With the exception of soil LA 98-983 (with very low carbon content) variation between K_{foc} values was less than between K_f values, indicating that organic carbon of soil content is an important factor in determining adsorption. Adsorption was not noted to correlate with any other soil parameter.

Table B.8. 93 Characteristics of soils used in an adsorption desorption study with ^{14}C-asulam sodium (Lewis 1999a)

Soil Code	PT 102	PT 103	SK961089	LA98-983	LA99-3	Emperor Lake
Origin	UK	UK	UK	US	US	UK
UK Particle size distribution						
Sand % (2000-63µm)	51	74	47	94	39	60
Silt % (63-2µm)	38	13	33	4	55	19
Clay % (<2µm)	11	13	20	2	6	21
Textural Class	Sandy Loam	Sandy Loam	Clay Loam	Sand	Sandy Silt Loam	Sandy Clay Loam
Organic Carbon (%)	2.5	1.3	5.4	0.1	2.5	6.1
Organic Matter (%)	4.3	2.2	9.3	0.2	4.3	10.5
pH:						
water	7.1	5.5	7.5	6.0	6.8	6.0
0.1M KCl	6.8	4.8	7.2	4.9	6.1	4.8
Cation exchange capacity (mEq/100g)	16.3	8.8	34.6	1.0	9.7	23.9
Moisture holding capacity (%)						
at pF 0 (0.001 bar) %	67.9	46.9	76.2	28.5	81.2	101.1
at pF 2.5 (0.33 bar) %	20.4	9.8	34.5	2.2	23.1	45.1
Extractable Cations (mEq/100g)						
Ca	10.52	4.70	25.54	0.10	7.7	5.26
Mg	1.18	0.30	1.16	0.00	3.2	0.88
K	1.47	0.11	0.40	0.01	0.39	0.19
Na	0.13	0.04	0.10	0.01	0.02	0.13
H	0.00	2.00	0.00	0.75	0.55	7.10
Calcium Carbonate Equivalent (%)	0.5	Not measured (pH <6.5)	20.6	Not measured (pH <6.5)	0.4	Not measured (pH <6.5)

Table B.8. 94 Freundlich constants for ^{14}C-asulam sodium on soils and sediment indicated

Soil or sediment	Organic carbon content (%)	pH	Experimental stage	K_f	K_{foc}	K_{fom}	1/n	r^2
			Adsorption	0.4	15.5	9.0	0.73	0.9997
PT102	2.5	7.1	Desorption 1	1.2	48.6	28.3	0.74	0.9990
			Desorption 2	3.3	131.1	76.2	0.80	0.9950
			Adsorption	0.3	23.4	13.8	0.82	0.9997
PT103	1.3	5.5	Desorption 1	0.7	53.3	31.5	0.78	0.9999
			Desorption 2	1.5	117.5	69.4	0.79	0.9992
			Adsorption	0.8	15.4	8.9	0.71	0.9994
SK961089	5.4	7.5	Desorption 1	2.5	46.9	27.3	0.79	0.9996
			Desorption 2	6.4	118.3	68.7	0.88	0.9944
			Adsorption	0.1	149.7	74.9	0.82	0.9996
LA98-983	0.1	6.0	Desorption 1	0.3	310.3	155.1	0.78	0.9988
			Desorption 2	0.7	695.5	347.7	0.78	0.9984
			Adsorption	0.6	25.5	14.8	0.73	0.9990
LA99-3	2.5	6.8	Desorption 1	1.6	65.9	38.3	0.72	0.9986
			Desorption 2	3.8	152.2	88.5	0.76	0.9968
			Adsorption	2.6	42.5	24.7	0.68	0.9904
Emperor lake	6.1	6.0	Desorption 1	5.6	92.4	53.7	0.73	0.9992
			Desorption 2	10.8	176.4	102.5	0.81	0.9966

(Lewis, 1999a)

This study has not previously been evaluated at EU level. It has been evaluated at member-state level. The conclusions presented are consistent with those from previously submitted studies and the EFSA conclusions report.

Report:

W. Völkel (2011a) [14C]-Asulam: Adsorption/Desorption on Soil. Innovative Environmental Services, Witterswill, Switzerland for AgriChem BV., Unpublished report No.: 152 01 013

Guidelines:

Modified EU Council Directive 91/414/EEC, as amended by Commission Directive 95/36/EC of July 1995, Section 7, Sub section 7.1.2

Deviations: None

GLP:

Yes

RMS opinion on study: fully acceptable. Please note the summary below is the applicant's. It is an accurate account of the report and considered acceptable by the RMS. The RMS has provided comments at the end of the study summary.

Executive Summary:

The adsorption/desorption behaviour of the test item ^{14}C-Asulam on soil was determined with five soils using the batch equilibrium method according to OECD Guideline No. 106. The following soils were used: Am Fischteich (soil I, silt loam), Am Hartschlössel (soil II, loam), Speyer 2.2 (soil III, loamy sand), Witterswil (soil IV, silty clay) and Speyer 6S (soil V, clay). The soils showed a range of different characteristics important for adsorption i.e. organic carbon content (OC), cation exchange capacity, pH and clay content.

Due to the expected high water solubility of the test item soil-to-solution ratios of 1:1 and 1:2 were tested in the preliminary test. The ratio of 1:1 was selected for the screening test.

The screening test was performed in order to investigate the adsorption/desorption behaviour of ^{14}C-asulam with time. For this purpose, a test concentration of 0.98 mg/L and the soil to aqueous phase ratio of 1:1 (5 g/5 mL) were used for all soils. The aqueous phase consisted of 0.01 M $CaCl_2$ solution. The soil/solution samples were incubated at 20 °C in the dark in sealed Teflon centrifuge tubes on a shaker to keep the soil in solution as a suspension. After 2, 5, 24 and 44 hours of agitation, aliquots of the aqueous phase were measured by Liquid Scintillation Counting (LSC).

The screening results showed that no adsorption equilibrium was reached after 44 hours. After 44 hours of adsorption, 41.2%, 78.4%, 20.4%, 42.9% and 18.7% of the applied amount was absorbed to soil I through V, respectively. The adsorption equilibrium not reachable, was not only due to the degradation of the test item, since in the supernatants of soils I, III and IV HPLC analyses showed more than 95% Asulam. There was a continuous increase in binding of asulam with time. A test on soil samples sterilized by gamma irradiation showed also that no equilibrium could be reached after 48 hours of adsorption.

All applied radioactivity was recovered in the control samples (96.5% to 100.6%) and remained constant throughout the incubation time. The amount of test item desorbed did not reach an equilibrium after 44 hours of desorption.

Degradation of the test item was observed in two of the soils during the adsorption phase. Therefore, the radioactivity measured in the supernatant solutions was corrected by the amount of asulam as analysed by HPLC. Moreover, for the desorption step, the radioactivity measured in the supernatant solutions was corrected for all soils by the amount of asulam analysed.

The mean values for the adsorption and desorption coefficients Kd and Kdes were 1.1 mL/g and 3.8 mL/g. The mean values for the adsorption and desorption coefficients related to the organic carbon content of the soils KOC and Kdes,OC for soils were 35 mL/g and 129 mL/g, respectively.

The higher coefficients for desorption indicated a partially irreversible sorption process.

The radioactive mass balance showed recoveries of 92.7% to 104.4% of applied. The radioactivity detected in the supernatant ranged from 23.9% and 81.5% and the radioactivity bound to soil from 16.2% to 60.9%. Extractable radioactivity was low, i.e. 0.6% and 7.9% for soil III and II. For the other soils no radioactivity could be extracted.

For the subsequent advanced test, the soil-to-solution ratio of 1:1 and a maximum agitation time of 48 hours (for both adsorption and desorption) and five different initial test item concentrations (0.977, 0.292, 0.099, 0.029 and 0.010 mg/L) covering two orders of magnitude were used.

The adsorption/desorption parameters resulting from the Freundlich isotherms are presented in Table B.8. 95 below.

Table B.8. 95 Asulam sodium Freundlich adsorption constants and K_{OC} values in four soils

Parameter		Soil				Mean
		Am Fischteich	Am Hart-schlösschen	Speyer 2.2	Witterswil	
K_F	(mL/g)	0.513	3.280	0.218	0.677	
K_{FOC}	(mL/g)*	24	67	16	17	31
K_{FOM}	(mL/g)	14	39	9	10	18
1/n		0.66	0.87	0.70	0.77	
r^2		0.9905	0.9995	0.9946	0.9999	
$K_{des, F}$	(mL/g)	6.953	4.975	0.470	3.124	
$K_{des, FOC}$	(mL/g)	328	101	34	79	135
$K_{des, FOM}$	(mL/g)	190	59	19	46	79
1/n		0.97	0.83	0.66	0.94	
r^2		0.9985	0.9995	0.9940	0.9958	

* As presented in report. Recalculation to 1 decimal place for consistency with equivalent study of Lewis gives values of 24.2, 66.5, 15.6 and 17.2

Materials and Methods

Test Material:

[14C]-Asulam sodium, purity 99.4%, Batch no.: 4006GAR001-1

Test Design:

The study consisted of three parts:

1. The preliminary test was performed with all soils at one test item concentration in order to determine:

- the optimal soil/solution ratio to be used in screening and advanced test,
- adsorption of the test item on the surfaces of the test vessels and the stability of the test item during the test period.

2. The screening test was performed with all five soils at one test item concentration and one soil to solution ratio in order to determine:

- the equilibration time for adsorption/desorption and the amount of test item adsorbed/desorbed at equilibrium,
- adsorption/desorption kinetics at a single concentration and determination of distribution coefficients Kd and Koc
- for all soils the pH of the aqueous phase was measured (before and after contact with each soil).
- a mass balance was performed (aqueous phases and soils were worked up directly after the adsorption step, two tubes per soil).

3. The advanced test was performed with four soils at five test item concentrations and one soil-to-solution ratio in order to determine the Freundlich adsorption/desorption isotherms to investigate the influence of the concentration on the extent of adsorption/desorption on soils.

Soil characteristics:

Parameters	Soil				
	I	II	III	IV	V
Site location:	Am Fischteich	Am Hart-schlösschen	Speyer 2.2	Witterswil	Speyer 6S
Batch:	03/09	08/09	F2.22308	09/08	F6S4108
Soil characteristics:					
- pH (0.01 M CaCl2)	5.74	4.60	6.05	7.36	7.20
- pH (water)	6.33	5.04	6.62	7.76	7.65
- Organic carbon (g/100 g soil) %	2.12	4.93	1.40	3.93	1.75
- Cation exchange capacity (mmol/100g soil)	17.44	27.21	8.04	40.21	22.0
- Nitrogen content %	0.22	0.39	0.13	0.45	0.15
- Organic matter* %	3.65	8.50	2.41	6.78	3.02
- C/N-ratio*	9.64	12.64	10.77	8.73	11.67
Soil type (according to USDA):	Silt loam	Loam	Loamy sand	Silty clay	Clay
Particle size analyses (mm)					
USDA:					
< 0.002 (clay) %	20.49	18.14	9.26	40.54	42.1
0.002-0.05 (silt) %	65.99	39.15	10.58	48.36	36.0
> 0.05 (sand) %	13.52	42.71	80.16	11.09	21.9

The entire stock of radiolabelled asulam was dissolved in acetone to give a stock solution. Various dilutions were prepared for the various tests. The radioactive content of each solution was checked by LSC. These were applied, after pre-equilibration of the soil (where appropriate) using different aliquot volumes to achieve the desired final concentrations as summarized below:

Test	Soil / aqueous phase ratio	Amount of soil [g]	Volume of aqueous phase for equilibration [mL]	Application solution identification	Volume of application solution [mL]	Final volume* [mL]
Preliminary	1:2	5	9	AS1	0.500	10
	1:1	5	4.5	AS1	0.250	5
Screening 1	1:1	10	9	AS2	0.400	10
Screening 2	1:1	10	9	AS3	0.800	10
Advanced	1:1	10	9	A-E	1.000	10

The test tubes were briefly shaken by hand, and then mechanically shaken on a rotary shaker in a temperature-controlled room. Control samples (duplicates) containing the test item in 0.01 M CaCl2 solution (i.e. without soil) were subjected to precisely the same steps as the test samples in all tests in order to check the stability of the test item in CaCl2 solution.

A blank run for each soil type with the same amount of soil and the same total aqueous phase volume (without test item) was subjected to the same test procedure. This served as a background control during the analysis to detect any contamination.

The achieved concentrations were as follows: preliminary test: 0.20 µg/mL; screening test: 0.98 µg/mL; advanced test (Freundlich isotherms): 0.98, 0.29, 0.10, 0.03 and 0.01 µg/mL.

Due to the expected high water solubility of the test item, the soil-to-solution ratios of 1:1 and 1:2 and an adsorption time of 24 hours were selected for the preliminary test. The adsorption was determined in the preliminary test using all five soils at a test item concentration of 0.20 mg/L (measured in the application solution). After 24 hours of shaking, duplicate tubes were sampled and the supernatants measured by LSC.

The adsorption/desorption kinetics were determined in the screening test using all five soils and the soil-to-solution ratio of 1:1 at a test item concentration of 0.98 mg/L (measured in the application solution). After 2, 4, 24 and 44 hours of shaking, duplicate tubes were sampled, and the supernatants measured by LSC. In addition, the supernatants from the longest interval were analysed by HPLC to determine the extent of degradation of the test item.

Due to degradation of the test item observed in the supernatant solutions, the adsorption kinetics test was repeated using two sterilized soils (gamma-irradiation). The same soil-to-solution ratio of 1:1 and a test item concentration of 0.75 mg/L were used. After 2, 4, 24 and 48 hours of shaking, duplicate tubes were sampled, and the supernatants measured by LSC. In addition, the supernatants from the 48-hour interval were analysed by HPLC. The pH of the aqueous phase was measured before and after (48 hours) contact with the soils.

The mass balance was carried out on all soils in the screening test directly after the adsorption phase at the ratio of 1:1 (two tubes per soil). After sampling, the supernatant was removed and the soil was extracted four times using acetonitrile/water (4:1; v/v). HPLC analysis was performed. Aliquots (about 0.1 g) of the remaining air-dried soil were then combusted in order to determine the radioactivity remaining in the soil.

To determine the influence of the concentration on the extent of adsorption/desorption (Freundlich isotherms), the advanced test was performed using four soils, five initial test item concentrations (0.98, 0.29, 0.10, 0.03 and 0.01 µg/mL), a soil-to-solution ratio of 1:1 and an agitation time of 48 hours. After 48 hours of shaking, duplicate tubes were sampled and the supernatants measured by LSC and analysed by HPLC.

Freundlich desorption isotherms were determined on the soils used in the adsorption isotherms experiment (five concentrations). After the adsorption step, the soil-solution mixture was centrifuged and the aqueous phase was removed. The volume of solution was replaced by an equal volume of 0.01 M CaCl2 without test item. The new mixture was agitated for further 48 hours. Thereafter, duplicate tubes were sampled and analysed as for the adsorption step.

HPLC was used for quantification of asulam. The HPLC system comprised a 5 µm Phenomenex C18 pre-column linked to a 250 mm x 4.6 mm, 5 µm Phenomenex Synerdie 4µ Hydro RP column which was attached to a radiodetector fitted with a liquid cell. The mobile phase was a gradient of 0.1% trifluoroacetic acid in water against 0.1% trifluoroacetic acid in acetonitrile.

Results and Discussion:

The purity of 14C-Asulam was determined to be 100% by HPLC analysis of the application solution before treatment. Its stability in 0.01 M CaCl2 solution was confirmed by HPLC analysis of control samples after 48 hours of agitation (at least 99.5% in the screening test and in the advanced test). However, the test item degraded when in contact with soil and showed that use of sterilized soil did not stop the

degradation. Therefore, for the advanced test soils were not sterilized, but the degree of degradation was taken into account to calculate the adsorption constants.

Preliminary test. Due to the expected high water solubility of the test item, the soil-to-solution ratio of 1:1 and 1:2 were selected at an adsorption time of 24 hours. The preliminary test results showed that adsorption of 14C-Asulam depended on the relative amount of soil i.e. it increased with the amount of soil. After 24 hours of adsorption, 41.8%, 77.9%, 22.2%, 34.7% and 39.7% of the applied amount was adsorbed to soils I through V, at the ratio 1:1. Corresponding values for the ratio 1:2 were 27.3%, 62.4%, 12.5%, 20.7% and 23.4% for soils I to V.

All of the applied radioactivity was recovered in the control samples (98.3% of applied). A soil to solution ratio of 1:1 was selected to obtain maximum adsorption, for the screening test.

Screening test. The screening test results at the soil to solution ratio of 1:1 showed that adsorption equilibrium was not reached after 44 hours of adsorption. After 44 hours of adsorption, 41.2%, 78.4%, 20.4%, 42.9 and 18.7% of the applied amount was adsorbed to soil I to V, respectively. The equilibrium which was not reached was not only due to degradation of the test item, since in the supernatants of soils I, III and IV HPLC analyses showed more than 95% asulam. There was a continuous increase in binding of Asulam with time.

A test on soil samples sterilized by gamma irradiation showed also that no equilibrium could be reached after 48 hours of adsorption. All of the applied radioactivity was recovered in the control samples (96.5% to 100.6%) and remained constant throughout the incubation time.

Formation of degradation products was observed in the supernatant solutions of soil II and V during the adsorption phase. Therefore, the radioactivity measured in the supernatant solutions was corrected by the amount of asulam as analysed by HPLC. The resulting Kd and KOC values are presented in Table B.8. 96 below.

Table B.8. 96 Asulam Kd and K_{OC} values from a screening test in five soils

Soil	I	II	III	IV	V
Koc (mL/g)	33	88	18	19	14
Mean I-V 35 mL/g					
Kd (mL/g)	0.7	4.3	0.3	0.8	0.2
Mean I-V 1.3 mL/g					

As for the adsorption step, the radioactivity measured in the supernatant solutions of the desorption step was corrected by the amount of asulam as analysed by HPLC.

Higher amounts of asulam were adsorbed after the desorption step than were observed in the corresponding soils after the adsorption step. No equilibrium was reached during adsorption and the binding of asulam to soils continued during the desorption step, therefore explaining the higher amounts of asulam adsorbed to soils. After the adsorption step all radioactivity was recovered from the soil samples with recoveries ranging from 92.7% to 104.4% for all soils. Amounts of radioactivity ranging between 23.9% and 81.5% of applied were detected in the supernatant. The complementary

amount was bound to soil (16.2%-60.9%). Extractable radioactivity was low, i.e. 0.6% and 7.9% for soils III and II and for the other soils no radioactivity could be extracted.

The pH of the aqueous phase was measured before and after contact with the soil at the soil-to-solution ratio of 1:1 Table B.8. 95. The pH of the 0.01 M $CaCl_2$ solution was 5.00. After 48 hours of incubation with $CaCl_2$ solution, the pH of soils I to V, was determined to be 7.20, 8.31, 7.26, 7.83 and 7.34, respectively.

The desorption Kd and Koc values are summarised in Table B.8. 97 below.

Table B.8. 97 Asulam Kd_{des} and K_{desoc} values from a screening test in five soils

Soil	I	II	III	IV	V
Kdesoc (mL/g)	315	157	53	71	50
Mean I-V 129 mL/g					
Kddes (mL/g)	6.7	7.8	0.7	2.8	0.9
Mean I-V 3.8 mL/g					

Advanced test. In the advanced test, soils I to IV were selected. The maximum recommended adsorption and desorption time of 48 hours were selected for the advanced test.
Recovered radioactivity in the control samples was not lower than 98.0%. HPLC analysis showed that the test item was stable in the aqueous phase throughout the 48-hour agitation period. HPLC analysis of the aqueous phase showed that the test item was not stable until the end of the desorption period, except for soil III. The percentages corresponding to asulam were used as correction factors to calculate the K_{FOC} values. For adsorption, the percent of asulam was not lower than 92.8%. For desorption, the percent of asulam in the supernatant varied between 72.6% and 96.5%. For consistency, adsorption and desorption values were corrected.
For all soils, adsorption decreased with increasing test item concentration. At the highest concentration of 0.977 mg/L, 41.1%, 79.9%, 20.6%, and 43.7% of the applied radioactivity was adsorbed to soils I, II, III and IV, respectively. At the lowest concentration, the corresponding values were 84.8%, 89.0%, 55.8% and 72.7% of applied.
The results obtained were evaluated by applying the linear Freundlich equation. The linear adsorption isotherm is graphically presented in Figure B.8. 34 to Figure B.8. 37, the resulting constants K_F, K_{FOC} and 1/n in Table B.8. 98.
Subsequent to 48 hours of adsorption, the desorption test was performed at the same initial test item concentrations as for adsorption. Similar behaviour was observed for the desorption process as for the adsorption process with desorption dependent on test item concentration for all soils. At the highest concentration of 0.977 mg/L, 62.7%, 81.6%, 23.7% and 56.5% of the applied radioactivity remained adsorbed for soils I to V, respectively. At the lowest concentration (0.010 mg/L), the corresponding values were 82.9%, 90.9%, 62.2% and 72.1% of applied for all the soils.
The results obtained were then evaluated by applying the linear Freundlich equation. The resulting constants Kdes, F, Kdes, FOC and 1/n values are presented in Table B.8. 98.

Figure B.8. 34 The Freundlich Adsorption Isotherm for Soil I

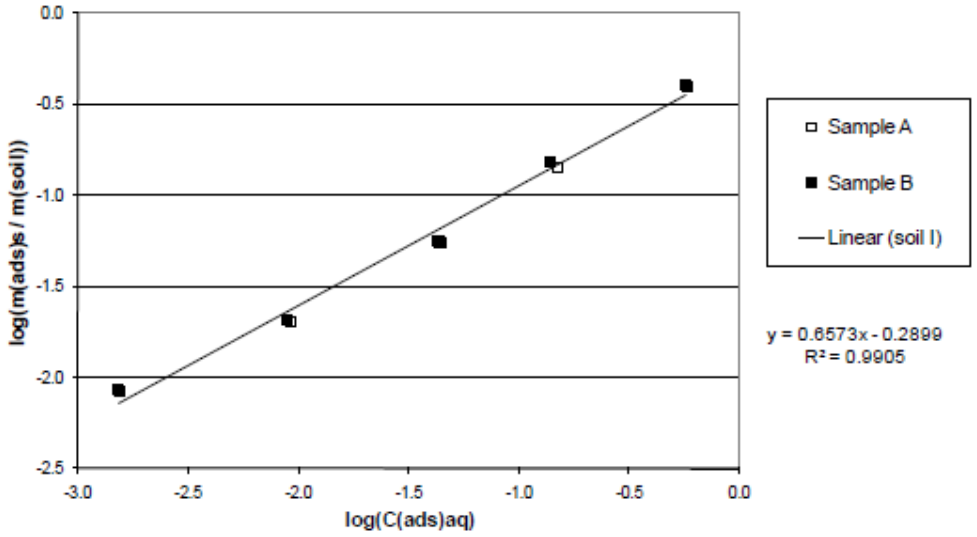


Figure B.8. 35 The Freundlich Adsorption Isotherm for Soil II

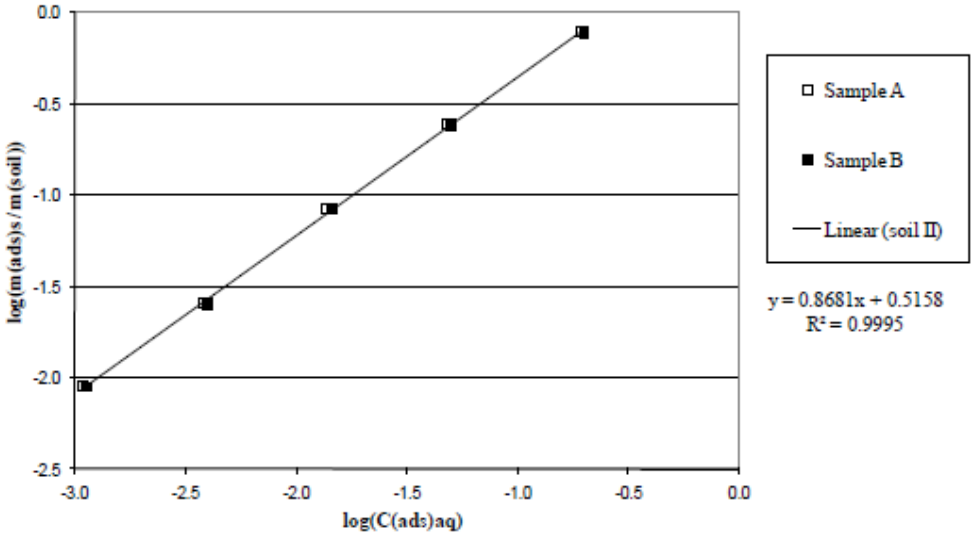


Figure B.8. 36 The Freundlich Adsorption Isotherm for Soil III

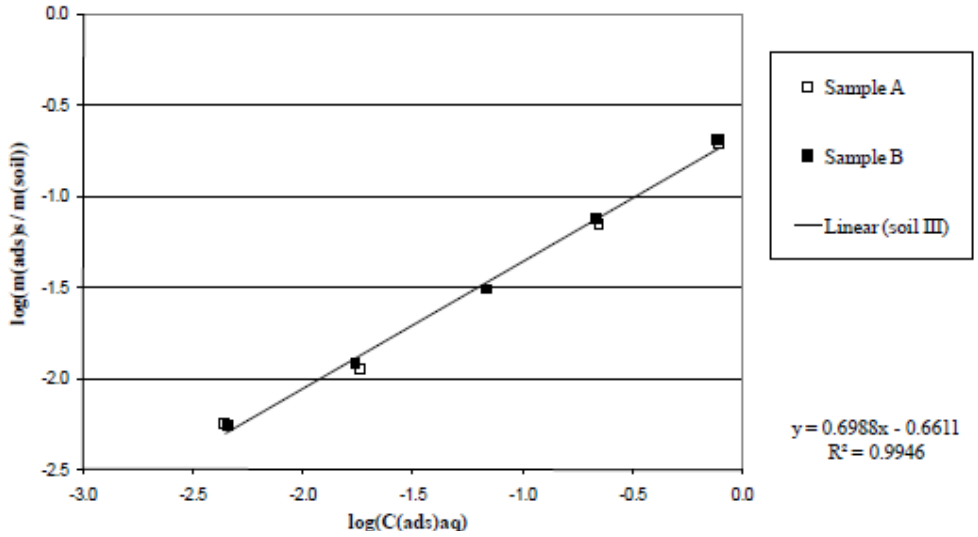


Figure B.8. 37 The Freundlich Adsorption Isotherm for Soil IV

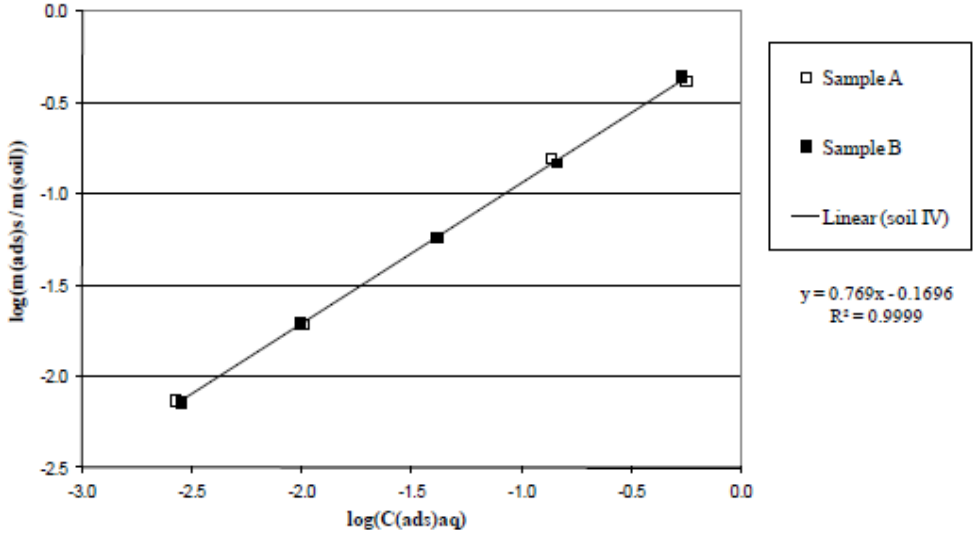


Table B.8. 98 Asulam adsorption/desorption parameters in four soils

Parameter		Soil				Mean
		Am Fischteich	Am Hart-schlösschen	Speyer 2.2	Witterswil	
K_F	(mL/g)	0.513	3.280	0.218	0.677	
K_{FOC}	(mL/g)*	24	67	16	17	31
K_{FOM}	(mL/g)	14	39	9	10	18
1/n		0.66	0.87	0.70	0.77	
r^2		0.9905	0.9995	0.9946	0.9999	
$K_{des, F}$	(mL/g)	6.953	4.975	0.470	3.124	
$K_{des, FOC}$	(mL/g)	328	101	34	79	135
$K_{des, FOM}$	(mL/g)	190	59	19	46	79
1/n		0.97	0.83	0.66	0.94	
r^2		0.9985	0.9995	0.9940	0.9958	

* As presented in report. Recalculation to 1 decimal place for consistency with equivalent study of Lewis gives values of 24.2, 66.5, 15.6 and 17.2

Conclusions:

The mean values for the adsorption and desorption Freundlich coefficients K_{FOC} and Kdes, $_{FOC}$ were 31 mL/g and 135 mL/g, respectively. The higher Freundlich isotherm coefficients for desorption indicated an irreversible sorption process.

(Völkel, 2011a)

RMS opinion on study

The study summary above has been evaluated by the RMS. The study was performed to an acceptable standard and to GLP and in accordance with the guidelines and the study was well reported. Mass balance was acceptable in the range (96.5% to 100.6% AR). The RMS has independently validated the Kf and Kfoc values calculated by the applicant and obtained similar results. The sorption values for asulam presented in Table B.8. 98 above are considered acceptable by the RMS.

This study has previously been evaluated at EU level. The conclusions are consistent with the EFSA conclusions report and no concerns were raised at EU level.

Report:

P. Lowden, N. Mahay (1999a) [^{14}C]-Asulam Sodium: Aged Desorption from Four Soils. Rhone-Poulenc Agriculture Ltd., Unpublished report No.: 201979, C015418

Guidelines:

Modified EU Council Directive 91/414/EEC, as amended by Commission Directive 95/36/EC of July 1995, Section 7, Sub section 7.1.2
Deviations: None

GLP: Yes

b) A batch equilibrium aged desorption study was conducted with asulam sodium in accordance with EC Directive 96/36/EC and OECD guideline 106 (1981) and to GLP.

^{14}C-asulam (radiochemical purity >98.1%, specific activity 740 MBq/mmole), as the sodium salt, in 75 mL 0.01M $CaCl_2$ was added to duplicate samples of 4 UK soils (15g dry weight) at equivalent concentrations of 5, 1, 0.2 and 0.04 mg a.s./L. Soil characteristics are presented in

Table B.8. 99. The desorption of ^{14}C-asulam was investigated following aging of the samples for periods of 0, 2 and 7 days. At the end of each aging period the soil samples were subjected to five successive desorption cycles (each of 24 h duration) with 0.01M $CaCl_2$ solution. Following the final desorption cycle one replicate from each soil at the highest concentration was extracted with acetonitrile:water (1:1 v/v) and aliquots removed for liquid scintillation counting (LSC).

Adsorption of ^{14}C-asulam sodium to glassware was investigated and no significant adsorption was found, the recovery of radioactivity being 97.2 % after 24h.
The stability of asulam sodium during the desorption period was demonstrated by HPLC in which asulam sodium was the only significant peak in chromatograms and that it represented all the desorbable material at all time points.

Overall the recovery of radioactivity was considered by the Rapporteur as acceptable, in the range 88.1 to 99.5%. The majority of recoveries from individual adsorption tubes was in the range 90 to 110% with the exception of 2 outliers (79.6 and 129.8%) both in the lowest concentration group but this was considered not to affect the validity of the study.

The Freundlich desorption coefficients $K_{f\ des}$ and $K_{foc\ des}$ were calculated for each desorption cycle, the values for the first of which are shown in Table B.8. 100. The values of $K_{f\ des}$ and $K_{foc\ des}$ were shown to increase with each successive desorption cycle from the first to the fifth for each aging period in all soils. It was also observed for Day 0, Day2 and Day7 aging phases that the 1/n values for $K_{f\ des}$ varied significantly from 1 for initial desorption but became closer to 1 for successive cycles, indicating a non-linearity for initial desorption stages with increasing linearity at the 4th and 5th desorption stages.

The increases in $K_{f\ des}$ and $K_{foc\ des}$ values with each desorption cycle, particularly at seven days, indicated that there may be different adsorption mechanisms at work, some of which resulted in very strong adsorption of some of the asulam applied to the soil. The effect of the aging period indicated that the potential mobility of asulam may be significantly reduced with time.

Table B.8. 99 Characteristics of soils used in aged desorption study with ^{14}C-asulam (Lowden and Mahay 1999e)

Soil Reference	98/26	98/27	98/28	98/31
Particle Size	%			
500µm - 2mm	3.98	8.78	14.99	15.55
250 - 500µm	6.47	14.43	46.76	27.82
106 - 250µm	10.38	24.93	25.14	17.01
63 - 106µm	4.59	1.10	2.86	2.87
20µ - 63µm	26.32	24.98	1.62	16.88
2 - 20µm	23.14	14.46	2.58	11.74
< 2µm	25.12	11.31	6.04	8.14
TOTAL	100.00	99.99	99.99	100.01
Textural Classification ADAS:				
Sand (63-2000 µm)	25.42	49.24	89.75	63.25
Silt (2-63 µm)	49.46	39.44	4.20	28.62
Clay (<2 µm)	25.12	11.31	6.04	8.14
Type	Clay loam	Sandy silt loam	Sand	Sandy loam
Organic carbon (%)	1.90	3.60	1.60	0.70
Organic matter (%)	3.28	6.21	2.76	1.21
Exchangeable cations				
Ca	7.8	4.5	3.1	2.6
Mg	1.0	0.9	0.1	0.2
Na	0.3	0.2	<0.05	0.1
K	0.8	1.2	0.2	0.2
Mn	0.1	<0.05	<0.05	<0.05
Total (mEq/100g)	10.0	6.8	3.4	3.1
pH in Water	7	6.1	5.8	6
pH in KCl	6.7	5.5	5.4	5.3
pH in $CaCl_2$	6.4	5.7	5.7	6.1

Table B.8. 100 Summary of Freundlich desorption coefficients in four soils after periods of aging for ^{14}C-asulam sodium in the first desorption cycle

Soil reference	Clay Loam 98/26	Sandy Silt Loam 98/27	Sand 98/28	Sandy Loam 98/31	Mean
Organic carbon (%)	1.9	3.6	1.6	0.7	
	0 days aging:				
$K_{f\ des\ 1}$	0.75	0.91	0.40	0.38	0.61
$K_{foc\ des\ 1}$	39	25	25	54	36
1/n	0.326	0.677	0.567	0.599	0.542
correlation	0.803	0.977	0.896	0.936	-
	2 days aging:				
$K_{f\ des\ 1}$	2.31	2.97	1.72	1.11	2.03
$K_{foc\ des\ 1}$	121	82	108	158	117
1/n	0.594	0.663	0.642	0.607	0.627
correlation	0.999	0.998	0.994	0.994	-
	7 days aging:				
$K_{f\ des\ 1}$	7.92	10.28	4.84	3.17	6.55
$K_{foc\ des\ 1}$	417	286	302	453	365
1/n	0.603	0.684	0.611	0.577	0.619
correlation	0.990	0.999	0.998	1.000	-

(Lowden and Mahay 1999e)

Adsorption and desorption of all relevant metabolites, degradation and reaction products

CA 7.4.2/01

This study has previously been evaluated at EU level. The conclusions are consistent with the EFSA conclusions report and no concerns were raised at EU level.

Report:
M.B. Simmonds (2002a) [^{14}C]-Sulphanilamide: Adsorption to and Desorption from Four Soils. Battelle AgriFood Ltd. For Aventis CropScience SA,
Unpublished report No.: CX02022, C026219

Guidelines:
EU95/36/EC, OECD 106
Deviations: None

GLP:
Yes

c) A batch equilibrium adsorption/desorption study was conducted with sulphanilamide in four soils to meet the requirements of Commission Directive 95/36/EC (1995), OECD 106 (1981 and 2000) and SETAC (1995) guidelines. The study was carried out to GLP and was considered acceptable by the Rapporteur.

Ring labelled ^{14}C-sulphanilamide (radiochemical purity >98%, specific activity 7.4 MBq/mg) in 0.01M CaCl2 was added to samples of four UK soils (15 g dry weight) at concentrations of 5, 1, 0.2 and 0.04 mg/L for each soil. Soil characteristics are presented in Table B.8. 101. Prior to treatment, the identity of sulphanilamide was confirmed by mass spectrometry by comparison against a certified reference standard. Treated samples were shaken in the dark at 20°C for 7 days. The equilibrium period was determined in an acceptable preliminary test. The preliminary test also confirmed that sulphanilamide was stable over the duration of the study, that minimal adsorption to glassware occurred and that the soil:water ratio of 1:5 resulted in an acceptable level of adsorption. After equilibration, the supernatant was removed by centrifugation and analysed by LSC. Adsorbed sulphanilamide was calculated as the difference between the amount of applied and that found in the aqueous phase. Following the adsorption phase, five desorption cycles were performed on each soil by re-suspending the soil in fresh 0.01 M CaCl2, equilibrating for 24 hours and analysing the supernatant by LSC. Radioactivity in the soil after the desorption steps was extracted with acetone, the soils were combusted and radioactivity determined by LSC. Selected samples were additionally analysed by HPLC to confirm that sulphanilamide was stable over the duration of the study. Material balance was considered by the Rapporteur to be acceptable with mean values from 97 to 100%.

The adsorption isotherms for each concentration were used to calculate Freundlich coefficients (Kf) and 1/n values for each soil, which are given in Table B.8. 102 for adsorption. Kfoc des values show that adsorption of sulphanilamide correlated with soil organic carbon content but no relationship was observed with soil pH. Correlation coefficients (r2) were acceptable ranging from 0.997 to 0.999. Desorption Kf values ranged from 4.54 to 145.36 and Kfoc values from 300 to 11791, increasing with each successive desorption cycle, indicating that adsorption was only partially reversible.

Table B.8. 101 Characterisation of soils used in an adsorption/desorption study with sulphanilamide (Simmonds, 2002a)

Soil Reference	01/01	01/03	01/07	01/09
Particle size distribution (µm)	%	%	%	%
500-2000	5.07	14.94	13.88	4.46
250-500	6.07	30.27	28.78	2.49
106-250	9.33	21.96	20.32	4.36
63-106	4.78	4.76	3.31	7.30
20-63	28.95	11.08	10.64	14.23
2-20	22.38	6.46	8.34	35.30
<2	23.42	10.53	14.75	31.87
Total	99.99	100.01	100.00	100.00
Textural classification ADAS:	Clay Loam	Sandy Loam	Sandy Loam	Calcareous Silty Clay Loam
Sand (63-2000µm)	27.27	72.70	67.03	19.60
Silt (2-63µm)	49.31	16.77	18.24	48.54
Clay (<2µm)	23.42	10.53	14.75	31.87

Organic Carbon (%) Organic Matter (%)	2.0 3.4	1.0 1.7	2.0 3.4	3.1 5.3
Cation Exchange Capacity (meq/100g)	21.5	3.3	33.5	83.5
pH Water 1M KCl 0.01M $CaCl_2$	7.9 6.9 6.9	5.0 4.0 4.2	7.8 7.6 7.4	8.2 8.0 7.6
Moisture holding capacity (%) Maximum @ 0.1 bar Maximum @ 0.3 bar	53.0 34.4 33.6	25.7 11.1 9.8	58.9 20.5 19.5	77.4 34.9 33.8
Soil Moisture (%)	4.86	5.16	17.27	30.15

Table B.8. 102 Adsorption coefficients for sulphanilamide in 4 UK soils

Soil reference	Organic carbon (%)	pH	K_f	1/n	Correlation coefficient	$K_{foc\ ads}$
Clay Loam (01/01)	2.0	7.9	3.59	0.716	0.997	179
Low pH Sandy Loam (01/03)	1.0	5.0	1.45	0.748	0.999	145
Sandy Loam (01/07)	2.0	7.8	2.48	0.705	0.999	124
Calcareous Silty Clay Loam (01/09)	3.1	8.2	4.07	0.673	0.998	131
		Arithmetic mean		0.711	-	145

(Simmonds, 2002a)

CA 7.4.2/02

This study has not previously been evaluated at EU level. It has been evaluated at member-state level. The conclusions are in line with those of the previously submitted study and the EFSA conclusions report.

Report:
A. Oddy and R. Brett (2008a) [^{14}C]-Sulfanilamide: Adsorption to and Desorption from Four Soils. Battelle AgriFood Ltd. For Aventis CropScience SA,
Unpublished report No.: OU/07/012

Guidelines:
EU95/36/EC, OECD 106
Deviations: None

GLP:
Yes

RMS opinion on study: fully acceptable. Please note the summary below is the applicant's. It is an accurate account of the report and considered acceptable by the RMS.

Executive Summary:
The adsorption/desorption characteristics of sulphanilamide were studied in four soils; a loamy sand, an acidic clay loam, an alkaline clay loam and an acidic loamy sand, using the batch equilibrium method.

The adsorption phase of the study was carried out using pre-equilibrated soils with [^{14}C]-sulphanilamide at concentrations of approximately 2.0, 0.67, 0.2, 0.07 and 0.02 mg L^{-1} in the dark and at 20 ± 2°C for 96 hours for all soils. The equilibration solution used was 0.01M aqueous $CaCl_2$, with a soil/solution ratio of 1:3 for all soils. The desorption phase of the study was carried out for 24 hours with fresh 0.01M aqueous $CaCl_2$ applied to pre-adsorbed soil for one desorption cycle.

The aqueous supernatant after adsorption and desorption was separated by centrifugation and analysed by LSC. After desorption, the soil was extracted with acetone. After extraction, the soil was combusted and the trapped CO_2 analysed by LSC. The adsorption parameters were calculated using the Freundlich isotherm. Sulphanilamide was shown to be stable for the duration of the definitive study for all soils.

The overall material balances in the definitive study were determined by LSC of the supernatants after adsorption, desorption and solvent extraction and combustion of the remaining soils. The overall material balance for individual samples was in the range of 93.2-97.9% for the LUFA Speyer 2.2 loamy sand (mean 95.2%), 92.9-96.3% for the Further Field clay loam (mean 95.1%), 93.7-95.3% for the Flint Hall clay loam (mean 94.6%) and 94.4-98.8% for the Warsop A1 acidic loamy sand (mean 96.3%).
In the definitive adsorption test, the amount of applied test material adsorbed ranged from 45.1 to 79.8% in the LUFA Speyer 2.2 loamy sand, 59.6 to 88.9% in the Further Field clay loam, 41.4 to 76.0% in the Flint Hall clay loam and 40.2 to 80.2% in the Warsop A1 loamy sand.

The calculated adsorption constants K_f of the Freundlich isotherms for the four test soils ranged from 2.20 to 4.44. A high degree of non-linearity was shown by the values of 1/n which ranged from 0.681 to 0.723 indicating very specific interactions and saturation of the adsorption sites. This was also demonstrated in the recovery of radioactivity in the supernatants where the proportion of radioactivity recovered in the adsorption supernatant was significantly lower at lower application rates. The adsorption K_{oc} values ranged from 98 to 278.

At the end of the desorption phase, the amount of test material desorbed, expressed as a percentage of the initial amount adsorbed, ranged from 14.6 to 6.9% for the in the LUFA Speyer 2.2 loamy sand, 8.4 to 4.0% in the Further Field clay loam, 12.5 to 6.0% in the Flint Hall clay loam and 12.9 to 5.7% in the Warsop A1 loamy sand.
The desorption K_{des} values ranged from 6.05 to 10.93 and were thus higher than the K_f values obtained in the adsorption phase, indicating stronger binding once adsorbed to soil.

Table B.8. 103 Key study data for sulphanilamide adsorption/desorption in four soils

Soil	LUFA Speyer 2.2 (07-022)	Further Field (07-024)	Flint Hall (07-028)	Warsop A1 (07-030)
Texture (USDA)	Loamy sand	Clay loam	Clay loam	Loamy sand
pH 0.01M $CaCl_2$	5.2	6.1	7.3	4.2
Organic Carbon (%)	1.9	1.6	2.5	0.9
K_f (mL g^{-1})	2.55	4.44	2.39	2.20
K_{oc} (mL g^{-1})	134	278	96	244
1/n (adsorption)	0.712	0.708	0.723	0.681
Correlation (R^2)	0.998	0.998	0.997	0.999
K_{des} (mL g^{-1})	6.09	10.93	6.05	6.33
K_{ocdes} (mL g^{-1})	321	683	242	703
Correlation (r^2)	0.997	0.997	0.996	0.998

The mean determined K_{oc} was 188 and the mean $K_{oc\ des}$ was 487. Thus, according to the McCall classification, sulphanilamide can be classified as being of medium mobility in soil. Once adsorbed, sulphanilamide will be less readily desorbed. Consequently, movement in the field is expected to be less than predicted by the adsorption data generated in this study.

Materials and Methods

Test Material:

[^{14}C]-Sulphanilamide, radiochemical purity < 99%, Batch no.: 2415MFO001-1

Test Design:

The adsorption/desorption characteristics of [^{14}C]-sulphanilamide in four contrasting UK agricultural soils was investigated. The soils were: LUFA Speyer 2.2 loamy sand (Germany, pH 5.2, 1.9% organic carbon), Further Field clay loam (UK, pH 6.1, 1.6% organic carbon), Flint Hall clay loam (UK, pH 7.3, 2.5% organic carbon) and Warsop A1 acidic loamy sand (UK, pH 4.2, 0.9% organic carbon). The soil characteristics are presented in below.

The adsorption/desorption tubes used throughout the study were thick walled borosilicate glass tubes with an external plastic coating. The tubes were approximately 125 mL capacity and sealed with a screw cap, which was lined with a Teflon seal. All tubes were accurately weighed before and after addition of soil (where appropriate) and then after addition of calcium chloride ($CaCl_2$) solution. Each tube was uniquely labelled.

The concentration of calcium chloride ($CaCl_2$) solution used throughout the study was 0.01 M and the solutions were all prepared using the dihydrate. The pH values of all solutions were measured before use. All solutions were shaken in the dark, on an end-over-end shaker (unless otherwise specified), at a temperature of 20 ± 2°C. Centrifugation was performed at 2000 rpm (830 RCF) and 20°C.

The test item, nominally 7.0 mg of [^{14}C]-sulphanilamide, was dissolved in 10 mL of water. A dilution of this stock solution was made by removing 0.1 mL and diluting to 10 mL with deionised water. Aliquots of this dilution were removed and counted by LSC to determine the exact concentration of the stock solution. The concentration of the stock solution was determined to be 0.68 mg/mL.

Treatment solutions for each phase of the preliminary and definitive studies were prepared by removing a suitable volume of the stock solution and diluting to the required volume with 0.01 M calcium chloride solution. The treatment solutions were prepared to ensure that the organic solvent content in any phase of the study would not exceed 0.1%.

Table B.8. 104 Soil characteristics

Soil Reference Batch ID	LUFA Speyer 2.2 07/022	Further Field 07/024	Flint Hall 07/028	Warsop A1 07/030
Source Geographic Location	LUFA, Grober Striet, Hanhofen, Rheinland-Pfalz, Germany	Boarded Barns Farm, Ongar, Essex, UK	Flint Hall, Royston, Herts UK	Site A1, Warsop, Nottinghamshire, UK
OS Map Reference	n/a	TL557047	TL365397	SK566696
Textural classification (USDA) Sand % (50-2000 µm) Silt % (2-50 µm) Clay % (<2 µm)	Loamy sand 83 8 9	Clay loam 22 47 31	Clay loam 26 40 34	Loamy sand 87 6 7
Textural classification (ADAS) Sand % (63-2000 µm) Silt % (2-63 µm) Clay % (<2 µm)	Loamy sand 84 8 8	Silty clay loam 19 50 31	Clay / Clay loam 23 42 35	Loamy sand 86 6 8
pH Deionised Water 0.01M $CaCl_2$ 1M KCl	5.8 5.2 5.2	6.7 6.1 5.8	8.2 7.3 7.3	5.1 4.2 3.9
Organic Carbon %	1.9	1.6	2.5	0.9
Organic Matter %	3.3	2.7	4.4	1.5
Cation Exchange Capacity (meq/100g)	7.7	17.1	15.8	5.3
% Soil Moisture	12.2	7.1	17.2	5.1
Water holding capacity pF 2.0 – WHC 0.1 bar pF 2.5 – WHC 0.33 bar	19.7 10.4	28.5 27.8	31.3 27.8	6.5 6.7
Bulk Density (fresh g/L)	980	854	802	1176

Aliquots (1 mL) of the prepared treatment solutions of [^{14}C]-sulphanilamide were dispensed using a calibrated pipette into sample tubes containing a pre-equilibrated slurry of soil and calcium chloride solution to achieve the target ratio and nominal concentrations as described in the following paragraphs. The exception was the preliminary experiment to measure adsorption to tubes, where the appropriate treatment solution was weighed directly into the tubes.
The dpm and µg applied to each tube were determined by performing dose checks during treatment. Aliquots of 1 mL of the appropriate treatment solution were applied directly to scintillation vials and counted by LSC.

Preliminary tests. Preliminary studies were carried out to check for adsorption to the tubes, to determine any background radioactivity in the soil, to determine the soil:

solution ratio to be used and to determine the time required for the compound to equilibrate between soil and water under both adsorption and desorption conditions. To check the adsorption of the test material to the tubes a 15 µL aliquot of the stock solution of [^{14}C]-sulphanilamide was added to approximately 500 mL of calcium chloride solution to give a treatment solution at a concentration of 0.02 mg/L. Aliquots of this treatment solution were weighed for analysis by LSC. Approximately 75 mL of the treatment solution was added to two tubes and the tubes were tightly capped, weighed and shaken for approximately 24 hours. Further aliquots of the solutions were then removed for analysis by LSC.

To determine the soil background radioactivity approximately 10 g oven dried equivalent (ode) of each soil was added to each of two tubes. Approximately 50 mL of calcium chloride solution was added to each tube and these were shaken for 24 hours. The tubes were then removed, weighed and centrifuged for 10 minutes. Accurately weighed aliquots of the supernatant were analysed by LSC.

To determine the optimal soil/solution ratio a treatment solution was prepared to allow treatment at a nominal concentration of 0.67 mg L^{-1} by diluting 0.99 mL of the stock solution to 25 mL with calcium chloride solution. For each soil, 4, 8 and 13.3 g oven dried equivalent (ode) portions of soil were added to single, uniquely numbered adsorption/desorption tubes. An appropriate volume of calcium chloride solution (39 mL less the moisture content of the soil) was added to each tube to give soil: solution ratios of approximately 1:10, 1:5 and 1:3 and the tubes were shaken overnight to pre-equilibrate (*ca* 16 hours) prior to treatment. Following pre-equilibration, 1 mL of the treatment solution was added to each tube. The tubes were capped tightly, shaken by hand to suspend the soil and then shaken for 24 hours. The tubes were then removed, centrifuged for 10 minutes, the supernatant decanted and the tubes reweighed. Weighed aliquots of the supernatant were taken for analysis by LSC.

To determine the adsorption equilibrium time a treatment solution was prepared to allow treatment at a nominal concentration of 0.67 mg/L by diluting 1.59 mL of the stock solution to 40 mL with calcium chloride solution. Six portions, weighing approximately 13.3 g ode, of each soil were weighed into uniquely numbered tubes. Calcium chloride solution (39 mL less the moisture content of the soil) was added and the mixture was shaken overnight to pre-equilibrate prior to treatment. Following pre-equilibration, 1 mL of the treatment solution was added to each tube. The tubes were shaken by hand to suspend the soil before being placed on an end-over-end shaker. One tube from each soil type was removed after 1, 4, 24, 48, 72, and 96 hours. At each time point, the tubes were centrifuged for 10 minutes, the supernatants removed and the tubes reweighed. Weighed aliquots of the supernatant were taken for analysis by LSC.

Following the adsorption phase, three sequential solvent extractions were carried out by adding *ca* 40 mL of acetone to each tube. The tubes were shaken to re-suspend the soil, weighed and placed back on the end-over-end shaker for *ca* 30 minutes. Following the addition of acetone in the final extraction step, the tubes were first placed in a sonic bath at 70ºC for *ca.* 30 minutes before replacing on the end-over-end shaker for a further 30 minutes. The tubes were then removed, re-weighed, centrifuged for 10 minutes, the supernatants removed and the tube re-weighed. Aliquots of each supernatant were weighed and the radioactivity determined by LSC.

All of the adsorption supernatants and solvent extracts (extracts 1-3 combined) were analysed by HPLC.

For determination of desorption equilibrium time further tubes (6 per soil) were prepared and treated as for the preliminary adsorption equilibrium time determination as described above. The tubes were shaken for 96 hours. The tubes were centrifuged for 10 minutes, the supernatants were removed by decantation and the tubes reweighed. Approximately 40 mL of fresh calcium chloride solution was added to each tube, which was re-capped, shaken by hand to re-suspend the soil, reweighed and then placed on an end-over-end shaker. One tube was removed for each soil after 1, 4, 24, 48, 72, and 96 hours. At each time point, the tubes were weighed, centrifuged for 10 minutes, the supernatants removed and the tubes reweighed. Aliquots of each supernatant were weighed and the radioactivity determined by LSC.
Selected adsorption supernatants, desorption supernatants and soil extracts were analysed by HPLC.

Definitive tests. Treatment solutions were prepared by dispensing 2.96 mL, 0.99 mL, 296 µL, 104 µL and 30 µL of the stock solution and diluting to 25 mL with calcium chloride. Uniquely labelled duplicate tubes were prepared for each soil at each of five concentrations. Approximately 13.3 g ode portions of each soil were weighed into pre-weighed tubes. The tubes were capped and re-weighed. Calcium chloride solution (37-38 mL depending on soil moisture) was added and the mixture was shaken for *ca* 16 hours to pre-equilibrate prior to treatment. Following pre-equilibration, 1 mL of the appropriate treatment solution, to allow treatment at nominal concentrations of 2.0, 0.67, 0.2, 0.07 and 0.02 mg/L [^{14}C]-sulphanilamide, was added to each tube. The soil: solutions were mixed and slurried for 96 hours on an end-over-end shaker. The tubes were removed from the shaker, weighed and centrifuged for 10 minutes. The supernatant solutions were removed by decantation and the tubes containing the soil pellets were reweighed. Aliquots of each supernatant were weighed and the radioactivity determined by LSC. An aliquot of the adsorption supernatant from selected samples was analysed by HPLC.

Following removal of the adsorption supernatant *ca* 40 mL of fresh calcium chloride solution was added to each tube, which was capped and weighed. Each tube was shaken by hand to re-suspend the soil and placed on an end-over-end shaker. After 24 hours, the tubes were removed, weighed and centrifuged for 10 minutes. The supernatant (desorbate 1) was removed and the tubes were reweighed. Aliquots of each supernatant were weighed and the radioactivity determined by LSC. An aliquot of the desorption supernatant from selected samples was analysed by HPLC.
Following the desorption cycle, all tubes were solvent extracted. Approximately 40 mL of acetone was added to each tube and the tubes were weighed and shaken to re-suspend the soil. The tubes were placed on the end-over-end shaker for 30 minutes, then removed, reweighed, centrifuged for 10 minutes and the supernatants decanted. Following removal of the supernatant, the tube and soil pellet were weighed to enable the weight of each supernatant to be calculated. Aliquots of each supernatant were weighed and the radioactivity determined by LSC. An aliquot of the solvent extract from selected samples was analysed by HPLC.

All supernatants, extracts and soil residues were assayed by LSC to determine the total recovery of radioactivity. The mass balance was calculated by comparison to the

applied radioactivity as determined from the appropriate dose check. Supernatants and extracts from the preliminary and definitive experiments were analysed by HPLC to confirm the stability of sulphanilamide in the test system. The HPLC system comprised a Kromasil KR100 5C1 250 x 4.6 mm i.d. column linked to a UV detector (270 nm) and a radiodetector fitted with a liquid cell. The mobile phase was water/acetonitrile (95:5 v/v) + 5mL/L formic acid.

To verify that all radioactive material injected onto the HPLC column was eluted, column recoveries were carried out for two samples, a 96 hour adsorption supernatant and the corresponding desorption supernatant. Representative 100 µL injections of these samples were made onto the HPLC column and the eluates collected in volumetric flasks (25 mL). Aliquots of the HPLC eluates and 200 µL injections collected directly from the autosampler were assayed for radioactivity by LSC.
All samples analysed during the study were profiled by HPLC within 2 days of their generation for the adsorption supernatants and within 12 days of their generation for the soil extracts. Analysis of the samples that had been stored for 12 days showed only parent material and therefore storage up to 12 days was acceptable. Samples were stored at < -15°C in the dark. The limit of quantification for the [^{14}C] HPLC method was derived from the magnitude of a peak that could be reliably identified in a chromatogram having a magnitude at least 2-3 times that of random events occurring in the detector (i.e. signal/ noise ratio ≥ 2). A chromatogram of an adsorption supernatant had such a peak that represented 0.0047 µg/g (ca 0.005 mg/L) of sulphanilamide. This chromatogram conforms to the criteria as set out above and thus an LOQ of 0.005 mg/L was achieved for the HPLC analysis in this study.

Results and Discussion
Preliminary tests.
The test systems were maintained in the dark and at a temperature of 20 ± 2°C throughout the study. The pH of the calcium chloride solutions used during this study was found to be in the range 7.0 to 7.1. Analysis of the samples that had been stored for 12 days showed only parent material and therefore no indication of instability in any sample within the study. Recovery of radioactivity from the tubes was quantitative with a mean recovery of 100.4%. No correction for adsorption to glassware was therefore required. Similarly the level of background radioactivity detected was negligible in all soils and therefore no correction was necessary.

At the soil/solution ratio of 1:5 two of the soils, LUFA Speyer 2.2 loamy sand and Further Field clay loam the acceptable range of 20 to 80% adsorbed was achieved, while the remaining soils, Flint Hall clay loam and Warsop A1 loamy sand achieved *ca* 19% and 16% adsorption to the soil, respectively. At the soil: solution ratio of 1:3, all of the soils achieved the acceptable range of 20 to 80% adsorbed. A ratio of 1:3 was therefore selected for each soil in the definitive phase.

In all four soils there was a gradual decrease in the quantity of radioactivity in the supernatants, without any sign of a genuine equilibrium up to the extended period of 4 days (96 hours). As HPLC analysis indicated compound stability at this time and for reasons of practicality, an adsorption equilibrium time of 4 days was selected. For desorption, there was very little change in the levels of radioactivity in solution between 1 hour and 48 hours in any of the soils tested. A desorption time of 24 hours for all soils was selected for the definitive study.

Definitive tests.
The recovery of radioactivity was quantitative, with all recoveries within the acceptable range of 90-110% of applied radioactivity. The overall material balance for individual samples was in the range of 93.2-97.9% for the LUFA Speyer 2.2 loamy sand (mean 95.2%), 92.9-96.3% for the Further Field clay loam (mean 95.1%), 93.7-95.3% for the Flint Hall clay loam (mean 94.6%) and 94.4-98.8% for the Warsop A1 acidic loamy sand (mean 96.3%).

The calculated adsorption coefficients and constants are shown in Table B.8. 105.

Table B.8. 105 Freundlich constants for sulphanilamide in four soils

Soil	Adsorption				Desorption			
	Kf (mL/g)	1/n	R^2	Koc (mL/g)	Kf (mL/g)	1/n	R^2	Koc (mL/g)
LUFA Speyer 2.2	2.55	0.712	0.998	134	6.09	0.747	0.997	321
Further Field	4.44	0.708	0.998	278	10.93	0.758	0.997	683
Flint Hall	2.39	0.723	0.997	96	6.05	0.748	0.996	242
Warsop A1	2.20	0.681	0.999	244	6.33	0.726	0.998	703
Mean	2.90	0.706	0.998	188	7.35	0.745	0.997	487

In the definitive adsorption test, the amount of applied test material adsorbed ranged from 45.1 to 79.8% in the LUFA Speyer 2.2 loamy sand, 59.6 to 88.9% in the Further Field clay loam, 41.4 to 76.0% in the Flint Hall clay loam and 40.2 to 80.2% in the Warsop A1 loamy sand.

For all soils the fit of log Cs1 *vs.* log Cw1 to a linear equation, was good with correlation coefficients of 0.997 or higher. The K_F values ranged from 2.20 in the Warsop A1 loamy sand to 4.44 in the Further Field clay loam.

The high degree of non-linearity as shown by the values of 1/n which ranged from 0.681 to 0.723 demonstrated very specific interactions and saturation of the adsorption sites. This was also clearly demonstrated in the recovery of radioactivity in the supernatants where the proportion of radioactivity recovered in the adsorption supernatant was significantly lower at lower application rates.

The values for Koc ranged from 96 in the Flint Hall clay loam to 278 in the Further Field clay loam, with a mean value of 188, indicating that sulphanilamide would be classified as ‘medium to high’ mobility in soil, according to the McCall classification. At the end of the desorption phase, the amount of test material desorbed, expressed as a percentage of the initial amount adsorbed, ranged from 14.6 to 6.9% in the LUFA Speyer 2.2 loamy sand, 8.4 to 4.0% in the Further Field clay loam, 12.5 to 6.0% in the Flint Hall clay loam and 12.9 to 5.7% in the Warsop A1 loamy sand.
K_{des} values obtained ranged from 6.05 in the Flint Hall clay loam to 10.93 in the Further Field clay loam. The values of 1/n for the desorption cycle were very similar to those obtained for the adsorption for each soil.

The desorption isotherms did not follow the adsorption isotherm in any soil, showing a clear hysteresis in the adsorption/desorption curve, suggesting that once adsorbed the sulphanilamide would be less readily desorbed. This was also demonstrated from the recovery in the supernatants, where a significant portion of the adsorbed radioactivity remained on the soil at the end of the desorption cycle. The pattern of the desorption isotherms suggests that the adsorption is therefore only partially reversible in all the soils.

The Kocdes values ranged from 242 in the Flint Hall clay loam to 703 in the low pH Warsop A1 loamy sand, with a mean of 487. These values were 2.4 to 2.9 times greater than the K_{oc} for adsorption indicating that, once adsorbed, sulphanilamide was less easy to desorb.

The radiopurity of the treatment solution was determined as 100.0% by HPLC. The identity was confirmed to be sulphanilamide by mass spectroscopy in comparison with the certified reference standard. Aqueous supernatants and solvent extracts were examined by HPLC. Sulphanilamide accounted for 100% of radioactivity in the HPLC radiochromatograms of all adsorption supernatants, desorption supernatants and solvent extracts analysed from all phases of the study. It was concluded that no significant degradation of sulphanilamide occurred over the duration of the study. No adjustment to the adsorption or desorption coefficients was therefore necessary.

Conclusions:
The K_{oc} values obtained ranged from 96 to 278 (mean 188). The Freundlich exponents displayed non-linearity with 1/n values ranging from 0.68 to 0.72, thus indicating an increased degree of adsorption at lower concentrations. The K_{ocdes} values ranged from 242 to 703 (mean 487). For all soils the K_{ocdes} values were higher than the corresponding adsorption K_{oc} value, indicating that once adsorbed to soil, sulphanilamide was harder to desorb.

The determined K_{oc} values indicate that sulphanilamide can be classified as medium mobility in soil. Once adsorbed, sulphanilamide will be less readily desorbed.

This study has not previously been evaluated at EU level. It has been evaluated at member-state level. The conclusions are in line with those of the previously submitted study and the EFSA conclusions report.

Report

Ir. H. Willems, (1998) Adsorption/Desorption of Methyl-benzene-sulfonyl-carbamate on Soil. NOTOX B.V., 's-Hertogenbosch, The Netherlands, Unpublished report No.: NOTOX project: 241403

Guidelines:

European Economic Community (EEC), Commission Directive 95/36/EC amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market. EEC publication no L172/8. 1995; Dutch Board for the Authorisation of

Agrochemicals (CTB). G.1.2 : Data on the mobility of the active ingredient and/or formulation and major metabolites in soil. 1995; OECD Guidelines for Testing of Chemicals. OECD 106 : Adsorption/desorption using a batch equilibrium method. October 1997 draft; US-EPA Pesticide Assessment Guidelines, Subdivision N : 163-1. Leaching and adsorption/desorption studies. 1982.

Deviations: None

GLP:

Yes

RMS opinion on study: fully acceptable.

A batch equilibrium adsorption/desorption study was conducted with methyl-benzene-sulfonyl-carbamate (MBSC) in three soils to meet the requirements of Commission Directive 95/36/EC (1995). The study was carried out to GLP and was considered acceptable by the Rapporteur.

Ring labelled ^{14}C- methyl-benzene-sulfonyl-carbamate (radiochemical purity 97.2%, specific activity 740 MBq/mmol) in 0.01M CaCl2 was added to samples of three European soils at an initial nominal concentration of 1.0, µg/L for each soil. Soil characteristics are presented in Table B.8. 106.

Table B.8. 106 Soil characteristics

Soil Reference Batch ID	Speyer 2.1 F13197	Speyer 2.2 F21995	Marknesse MARK 010492
Source Geographic Location	Rheinland-Pfalz / Rheinzabern	Rheinland-Pfalz / Hanhofen	Noordoost polder, Marknesse, The Netherlands
Textural classification (USDA) Sand % (50-2000 µm) Silt % (2-50 µm) Clay % (<2 µm)	Sand 89.5 9.3 2.5	Loamy sand 79.02 13.95 7.03	Silt loam 15.5 58.1 26.4
pH Water $CaCl_2$ KCl	Not determined 6.0 Not determined	Not determined 5.6 Not determined	Not determined 7.2 7.5
Organic Carbon %	0.59	2.32	2.5
Organic Matter %	1.02	3.99	4.4
Cation Exchange Capacity (meq/100g)	4	10.9	15.8
Water holding capacity (%) pF 2.5 – WHC 0.33 bar pF 2.0 – WHC 15 bar	29 Not determined Not determined	48 Not determined Not determined	Not determined Not determined Not determined

The treated soil samples were placed in 50 mL polypropylene tubes plus approximately 22.5 mL of 0.01 M CaCl2 solution and placed on a shaker overnight for pre-equilibration. The amounts of soil used, soil/solution ratio concentration and sampling times for the adsorption and desorption is presented in Table B.8. 107 below.

Table B.8. 107 Experimental details adsorption-desorption kinetics

Soil	Dry weight (g)	Initial conc. (µg/mL)	Soil:solution ratio	Sampling times adsorption (hr)	Sampling times desorption (hr)
Speyer 2.1	24.88	1.07	1:1	4, 7, 24, 48	-
	24.86	1.07	1:1	4, 7, 24, 48	-
Speyer 2.2	24.69	1.01	1:1	4, 7, 24, 48	-
	24.64	1.01	1:1	4, 7, 24, 48	2.75, 5, 21, 27.5
Marknesse	24.37	1.0	1:1	4, 7, 24, 48	-
	24.38	1.0	1:1	4, 7, 24, 48	2.75, 5, 21, 27.5

For each soil, a blank was included containing 0.01 M $CaCl_2$ without test substance. Two controls were also included with 0.01 M $CaCl_2$ with labelled methyl-benzene-sulfonyl-carbamate without soil to evaluate the adsorption to the centrifuge tube and stability of the test substance. No significant adsorption was found with contact for up to 48 h with recovery of radioactivity between 99 and 101% AR. The stability of the test substance (90 – 100%) was demonstrated by the TLC chromatograms of the supernatants at the end of the experiments.

The centrifuge tubes were sealed and placed on a shaker at 20 ± 2°C. At the sampling times in Table B.8. 107 the samples were removed and centrifuged at 3100 g for 5 min at 20°C. the radioactivity in the supernatant was determined by LSC. Activity in the blanks and controls was similarly determined by LSC. The adsorption phase was conducted in duplicate for each soil.

At the end of the adsorption phase, the supernatant was removed and replaced with an equal volume of fresh 0.01 M $CaCl_2$. The desorption phase was conducted on one sample of the Speyer 2.2 and Marknesse soils. The other sample was used for the determination of the mass balance in the adsorption phase. Desorption of the Speyer 2.1 soil was not determined as no adsorption took place.

The results of the adsorption are presented in Table B.8. 108. It is noted by the RMS that adsorption of methyl-benzene-sulfonyl-carbamate is very low (1.7 – 8.0%) equilibrium being established after 48 h. Due to the low adsorption, no adsorption-desorption isotherms were determined. Consequently it was not possible to determine the Freundlich adsorption coefficient, the adsorption being determined at only a single concentration. The results for the desorption phase are presented in Table B.8. 109 although, due to the low adsorption of methyl-benzene-sulfonyl-carbamate, the desorption is somewhat questionable. Desorption was not conducted on Speyer 2.1 soil.

Table B.8. 108 Results of adsorption experiment

Soil	% adsorbed [1]			
	2.75 h	7 h	24 h	48 h
Speyer 2.1	0.00	1.41	1.58	1.68
Speyer 2.2	6.24	6.28	7.05	8.01
Marknesse	1.13	1.77	1.50	2.28

[1] mean of duplicate samples

Table B.8. 109 Results of the desorption phase

Soil	% desorbed			
	2.75 h	5 h	21 h	27.5 h
Speyer 2.1	-	-	-	-
Speyer 2.2	3.41	16.30	6.06	9.36
Marknesse	0.00	0.00	0.00	0.00

The adsorption values at equilibrium (kd, koc and kom) are presented in Table B.8. 110.

Table B.8. 110 Adsorption parameters for methyl-benzene-sulfonyl-carbamate adsorption on soil

Soil	Kd (mL/g)	Kom (mL/g)	Koc (mL/g)
Speyer 2.1	0.015	1.70	2.93
Speyer 2.2	0.090	2.25	3.88
Marknesse	0.025	0.89	1.53
	Arithmetic mean	1.61	2.78

Methyl-benzene-sulfonyl-carbamate is considered highly mobile in soil.

(Willems, 1998)

B.8.2.2 Column leaching

This study has previously been evaluated at EU level. The conclusions are consistent with the EFSA conclusions report and no concerns were raised at EU level.

Report:
G.L. Reeves, D.A. England, E.A. Savage (1988a) Herbicides: Asulam-[^{14}C]: Leaching Study with Four Soils. Rhone-Poulenc Agriculture Ltd, Ongar, Essex, England
Unpublished report No.: D.Ag. 1118

Guidelines:
USEPA (= EPA) 163-1
Deviations: None

GLP: Yes

a) A column leaching study was performed to satisfy EPA Guidelines, Pesticide Assessment Guidelines, Subdivision N, Series 163-1 (1982). The study was carried out to GLP and was considered acceptable by the Rapporteur.

Duplicate segmented 30 cm glass columns were packed with four different soils (dried and 2 mm sieved), a sand, a clay loam, a sandy clay loam and a sandy loam, and pre-wetted with water. The soil characteristics are presented in Table B.8. 111 below.

Table B.8. 111 Characterisation of soils used in a column leaching study with ^{14}C-asulam sodium (Reeves, England and Savage 1988a)

Texture classification	Sand	Clay Loam	Sandy Clay Loam	Sandy Loam
Origin	Icklingham Suffolk England	Tawney Common Essex England	Dunmow Essex England	Manningtree Essex England
% Sand	92	48	57	68
% Silt	2	27	24	22
% Clay	6	25	19	10
% Organic Matter	0.84	3.56	2.58	2.19
% Organic Carbon	0.48	2.07	1.50	1.27
pH (water)	7.23	7.26	7.10	7.11
Cation exchange capacity (meq/100 g)	4.2	23.8	16.3	11.8
Maximum moisture holding Capacity	19.1	29.1	24.7	24.6
Moisture holding capacity (%at 0.33 bar)	13.1	28.1	23.4	19.9
Bulk density	1.73	1.24	1.28	1.41
Microbial counts (per g soil):				
Bacteria	2.0×10^7	5.0×10^8	2.9×10^7	3.4×10^7
Fungi	1.9×10^4	2.1×10^4	3.1×10^4	1.8×10^4
Actinomycetes	3.7×10^5	7.0×10^5	6.4×10^5	4.9×10^5

Stock solutions of ring labelled ^{14}C-asulam (radiochemical purity >98%, specific activity 12.5mCi/mmole were prepared in aqueous solution in equimolar sodium hydroxide to form the sodium salt and dosed onto each column at a rate equivalent to 4.65 kg/ha. Columns were then eluted with deionized water (576 mL) at a rate which did not exceed its rate of infiltration at the soil surface and the leachate collected. Radioactivity in the leachate was quantified by LSC and analysed by HPLC.

Following elution, all columns were sectioned into 6 cm sections and soil was extracted firstly with acetone/water followed by re-extraction twice with acetone. The extracts were combined and aliquots analysed by LSC. The solvent extracts were not analysed further as there was insufficient radioactivity. Unextracted soil radioactivity was quantified by combustion and LSC.

Mean recovery of radioactivity following elution is summarised in Table B.8. 112. Total recovery was acceptable within the range 90-100% AR. Only one significant radioactive component was indicated to be asulam.

Table B.8. 112 Distribution of radioactivity in a column leaching study on four soil types.

Soil	% applied radioactivity recovered in:						
	Leachate	Soil sections (cm)					Total
		0-6	6-12	12-18	18-24	24-30	
Sand:							
Column A	98.38	0.10	0.08	0.05	0.06	0.09	98.76
Column B	97.32	0.20	0.11	0.09	0.07	0.11	97.89
Clay Loam:							
Column A	95.69	0.36	0.25	0.36	0.45	0.58	97.70
Column B	93.25	0.22	0.15	0.18	0.22	0.47	94.50
Sandy Clay Loam:							
Column A	88.72	0.76	0.67	1.06	0.90	1.62	93.74
Column B	92.21	1.02	0.39	0.40	0.39	0.88	95.40
Sandy Loam:							
Column A	91.35	0.75	0.67	0.48	0.66	0.82	94.73
Column B	92.31	0.69	0.49	0.28	0.29	1.37	95.44

(Reeves, England and Savage 1988a)

b) An aged column leaching study was performed to satisfy EPA Guidelines (Pesticide Assessment Guidelines, Subdivision N, Series 163-1).

Duplicate segmented 30 cm glass columns were packed with four different soils (dried and 2 mm sieved: a sand, a clay loam, a sandy clay loam and a sandy loam) and pre-wetted with water. The soil characteristics are presented in Table B.8. 111 above.

Stock solutions of ring labelled ^{14}C-asulam (radiochemical purity >98%, specific activity 12.5mCi/mmole) were prepared in aqueous solution in equimolar sodium hydroxide to form the sodium salt. A sample of each partially air-dried soil was adjusted to 75% of the 0.33 bar moisture holding capacity and dosed at a rate equivalent to 4.4 kg/ha. The treated soils were then incubated in the dark at 20 ± 2°C under aerobic conditions.

Leaching columns were prepared but with a portion (approximately 20g) of soil removed from the top of each. This was replaced by 20g air–dried equivalent of the treated soil which had been incubated for approximately one half-life (7 days for sandy clay loam and sandy loam, 10 days for clay loam and 31 days for sand) (see Table B.8. 113). Columns were then eluted with distilled water (576 mL) at a rate which did not exceed its rate of infiltration at the soil surface and the leachate collected. Soil columns were divided into 6 cm layers and radioactivity quantified by LSC following solvent extraction (acetone/water followed by acetone) followed by combustion and LSC to determine bound residues. Radioactivity in the leachate was quantified by LSC and analysed by HPLC.

The mean recovery of radioactivity from the incubated soils is shown in Table B.8.37. The decrease in total recovery of radioactivity from soil samples with time is attributed to loss of asulam or its metabolites as volatiles or CO_2.

Mean recovery of radioactivity following elution is summarised in Table B.8. 114. HPLC and TLC analysis showed that the radioactivity found in the leachate was associated with asulam and no other significant components. The majority of the radioactivity remaining on the column after leaching was bound residue. Solvent extracts of the soil layers that contained sufficient radioactivity were shown by HPLC to contain asulam as a major component and sulphanilamide as a minor component.

Table B.8. 113 Recovery of Applied radioactivity from the incubated soil samples

Soil	Time (days)	% applied radioactivity in:		
		Solvent Extracts	Soil Residue	Total
Sand	0	125.36	0.88	126.24
	3	94.29	4.04	98.33
	6	87.67	8.69	96.36
	31	57.34	25.26	82.60
Clay Loam	0	104.00	3.36	107.36
	3	83.02	16.32	99.34
	6	69.69	28.62	98.31
	10	53.78	40.89	94.67
Sandy Clay Loam	0	117.60	4.15	121.75
	3	78.36	18.16	96.52
	6	58.58	33.11	91.69
	7	54.56	35.61	90.17
Sandy Loam	0	102.07	1.60	103.67
	3	73.45	26.81	100.26
	6	56.82	41.78	98.60
	7	52.76	35.90	88.66

Table B.8. 114 Mean distribution of radioactivity from eluted aged columns of four soil types.

Soil	% applied radioactivity recovered in:						
	Leachate	Soil sections (cm)					Total
		0-6	6-12	12-18	18-24	24-30	
Sand:							
Column A	76.96	15.82	0.44	0.34	0.36	0.43	94.34
Column B	76.71	12.35	0.47	0.51	0.41	0.41	90.86
Clay Loam:							
Column A	52.25	18.88	1.90	3.13	1.76	9.02	86.94
Column B	55.19	18.72	1.31	1.43	3.39	4.81	84.85
Sandy Clay Loam:							
Column A	51.97	42.69	0.87	1.28	0.76	0.93	98.50
Column B	47.01	38.69	1.13	1.04	1.15	2.36	91.39
Sandy Loam:							
Column A	60.04	26.76	1.01	1.64	1.48	2.95	93.87
Column B	61.21	33.08	3.52	2.01	0.71	1.37	101.90

(Reeves, England and Savage 1988a)

B.8.2.3 Summary & assessment - Adsorption, desorption and mobility in soil

Batch adsorption was conducted in two studies with asulam sodium on 8 soils, a sand and a sediment (pH 4.6 – 7.5; organic matter content 0.2 – 10.5%). The K_{foc} values ranged from 15.4 to 149.7 mL/g (mean 25.4 mL/g) and 1/n values were in a range of 0.68 to 0.87 (mean 0.75). With the exception of the sand (with very low carbon content) variation between K_{foc} values was less than between K_f values, indicating that organic carbon of soil content is an important factor in determining adsorption. Adsorption was not noted to correlate with any other soil parameter. For the purposes of calculating mean values for input to computer models, the values for the sand and the sediment were excluded by the Notifier giving a mean K_{foc} of 25.4 mL/g and 1/n value of 0.75.

A batch equilibrium aged desorption study was conducted with asulam sodium on four UK soils (pH 5.8 – 7.0; organic matter 1.2 – 6.2%) over seven days. The $K_{f\ des}$ and $K_{foc\ des}$ values increased at each desorption cycle particularly at seven days indicating that different adsorption mechanisms may be at work, some of which resulted in very strong adsorption of some of the asulam applied to the soil. The effect of the aging period indicated that the potential for mobility of asulam may be significantly reduced with time.

Batch adsorption studies were also conducted with metabolite sulphanilamide on 8 soils. K_{foc} values showed that adsorption of sulphanilamide correlated with soil organic carbon content but no relationship was observed with soil pH. The K_{foc} values ranged from 124 to 179 ml/g (mean 145 mL/g) and 1/n values were in the range 0.673 to

0.748 (mean 0.711). Desorption results indicated that adsorption may not be completely reversible.

A batch adsorption study was conducted on metabolite methyl-benzene-sulfonyl-carbamate (MBSC) on four soils. Due to the low adsorption, the study was conducted at a single concentration only and no adsorption isotherms were determined. The Koc values ranged between 1.53 and 3.88 mL/g (mean 2.8 mL/g). Due to the low adsorption of methyl-benzene-sulfonyl-carbamate on soil, desorption was not considered reliable.

It is considered by the Rapporteur that the batch sorption results are appropriate to allow assessment of leaching potential to groundwater by standard FOCUS modelling approaches.

A column leaching study was conducted with asulam sodium on four UK soils (pH 7.10 – 7.26; organic matter 0.84 – 3.56%). The percentage of AR found in the leachates ranged from 90 to 98%. The radioactivity remaining on the soil was distributed throughout the columns mainly in the form of bound residue. There was insufficient radioactivity in solvent extracts of the soil to perform chromatographic analysis. Analysis of the leachates from all four soils showed only the presence of asulam indicating a high mobility in these soil types.

An aged column leaching study was conducted with asulam sodium on four UK soils discussed above. The amount of AR in the leachate was less than for the unaged leaching study ranging from 49 to 77%. The majority of the radioactivity remaining on the columns was in the top 6 cm layer of the soil mainly in the form of bound residue. Analysis of the leachates from all four soils showed asulam as the main component. Solvent extracts of the soil layers containing sufficient radioactivity also showed the presence of asulam and a minor component suggested to be sulphanilamide. The results suggested medium to high mobility for previously aged asulam in these soil types.

Soil adsorption/desorption (Annex IIA, point 7.1.2)

Asulam ‡							
Soil Type	OC %	Soil pH	Kd (mL/g)	Koc (mL/g)	Kf (mL/g)	Kfoc (mL/g)	1/n
PT102 Sandy loam	2.5	7.1			0.3879	15.5	0.728
PT103 Sandy Loam	1.3	5.5			0.3044	23.4	0.822
SK961089 Clay loam	5.4	7.5			0.832	15.4	0.712
LA98-983 Sand*	0.1	6			0.1497	149.7	0.817
LA99-3 Sandy silt loam	2.5	6.8			0.6379	25.5	0.733
Emperor Lake (sediment)*	6.1	6			2.59	42.5	0.684
Soil I, Silt loam	2.12	5.74			0.513	24.2	0.660

Soil II, Loam	4.93	4.6			3.28	66.5	0.870
Soil III, Loamy sand	1.4	6.05			0.218	15.6	0.700
Soil IV, Silty clay	3.93	7.36			0.677	17.2	0.770
Arithmetic mean/median						25.4	0.75
pH dependence, Yes or No			No				

* sand and sediment excluded from the mean calculation

Sulphanilamide ‡							
Soil Type	OC %	Soil pH	Kd (mL/g)	Koc (mL/g)	Kf (mL/g)	Kfoc (mL/g)	1/n
Clay Loam (01/01)	2	7.9			3.59	179	0.716
Low pH Sandy Loam (01/03)	1	5.0			1.45	145	0.748
Sandy Loam (01/07)	2	7.8			2.48	124	0.705
Calcareous Silty Clay Loam (01/09)	3.1	8.2			4.07	131	0.673
LUFA Speyer 2.2, Loamy sand	1.9	5.8			2.55	134	0.712
Further Field, Clay loam	1.6	6.7			4.44	278	0.708
Flint Hall, Clay loam	2.5	8.2			2.39	96	0.723
Warsop A1, Loamy sand	0.9	5.1			2.2	244	0.681
Arithmetic mean/median					2.90	166	0.708
pH dependence (yes or no)			No				

Methyl-benzene-sulfonyl-carbamate (MBSC) ‡							
Soil Type	OC %	Soil pH	Kd (mL/g)	Koc (mL/g)	Kf (mL/g)	Kfoc (mL/g)	1/n
Speyer 2.1, Sand	0.59	6.0	0.015	2.93	-	-	-
Speyer 2.2, Loamy sand	2.32	5.6	0.090	3.88	-	-	-
Marknesse, Silt loam	2.5	7.2	0.025	1.53	-	-	-
Arithmetic mean				2.8	-	-	1.0*
pH dependence (yes or no)			No				

FOCUS default used in modelling

Mobility in soil (Annex IIA, point 7.1.3, Annex IIIA, point 9.1.2)

Column leaching ‡	Elution (mm): 576 mL on 4 soils Time period (d): Rate which did not exceed infiltration capacity of each soil (time not specified in report)
	Leachate: 90.5 to 97.9% total residues/radioactivity in leachate indicated to be asulam only 0.115 to 0.89 % total residues/radioactivity retained in top 6 cm Koc (When it has not been possible to determine it by batch sorption experiments).
Aged residues leaching ‡	Aged for (d): 7 to 31 d Time period (d): Rate which did not exceed infiltration capacity of each soil Elution: 576 mL
	Analysis of soil residues post ageing (soil residues pre-leaching): *25.26 to 40.89* % active substance >x % total residues/radioactivity retained in top x cm
	Leachate: 49.5 to 76.8% total residues/radioactivity in leachate present as asulam 14.1 to 40.7 % total residues/radioactivity retained in top 6 cm
Lysimeter/ field leaching studies ‡	None submitted and none required

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

The predicted environmental concentration in soil has been calculated for asulam and the major soil metabolite for the representative uses on spinach and flower bulbs. For both uses the application rate according to the GAP is 2.4 kg/ha. Zero interception is assumed.

The following assumptions have been made when calculating the soil PEC values for asulam below for single annual use by the Rapporteur (see Table B.8. 115):

- One application of asulam at a rate of 2400 g a.s./ha to spinach/flower bulbs with zero crop interception (appropriate for pre-emergence use).
- Soil bulk density of 1.5 g/cm^3 dry weight
- Equal distribution in the top 5 cm soil layer
- Single first order DT_{50} for asulam of 11.2 days (longest first order laboratory DT_{50} with acceptable model fit at 20°C)

Table B.8. 115 Soil PEC values for asulam in a 5 cm soil layer following a single application of 2400 g a.s./ha to spinach/flower bulbs (DT_{50} 11.2 days)

Days after appl'n	PECsoil (mg/kg)	Time weighted average (mg/kg)
0	3.200	-
1	3.008	3.103
2	2.827	3.010
4	2.498	2.835
7	2.075	2.597
14	1.345	2.141
21	0.872	1.791
28	0.566	1.520
42	0.238	1.140
50	0.145	0.987
100	0.007	0.516

The only major soil metabolite for asulam is sulphanilamide. For calculating the soil PEC value for sulphanilamide, the following assumptions were made:

- A worst case 100% conversion of asulam to sulphanilamide
- The equation to calculate the initial PECsoil value for asulam was factored for molecular weight differences (asulam 230.2, sulphanilamide 172.2) and maximum formation in laboratory soil studies 17.2%.

On the basis of the maximum PECsoil for parent asulam of 3.20 mg/kg, this corresponds to a maximum PECsoil value of 0.41 mg/kg for sulphanilamide.

Parent: Asulam Method of calculation	DT_{50} (d): 11.2 days Kinetics: SFO Field or Lab: representative worst case from lab studies.
Application data	Crop: spinach/flowering bulbs Depth of soil layer: 5 cm. Soil bulk density: 1.5g/cm^3 % plant interception: Pre-emergence therefore no crop interception Number of applications: 1 Interval (d): *n/a* Application rate(s): 2400 g as/ha

PEC$_{(s)}$ (mg/kg)	Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial	3.200		x	
Short term 24h	3.008	3.103	x	x
2d	2.827	3.010	x	x
4d	2.498	2.835	x	x
Long term 7d	2.075	2.597	x	x
28d	0.566	1.520	x	x
50d	0.145	0.987	x	x
100d	0.007	0.516	x	x
Plateau concentration	Not calculated			

Metabolite: Sulphanilamide Method of calculation	Molecular weight relative to the parent: 172.2/230.2 DT_{50} (d): not used, initial value only calculated Kinetics: SFO Field or Lab: representative worst case from lab studies.
Application data	Application rate assumed: 308.8 g as/ha (assumed Met I is formed at a maximum of 17.2 % of the applied dose)

PEC$_{(s)}$ (mg/kg)	Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial	0.41		x	
Short term	x	x	x	x
2d	x	x	x	x
4d	x	x	x	x
Long term	x	x	x	x
28d	x	x	x	x
50d	x	x	x	x
100d	x	x	x	x
Plateau concentration	Not calculated			

B.8.4 Fate and behaviour in water (IIA 7.2.1, IIIA 9.2.1, 9.2.3)

B.8.4.1 Aqueous hydrolysis

This study has previously been evaluated at EU level. The conclusions are consistent with the EFSA conclusions report and no concerns were raised at EU level.

Report:

M. Gohdes (1989a) Hydrolysis of [^{14}C]-Asulam in Buffered Aqueous Solutions. Hazleton Laboratories America Inc, Madison, Wisconsin, USA for Rhone-Poulenc Agro., Unpublished report No.: HLA 6224-122 (R000015)

Guidelines:

USEPA (=EPA) 163-1
Deviations: None

GLP:

Yes

a) A sterile aqueous hydrolysis study was conducted with ^{14}C-asulam in accordance with EPA guideline N 161-1 (1982) and in accordance with GLP. The study was considered acceptable by the Rapporteur.

A preliminary study was conducted to evaluate analytical procedures, rate of degradation and material balance under test conditions, these conditions were then used as a basis for experimental conditions in the definitive study reported below.

Sterile aqueous buffer solutions (pH 5, 7 and 9) were prepared containing ^{14}C-asulam (radiochemical purity 94.7%; specific activity 10.64 mCi/mmol) at a concentration of 4.9µg/mL. Duplicate aliquots were analysed by liquid scintillation counting (LSC) to verify sample homogeneity. The difference from the mean concentration was 0.4% (pH5), 0.2% (pH7) and 0.1% (pH9). Samples of each treated buffer were incubated at 24 to 26°C in the dark in sealed vials (12 for analysis and 4 for pH determination). Duplicate samples were analysed by HPLC and LSC at days 0, 6, 12, (day 13 for pH9), 20, 24 and 31.

The recovery of radioactivity applied to the samples relative to day 0 throughout the 31 day period for all pH's was acceptable in the range from 100.8 to 106.0%. The pH of samples throughout the study period did not vary by more than 0.06 pH units.

HPLC analysis of the buffer solutions showed ^{14}C-asulam as the major component plus 4 minor components designated as regions 1 to 4. Throughout the 31 day period, the mean radioactivity for region 1 reached a maximum of 4.7% in pH 5 samples but was not detected for pH 7 and 9 samples and for HPLC regions 2, 3 and 4, the mean radioactivity did not exceed 1.4% throughout the study for all pH's. The mean HPLC results are presented in Table B.8. 116. The results indicated that asulam was stable to hydrolysis at all pH values tested over 31 d (based on levels not declining by more

than 10% over the study duration). There was insufficient degradation to calculate DT_{50}'s.

Table B.8. 116 Mean distribution of radioactivity expressed as percentage of applied radioactivity in buffer solutions at pH 5, 7 and 9 containing ^{14}C-asulam

Time (days)	Region 1	Asulam	Region 2	Region 3	Region 4
pH 5					
0	nd	96.8	nd	0.8	0.3
6	1.2	94.5	0.3	0.9	nd
12	1.8	94.4	0.6	0.6	nd
20	3.2	93.9	nd	0.6	nd
24	3.6	90.9	0.3	0.3	0.3
31	4.7	92.2	1.0	0.5	0.6
pH 7					
0	nd	93.8	nd	0.9	0.7
6	nd	94.7	0.3	1.3	nd
12	nd	94.8	0.4	1.1	0.7
20	nd	93.7	nd	0.9	0.5
24	nd	92.8	nd	0.7	0.5
31	nd	94.5	nd	0.9	0.5
pH 9					
0	nd	95.9	nd	0.9	0.5
6	nd	96.9	0.4	1.2	nd
13	nd	96.1	0.2	1.3	nd
20	nd	93.1	nd	1.1	0.6
24	nd	96.1	nd	1.0	0.5
31	nd	95.1	nd	1.1	0.2

nd: not detected

(Gohdes, 1989a)

B.8.4.2 Aqueous photolysis

This study has previously been evaluated at EU level. The conclusions are consistent with the EFSA conclusions report and no concerns were raised at EU level.

Report:

E.A. Mills, M.B. Simmonds (2003a) [^{14}C]-Asulam: Aqueous Photolysis and Quantum Yield at pH 9 and pH 4. Battelle AgriFood Ltd, Ongar, Essex, England
Unpublished report No.: CX/02/057 (C031419)

Guidelines:

EU 94/37, EPA 161-2
Deviations: None

GLP:

Yes

a) The aqueous photolysis of asulam was studied according to EPA (1998), OECD (Draft 2000), and SETAC (1995) guidelines and to GLP. The study was considered acceptable by the Rapporteur.

Sterile aqueous buffer solutions at pH9 and pH 4 were prepared containing ^{14}C-asulam (radiochemical purity >98%, specific activity 5.617 MBq/mg) at concentrations of 0.607 µg a.s./mL and 0.604 µg a.s./mL respectively. Aliquots (25 ml) were incubated at 25±2°C in glass photolysis vessels fitted with traps for organic volatiles (ethylene glycol) and CO_2 (2M KOH). Single vessels were either kept in the dark or irradiated continuously with artificial light from a xenon arc lamp (wavelengths filtered below 290 nm) for up to 54 hours (pH9) and up to 26 hours (pH4). The intensity of light was compared to natural sunlight and 1 d (24h) of artificial was reported to be equivalent to 1.79 d (pH9) and 1.78 d (pH4) of summer sunlight at 50°N in the UK. Samples were taken at the start and at representative time points up to 54 h for pH 9 and 26 h for pH4. Radioactivity was quantified directly by LSC and analysed by HPLC with confirmatory analysis of metabolites by LC-MS and TLC. The photolysis of an actinometer solution consisting of 4-nitroacetophenone incubated in the presence of pyridine was also investigated under identical conditions in order to calculate the quantum yield of asulam.

Mean recoveries were acceptable. At pH 9, recoveries were 98.7% in irradiated samples and 98.5% in dark controls. At pH 4, mean recoveries in irradiated samples was 100.2% and 99.7% in dark controls. The results of analysis for the pH 9 samples are summarised in Table B.8. 117 and for the pH 4 samples in Table B.8. 118.

In the irradiated samples at pH 9, levels of asulam declined to 17.8% AR in 51.8 h (equivalent to 3.9 days of natural UK summer sunlight). A major photodegradate reached a maximum of 24.2% after 46 h and was identified by LC-MS as N-(4-aminophenyl)formamide. One other photodegradate reached 11.9% AR but was reported as being 9% by the Notifier from a ModelManager fit to observed data which was performed due to the scatter of the results. This component was tentatively identified by LC-MS as (4(4-methoxycarbonylaminophenyl)aminophenyl)carbamic acid. The remaining degradation products were all below 10% AR (see Table B.8. 117). No significant degradation of asulam occurred in the pH 9 dark control samples.

In the irradiated samples at pH 4, levels of asulam declined to a minimum of 21.0% AR in 25.8 h (equivalent to 1.9 days natural UK summer sunlight). One major photodegradate reached 55.5% AR after 20.1 hours and was identified by LC-MS and confirmed by TLC as sulphanilic acid. The remaining degradation products were all below 10% AR (see Table B.8.44). One minor degradation product was observed in dark controls as <1% AR.

No significant quantities of volatile products were collected in either irradiated samples or the dark controls at either pH.

At both pH values, the DT_{50} and DT_{90} values for the photolytic degradation of asulam were calculated using Microsoft Excel assuming single first order kinetics and are summarised in Table B.8. 119.

The DT50 values for the five major photodegradates at pH 9 and for sulphanilic acid at pH 4, calculated by the Notifier using ModelManager are shown Table B.8. 120. The observed experimental values were fitted using assuming single first order kinetics. However the 95% confidence limits were very wide and the DT_{50} values predicted were the maximum the model allowed and therefore considered unreliable by the Rapporteur.

The quantum yield of asulam was calculated to be 0.157 at pH 9 and 0.114 at pH4.

Table B.8. 117 Composition of radioactivity by HPLC irradiated samples pH 9

	% Applied radioactivity											
Time irradiated (hours)	Sample water	Asulam	Sulphanilic acid rrt = 0.19	rrt = 0.23	rrt = 0.41 *	rrt = 0.75	rrt = 0.78	rrt = 0.83	rrt = 0.85	rrt = 0.88	rrt = 0.93 **	Total remaining (each <4%)
0	96.8	96.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	96.8	96.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	98.7	98.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	98.2	98.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.3	97.6	96.5	0.0	0.0	0.7	0.4	0.0	0.0	0.0	0.0	0.0	0.0
5.2	97.1	88.3	0.0	2.6	1.2	1.2	0.8	0.0	0.2	0.0	1.5	1.4
6.7	99.5	92.3	0.0	1.7	1.1	1.1	0.4	0.0	0.0	0.0	1.3	1.0
7.8	99.5	89.2	0.6	2.2	2.3	0.6	0.0	0.0	0.0	0.0	2.9	1.7
14.2	101.4	64.4	2.9	4.1	14.3	2.9	3.4	2.1	0.5	0.0	3.4	3.4
21.0	100.0	53.6	1.3	3.5	12.9	3.6	5.3	2.3	3.5	1.6	7.7	4.7
26.7	97.6	37.9	4.4	4.3	15.1	6.3	5.5	3.5	3.4	1.9	5.0	10.3
32.2	98.0	32.3	3.9	4.7	15.8	7.1	6.9	2.0	4.1	1.5	6.4	13.0
39.7	100.1	29.4	3.4	3.6	15.2	5.7	8.5	5.2	5.3	3.2	9.3	11.3
46.3	96.4	20.0	3.7	3.2	24.2	7.9	5.4	4.2	7.0	4.3	6.2	10.3
51.8	100.7	17.8	3.4	5.2	14.5	7.0	8.8	3.6	7.2	5.1	11.9	16.3

rrt = relative retention time
* identified as N-(4-aminophenyl)formamide by LC/MS
** tentatively identified as (4(4-methoxycarbonylaminophenyl)aminophenyl)carbamic acid

Table B.8. 118 Composition of radioactivity by HPLC irradiated samples pH 4

	% Applied radioactivity							
Time irradiated (hours)	Sample water	Asulam	Sulphanilic acid rrt = 0.19	rrt = 0.75	rrt = 0.78	rrt = 0.88	rrt = 0.93	Total remaining each <3%
0	98.9	98.9	0.0	0.0	0.0	0.0	0.0	0.0
0	97.6	97.2	0.4	0.0	0.0	0.0	0.0	0.0
0	101.7	101.7	0.0	0.0	0.0	0.0	0.0	0.0
0	100.8	100.4	0.4	0.0	0.0	0.0	0.0	0.0
1.3	100.2	94.3	3.4	0.0	0.6	0.0	1.9	0.0
2.2	100.3	88.7	6.9	0.0	0.7	2.0	1.5	0.4
3.8	102.6	87.2	10.2	0.0	0.0	2.0	2.8	0.4
6.2	100.6	67.8	20.6	0.0	1.5	6.7	2.0	1.9
8.2	93.5	55.9	25.5	0.1	2.0	2.3	3.4	4.3
12.2	101.4	38.9	42.6	1.5	4.2	2.8	4.1	7.4
15.1	102.1	38.1	43.0	2.3	4.9	0.5	5.3	8.0
17.6	102.1	29.5	50.6	2.2	2.9	2.4	5.7	8.7
20.1	99.2	21.0	55.5	3.7	2.4	3.9	5.4	7.2

25.8	101.0	24.0	53.1	3.0	3.5	2.7	6.0	8.5

rrt = relative retention time

Table B.8. 119 Calculated DT50 and DT90 values for asulam in irradiated aqueous solution at pH 9 and pH 4 assuming single first order kinetics

System	Irradiated in Suntest		Natural Sunlight*	
	DT_{50}	DT_{90}	DT_{50}	DT_{90}
Irradiated pH 9 buffer	20.9 hours, 0.870 days	2.89 days	1.56 days	5.17 days
Irradiated pH 4 buffer	10.6 hours, 0.440 days	1.46 days	0.781 days	2.59 days

* 1 day Suntest = 1.79 summer sunlight days 52°N at pH 9
= 1.78 summer sunlight days 52°N at pH 4

Table B.8. 120 DT50 for photodegradates calculated using ModelManager

	DT_{50} (hours)	DT_{50} 95% confidence limits (hours) *	
pH 9 study			
rrt = 0.41	1.48E+02	-4.52E+02	7.49E+02
rrt = 0.75	1.39E+07	-3.47E+12	3.47E+12
rrt = 0.78	1.39E+07	-4.77E+12	4.77E+12
rrt = 0.85	1.39E+07	-6.85E+12	6.86E+12
rrt = 0.93	1.39E+07	-5.07E+12	5.07E+12
Sulphanilic acid	1.41E+02	-5.22E+02	8.04E+12
pH 4 study			
Sulphanilic acid	1.39E+07	-4.14E+12	4.14E+12

* due to wide confidence limits, values may be unreliable

(Mills and Simmonds 2003a)

An additional aqueous photolysis study has been performed in sterile natural surface water from a UK reservoir (see study summary (d) below). In natural surface water, none of the major (i.e. >10%) metabolites identified in the photolysis study performed in sterile buffer above were formed in significant amounts. The Notifier has proposed that the results from the natural water study are more relevant than from the sterile buffer study for the purposes of the exposure assessment, since the effects of indirect phototransformation are considered alongside those of direct phototransformation in the natural water study. See the Summary and Assessment section for further consideration of the two aqueous photolysis studies.

b) The environmental photolytic half-life of asulam was calculated from the Quantum Yield, light absorbance data for asulam and solar light intensities determined in the aqueous photolysis study (Mills and Simmonds, 2003a) according to the method describe in ECETOC Technical Report No. 12(1984), UBA Test Guidelines (1990) and the Spectral irradiance data of Frank and Kopffler (1988). The factors which determine the sunlight intensity at the water surface include:

- Latitude
- Time of day
- Ozone layer thickness

- Cloud cover
- Concentration of aerosol and solid particles in air
- Presence of air borne gaseous contaminants

This method estimated the half-life for asulam when dissolved in the top few millimetres of a natural aquatic system using solar irradiance data appropriate to central European latitudes (52°N). Based on these assumptions, the half-life values for asulam for each month at pH 4 and pH 9 are summarised in Table B.8. 121.

The factors which determine the penetration of light into the water include:

- Refractive index of the water
- Light attenuation of the water
- The concentration of suspended solids
- The vegetation cover
- Depth of the water system

These factors are not considered and therefore the results are only valid for direct phototransformation in the top few millimetres of a natural aquatic system.

Table B.8. 121 Photolytic half-lives of asulam sodium at pH 4 and pH 9 in the top millimetres of natural aquatic systems (Central European latitude 52°N)

Half-lives in hours, by Month					
Month	pH 4	pH 9	Month	pH 4	pH 9
Jan	76	135	Jul	8	13
Feb	38	65	Aug	8	13
Mar	19	31	Sep	13	21
Apr	11	18	Oct	25	41
May	8	14	Nov	61	106
Jun	7	12	Dec	119	213

(Lowden 2004a)

c) The environmental photolytic half-life of asulam was calculated from the Quantum Yield, light absorbance data for asulam and solar light intensities determined in the aqueous photolysis study (Mills and Simmonds, 2003a) according to the method describe in ECETOC Technical Report No. 12(1984) and the UBA Test Guidelines (1990). The GCSOLAR program of Zepp and Cline was used to generate data 40°, 50° and 60°N by season, at the surface and at depths of 30 and 100cm in the water body.

The factors which determine the sunlight intensity at the water surface and penetration of light into water are summarised in section B.8.4.2 (b).

The GCSOLAR program has the capability to determine real half-lives according to the properties of the water (refractive index, light absorption and depth). The program does not account for cloud cover, concentration of pollutants in air, or suspended solids and vegetation cover in the water.

The results for photolysis half-lives for asulam are summarised for pH 4 in Table B.8. 122 and for pH 9 in Table B.8. 123.

Table B.8. 122 Photolytic half-lives of asulam (pH 4) at the surface and at depths of 30cm and 100cm depth at various European latitudes

Latitude	Season	Half-life (days) in water body depth of:		
		0 cm	30 cm	100 cm
40°N	Spring	0.431	1.94	6.13
	Summer	0.307	1.45	4.60
	Autumn	0.785	3.56	11.3
	Winter	1.660	6.77	21.3
50°N	Spring	0.586	2.49	7.84
	Summer	0.362	1.66	5.24
	Autumn	1.450	6.04	19.0
	Winter	4.090	14.9	46.1
60°N	Spring	0.823	3.32	10.4
	Summer	0.444	1.96	6.19
	Autumn	3.29	12.3	38.5
	Winter	13.6	44.0	135

Table B.8. 123 Photolytic half-lives of asulam (pH 9) at the surface and at depths of 30cm and 100cm depth at various European latitudes

Latitude	Season	Half-life (days) in water body depth of:		
		0 cm	30 cm	100 cm
40°N	Spring	0.703	3.34	10.7
	Summer	0.501	2.48	7.96
	Autumn	1.29	6.18	19.8
	Winter	2.78	12.1	38.5
50°N	Spring	0.965	4.36	13.9
	Summer	0.591	2.85	9.13
	Autumn	2.42	10.7	34.2
	Winter	7.19	27.5	86.8
60°N	Spring	1.38	5.92	18.9
	Summer	0.728	3.40	10.9
	Autumn	5.68	22.6	71.5
	Winter	24.9	84.0	261

(Lowden 2004b)

d) The aqueous photolysis of asulam was studied according to Japanese J MAFF 2-6-2 (2001) guidelines, followed guidance notification13 Seisan No. 3986 (2001) and to GLP. The study was considered acceptable by the Rapporteur.

The study was conducted in natural water (UK reservoir pond, pH 7.8) which was sterilised by filtration through a sterile 0.22µm membrane filter and dosed with ^{14}C-asulam (radiochemical purity >98%, specific activity 5.16 MBq/mg) at a nominal concentration of 1 µg a.s./mL. Aliquots (35 ml) were incubated at 25±2°C in sealed glass photolysis vessels. Single vessels were either kept in the dark or irradiated continuously with artificial light from a xenon arc lamp (wavelengths filtered to remove wavelengths below 290 nm). The intensity of light was compared to natural sunlight and 1 d (24h) of artificial was reported to be equivalent to 5.01 d of natural spring sunlight at 35°N in Tokyo, Japan which is comparable with Southern Europe (e.g. Athens 37°N). Samples were taken at the start and at intervals up to 145.7 h for irradiated samples and up to 169.6 h for non-irradiated samples. Duplicate samples were taken for the initial and last timepoints and single samples for the other timepoints. Radioactivity was quantified by LSC and analysed by HPLC with confirmatory analysis by HPLC and TLC.

Mean recoveries were acceptable at 100% in the irradiated samples and 103% in dark controls. For the irradiated samples carbon dioxide levels determined from the loss in dissolved radioactivity after precipitation with barium chloride, ranged up to 27.4% AR and 1.1% in the dark controls. After precipitation of CO_2 with barium chloride, the remaining radioactivity in the samples was analysed by HPLC with selected samples analysed by HPLC and TLC as confirmation. The levels of asulam declined rapidly reaching <1% AR at the end of the irradiation period. Many photodegradates were formed, all less than 10% AR and the results of analysis are presented in Table B.8. 125.

Three photodegradates exceeded 5% radioactivity, reaching maximum values of 6.4% (rrt=0.25), 5.8% (rrt=0.38) and 5.0% (rrt=0.22). Six photodegradates showed maximum values between 3 and 5% AR. A further 11 photodegradates showed values between 2 and 3% and 27 more photodegradates were between 1 and 2% AR. The formation of metabolites in this study was noted by the Rapporteur to be in contrast to the study performed in sterile buffer where a number of major metabolites were formed in the presence of light (see study (a), Mills and Simmonds, 2003a above). The Notifier has proposed that the results of this study performed in natural water is more relevant for the purposes of the exposure assessment since the effects of indirect phototransformation are considered alongside those of direct phototransformation.

No significant degradation of asulam was found in the dark control samples.

The half-life and DT_{90} values for the photolytic degradation of asulam were calculated assuming single first order kinetics and are summarised in

Table B.8. 124. The correlation coefficient for the experimental data was 0.997.

Table B.8. 124 Calculated DT50 and DT90 values for asulam in irradiated natural water for a period of 145.7 hours (equivalent to 30.43 days natural Japan spring sunlight)

	Experimental days	Environmental days*
DT50	0.84	4.21
DT90	2.79	13.98

* 1 day suntest = 5.01 days sunlight (Japan)

Table B.8. 125 Composition of radioactivity in irradiated natural water samples fortified with ^{14}C-asulam at 1 µg/mL after various periods of exposure to light (and removal of carbon dioxide)

Test Point	Sample Number	Suntest Hours	% applied radioactivity in $BaCl_2$ Extract	% applied radioactivity represented by peak no. (rrt)				
				1 (Asulam) (1.0)	2 (0.20)	3 (0.22)	4 (0.23)	5 (0.25)
initial	1	0.0	102.92	98.3	0.9	0.2	0.0	0.0
initial	2	0.0	102.07	98.7	0.0	0.1	0.0	0.0
initial	3	0.0	101.19	97.9	0.4	0.3	0.0	0.0
initial	4	0.0	98.81	95.8	0.5	0.0	0.0	0.0
initial	5	0.0	103.80	99.3	0.1	0.7	0.1	0.0
initial	6	0.0	100.91	96.7	0.1	0.3	0.0	0.0
1.5 h	7	1.3	101.52	95.0	0.2	0.3	0.1	0.3
4 h	8	3.6	101.00	92.9	0.7	0.8	0.3	0.1
8 h	9	7.4	100.45	86.0	1.6	1.3	0.5	0.5
10 h	10	9.2	97.30	79.2	2.1	1.2	0.8	0.6
15 h	11	15.0	94.82	61.5	2.4	2.5	1.2	2.3
19 h	12	18.3	89.80	48.2	3.4	4.1	1.8	1.8
23 h	13	22.4	87.87	43.1	3.8	3.3	1.6	2.6
30 h	14	28.3	89.60	42.0	3.7	3.3	1.7	2.1
39 h	15	38.2	79.68	20.2	4.5	5.0	3.7	3.5
49 h	16	45.2	80.28	18.1	3.8	2.9	3.4	3.6
57 h	17	52.5	78.24	17.0	3.9	3.5	2.3	3.6
68 h	18	69.5	77.11	11.2	3.6	2.1	2.5	3.8
78 h)	19	77.7	77.35	4.0	2.9	3.1	2.3	4.3
92 h	20	94.2	73.76	3.5	2.1	3.0	3.2	4.3
102 h	21	101.7	76.38	3.3	1.9	2.8	2.8	5.2
120 h	22	119.2	75.50	1.1	2.8	2.8	2.3	5.6
140 h	23	139.1	71.61	0.7	2.9	2.9	2.6	4.6
147 h	24	145.7	71.56	0.8	2.1	2.3	2.2	5.1
147 h	25	145.7	68.96	0.9	1.5	3.4	2.0	6.4
maximum level					**4.5**	**5.0**	**3.7**	**6.4**

Table B.8. 125 (Cont'd) Composition of radioactivity in irradiated natural water samples fortified with ^{14}C-asulam at 1 µg/mL after various periods of exposure to light (and removal of carbon dioxide)

Test Point	Sample Number	Suntest Hours	% applied radioactivity represented by peak no. (rrt)					
			6 (0.27)	11 (0.38)	12 (0.39)	16 (0.46)	54 (1.30)	Total remainder*
initial	1	0.0	0.0	0.0	0.0	0.0	0.0	3.5
initial	2	0.0	0.0	0.0	0.0	0.0	0.0	3.2
initial	3	0.0	0.0	0.0	0.0	0.0	0.0	2.7
initial	4	0.0	0.0	0.0	0.0	0.0	0.0	2.5
initial	5	0.0	0.0	0.0	0.0	0.0	0.1	3.4
initial	6	0.0	0.0	0.0	0.0	0.0	0.0	3.7
1.5 h	7	1.3	0.0	0.0	0.0	0.0	0.4	5.2
4 h	8	3.6	0.0	0.1	0.0	0.0	0.7	5.4
8 h	9	7.4	0.1	0.4	0.0	0.0	1.2	8.7
10 h	10	9.2	0.4	0.5	0.1	0.0	2.1	10.3
15 h	11	15.0	1.2	1.1	0.4	0.4	2.2	19.7
19 h	12	18.3	1.4	1.3	1.4	0.4	2.8	23.3
23 h	13	22.4	2.0	1.6	1.2	0.7	3.3	24.6
30 h	14	28.3	2.2	1.3	1.7	0.3	2.7	28.4
39 h	15	38.2	1.3	2.2	2.0	1.1	2.9	33.3
49 h	16	45.2	1.6	3.1	2.4	0.7	3.6	37.2
57 h	17	52.5	1.9	4.5	1.9	1.1	2.8	35.8
68 h	18	69.5	2.0	5.2	3.4	1.6	2.6	39.0
78 h	19	77.7	1.9	5.8	3.9	2.3	2.1	44.6
92 h	20	94.2	2.2	4.7	3.1	3.0	3.5	41.3
102 h	21	101.7	2.1	5.0	4.3	1.5	3.9	43.7
120 h	22	119.2	2.9	4.9	3.8	2.2	2.7	44.3
140 h	23	139.1	3.0	4.6	4.3	2.5	2.7	40.8
147 h	24	145.7	2.2	5.4	4.2	2.7	3.1	41.5
147 h	25	145.7	1.9	4.2	2.5	1.6	2.9	41.8
maximum level			**3.0**	**5.8**	**4.3**	**3.0**	**3.9**	

* each < 3% applied radioactivity.
Total of 59 photodegradates

(Mills and Caine 2004a)

This study has not previously been evaluated at EU level. It has been evaluated at member-state level. The conclusions presented, in conjunction with those from CA 7.6/03, are similar to those of the previously submitted study and the EFSA conclusion report.

Report:
E. Mills (2007a) Asulam: [^{14}C]-Aqueous Photolysis and Quantum Yield in Buffers at pH 4 and 9. Battelle UK Ltd, Ongar, Essex, England for AgriChem BV, Unpublished report No.: OU/07/008.

Guidelines:
EU 94/37
Deviations: None

GLP:
Not relevant

RMS opinion on study: fully acceptable. Please note the summary below is the applicant's. It is an accurate account of the report and considered acceptable by the RMS.

The photolysis of [^{14}C]-asulam in aqueous solution at pH 4 and at pH 9 was investigated. The study was conducted, under sterile conditions, at 25 ± 2°C, with continuous illumination under artificial sunlight. The artificial sunlight was provided by a xenon arc lamp with filters to cut off any radiation below 290 nm. These conditions were maintained throughout the study. Dark (non-irradiated controls) were also run.

The illumination was continued for just greater than two half-lives at each pH. The initial concentration of asulam was 0.6 mg L^{-1} with 0.3% acetonitrile present as a co-solvent. The solutions were dispensed into photolysis vessels that were connected to traps for volatile compounds and carbon dioxide. At pH 4 samples were taken at intervals from 1 hour to 26 hours irradiation and non-irradiated samples were taken at intervals from 4 hours to 55 hours incubation. At pH 9 samples were taken at intervals from 3 hours to 49 hours irradiation and non-irradiated samples were taken at intervals from 3 hours to 72 hours incubation.

At each sampling point the whole test solution in one vessel was taken. The traps were also removed and sampled at this time. Sterility aliquots were taken from irradiated and non-irradiated test solutions at intervals from 4 hours to 54/55 hours during each study. Sterility aliquots were taken from irradiated and non-irradiated test solutions at intervals from 3/4 hours to the last test point during each study.

Actinometry was conducted with solutions containing approximately 1.8 x 10^{-6} M or 1.7 x 10^{-6} M 4-nitroaceto phenone and approximately 0.2 M pyridine. Aliquots were taken from both irradiated and non-irradiated actinometer solutions at intervals from 4 hours to approximately 77 hours. Sterility checks were performed at designated intervals for both irradiated and non-irradiated test solutions.

The samples were examined on an HPLC system and the actinometer samples on a separate system. Sample solutions from each study pH were selected for concentration and liquid chromatography-mass spectrometry (LC/MS) analysis.

At pH 4 the irradiation was continued for 25 hours which is equivalent to 2.0 days of natural summer sunlight (EU). The radioactivity recovered from the samples ranged from 96.8% to 107.5% with a mean of 102.6% of applied radioactivity from 100.1% to 108.8% with a mean of 104.3% of applied radioactivity for irradiated and non-irradiated samples respectively. No significant quantities of volatile product were collected in either the irradiated experiment or the non-irradiated experiment.
HPLC analysis showed that the amount of asulam present in the irradiated samples declined to a minimum of 13.5% of applied radioactivity during 25 hours irradiation. One major photodegradate was found reaching 63.9 % of applied radioactivity. This was confirmed to be sulphanilic acid. Several other photodegradates were produced during this irradiation time, four of which reached between 3.7% and 9.8% of applied radioactivity. No significant degradation of asulam was observed in the non-irradiated system. There was no evidence for further degradation of the observed photodegradates.

At pH 9 the irradiation was continued for 47 hours which is equivalent to 3.7 days of natural summer sunlight (EU). For the irradiated samples the individual sample recoveries ranged from 99.9% to 107.6% with a mean of 104.1% of applied radioactivity. For non-irradiated samples the individual sample recoveries ranged from 100.2% to 106.7% with a mean of 104.0% of applied radioactivity.
No significant quantities of volatile products were collected in either the irradiated experiment (<0.2% of applied radioactivity) or the non-irradiated experiment (<0.1% of applied radioactivity). HPLC analysis showed that the amount of asulam present in the irradiated samples declined to 24.0% of applied radioactivity after 47 hours irradiation.

A number of photodegradates were observed in the irradiated samples. Seven of these exceeded 4% of applied radioactivity. The second most abundant photodegradate reached a maximum of 15.4% and was identified as N-(4-aminophenyl)formamide. Sulphanilic acid, a known metabolite of asulam, was confirmed to be present at levels up to 18.9% of applied radioactivity. There was no evidence for further degradation of the observed photodegradates. No significant degradation of asulam was observed in the non-irradiated system.

At both pH values, the half-life and DT_{90} values for the decline of asulam in the irradiated experiment using Microsoft Excel and assuming first order kinetics are presented in Table B.8. 126.

Table B.8. 126 Calculated DT50 and DT90 values for asulam in irradiated aqueous solution at pH 9 and pH 4 assuming single first order kinetics

System	Chi2 error	Irradiated in Suntest		Natural Sunlight*	
		DT_{50}	DT_{90}	DT_{50}	DT_{90}
Irradiated pH 9 buffer	5.4	20.7 hours, 0.863 days	2.87 days	1.64 days	5.44 days
Irradiated pH 4 buffer	11.1	6.8 hours, 0.284 days	0.944 days	0.537 days	1.48 days

* 1 day Suntest = 1.89 summer sunlight days 52°N at pH 9
= 1.90 summer sunlight days 52°N at pH 4

The quantum yield for asulam was calculated as 0.168 at pH 4 and 0.0612 at pH 9.

The RMS considers the study acceptable. The DT50/DT90 values calculated by the applicant are comparable with the ones in Mills and Simmonds 2003a (see Table B.8. 119).

This study has not previously been evaluated at EU level. It has been evaluated at member-state level. The conclusions presented are similar to those from the previously submitted report and the EFSA conclusions report.

Report:
E. Mills (2009a) Asulam: [^{14}C]-Aqueous Photolysis at pH 4 and 9: Identification of Photolytic Degradates. Battelle UK Ltd, Ongar, Essex, England for AgriChem BV. Unpublished report No.: OU/08/001.

Guidelines:
EU 94/37
Deviations: None

GLP:
Yes

RMS opinion on study: fully acceptable. Please note the summary below is the applicant's. It is an accurate account of the report and considered acceptable by the RMS.

The photolysis of [^{14}C]- in aqueous buffer solutions (0.01M acetate, pH 4; 0.01M glycine, pH 9) at an initial concentration of 0.6 mg L^{-1} has been investigated. The study was conducted, under sterile conditions, at 25 ± 2°C, with continuous irradiation under artificial sunlight for a period of up to 2 days. The artificial sunlight was provided by a xenon arc lamp with filters to cut off any radiation below 290 nm. This is a supplementary study to clarify quantification of four metabolites found at between 5% and 10% of applied radioactivity in a previous study (Mills, 2007a) Battelle Report OU/07/008, September 2007.

The test vessels were connected to traps for the collection of CO_2 and organic volatiles. Irradiation was continued for 23 hours (equivalent to 2.3 days natural sunlight) at pH 4 and for 46 hours (equivalent to 4.5 days natural sunlight) at pH 9. The initial concentration of asulam was 0.6 mg L^{-1}, with 0.1% acetonitrile present as a co-solvent. Further samples were prepared at three and at five times the concentration to aid identification of degradates. The samples were analysed directly and the asulam residues were quantified by HPLC. Identification and quantification of the transformation products was performed by HPLC (in comparison to certified analytical standards). Selected samples at pH 9 were collected in fractions and re-analysed using a different HPLC method.

The overall mean recovery of applied radioactivity was 103.8% (range 97.6 to 109.8%) at pH 4 and 102.0% (range 99.6 to 108.5%) at pH 9.

At pH 4, the parent compound decreased from 93.9% of the applied amount at time zero to 30.2% (single sample 19.1%) of the applied at the end of the irradiation. The only major photodegradate (i.e. >10% of applied radioactivity) reached a maximum of

61.3% and had been identified as sulphanilic acid in the previous study. No other photodegradates reached 4%. In particular the degradate of interest, rrt = 0.95, which reached between 5% and 10% in the previous study, was shown to be very minor and so no further identification of this component was attempted.

At pH 9, the parent compound decreased from 96.5% of the applied amount at time zero to 38.1% (single sample 30.3%) of the applied at the end of the irradiation. The unidentified degradates of interest were reaching plateau values at the end of the irradiation. The two major photodegradates (i.e. >10% of applied radioactivity) reached maxima of 18.4% (sulphanilic acid) and 10.6% (N-(4-aminophenyl)formamide). A third photodegradate, at rrt = 0.50, reached 6.2% (benzenesulphonic acid). The identification of all three was shown by spiking samples with cold reference standards and had been previously confirmed.

The three degradates of interest, which reached between 5% and 10% in the previous study were rrt = 0.31, 0.88 and 1.1. In this study they reached 6.1%, 4.1% and 7.2% respectively. These three regions of radioactivity were investigated further. Each region was isolated by HPLC with fraction collection using the usual analytical method. The fraction collected samples were further analysed by HPLC using a different method. The results showed that for each region, the radioactivity was composed of multiple components. The maxima for the largest component in each region rrt = 0.31, 0.88 and 1.1, were calculated to be respectively 2.2%, 1.4% and 3.0% applied radioactivity. Thus no unidentified component in the pH 9 samples reached 4% and all considered of minor significance by the applicant and no further attempt at identification was made.

This study has not previously been evaluated at EU level. It has been evaluated at member-state level. It is based on data from an additional photolysis study and supersedes the previously submitted report.

Report:
P. Lowden (2007a) Asulam: Estimation of Environmental Photolytic Half-life in Water (Model Calculation According to Frank and Klöpffer). Battelle UK Ltd, Ongar, Essex, England. Unpublished report No.: OU/07/009A.

Guidelines:
EU 94/37
Deviations: None

GLP:
Not relevant

RMS opinion on study: fully acceptable. Please note the summary below is the applicant's. It is an accurate account of the report and considered acceptable by the RMS.

Executive Summary:
The photolytic half-lives of asulam sodium using the quantum yield values obtained at pH 4 and pH 7 in the study of Mills ([^{14}C]-Asulam : Aqueous Photolysis and Quantum Yield at pH 9 and pH 4, Battelle UK Ltd Report No. OU/07/008) were calculated using

the light absorption spectra obtained at these pHs and the Solar photon irradiance data appropriate to the climatic situation in Central Europe and the adjacent North Sea, as published by Frank and Klöpffer. The calculations showed that the half-lives would vary with pH and according to the time of year, ranging from 4 to 5 hours in June up to 29 to 38 hours in December.

Materials and Methods

Test Material:
Asulam, purity not relevant, Batch no.: not relevant

Test Design:
The environmental half-life of asulam in the top millimetres of natural aquatic systems was estimated according to methods that permit calculation of a pseudo first-order rate constant for direct phototransformation from:

- The experimentally determined quantum yield
- The light absorption spectrum of the compound in aqueous solution at above 290 nm
- The solar light intensity in the spectral range which coincides with the light absorption spectrum of the compound

Solar photon irradiance data appropriate to the climatic situation in Central Europe and the adjacent North Sea, as published by Frank and Klöpffer, were used in this study.

Results and discussion:
The photolytic half-life of asulam was shown to range from the equivalent of 4 hours (pH 4) or 5 hours (pH 9) in June to 38 hours (pH 4) or 29 hours (pH 9) in December. The results in full are presented in Table B.8. 127.

Table B.8. 127 Photolytic half-lives of asulam sodium at pH 4 and pH 9 in the top millimetres of natural aquatic systems (Central European Latitude 52° North)

Half-lives in hours, by Month											
Month	pH 4	pH 9	Month	pH 4	pH 9	Month	pH 4	pH 9	Month	pH 4	pH 9
Jan	25	20	Apr	5	5	Jul	4	5	Oct	11	10
Feb	14	12	May	5	5	Aug	4	5	Nov	22	18
Mar	8	8	Jun	4	5	Sep	6	7	Dec	38	29

Conclusions:
It can be concluded that asulam in the top few millimetres of aquatic systems will undergo degradation by direct photolytic processes under the conditions prevailing in Central Europe. The DT_{50} would vary with pH and according to the time of year, ranging from 4 to 5 hours in June up to 29 to 38 hours in December.

This study has not previously been evaluated at EU level. It has been evaluated at member-state level. It is based on data from an additional photolysis study and supersedes the previously submitted report.

Report:
P. Lowden (2007b) Calculated Theoretical Lifetime in the Top Layer of Aqueous Systems and the Real Lifetime of Asulam (GC Solar). Battelle UK Ltd., Unpublished report No.: OU/07/009B

Guidelines:
EU 94/37
Deviations: N/A

GLP:
Not relevant

RMS opinion on study: fully acceptable. Please note the summary below is the applicant's. It is an accurate account of the report and considered acceptable by the RMS.

Executive Summary:
The photolytic half-lives of asulam sodium using the quantum yield values obtained at pH 4 and pH 7 in the study of Mills ([^{14}C]-Asulam : Aqueous Photolysis and Quantum Yield at pH 9 and pH 4, Battelle UK Ltd Report No. OU/07/008) were calculated using the GC Solar program of Zepp & Cline.

The results indicate that asulam in the top few millimetres of aquatic systems will undergo degradation by direct photolytic processes under the conditions prevailing in Central Europe, with half-lives of < 0.5 day in summer, regardless of latitude or pH. The half-lives in Spring will be < 0.5 days and those in autumn < 1.0 days except for at latitude 60° N at which they may increase to just under 1.5 days.

Real half-lives in natural aquatic systems will vary according to the depth of the water body. For water bodies of 30 cm depth the half-life will be up to 0.6 days in summer and up to 1 day in spring. Autumn half-lives would vary from about a day up to about 3 days, depending on latitude. For water bodies with a depth of 100 cm the half-life will be up to 2 days in summer and up to 2½ days in spring. Autumn half-lives would vary from about 1½ days up to just over a week, depending on latitude and pH.
From the results generated it can be concluded that, although asulam will be rapidly degraded by light in the top few millimetres of an aquatic system, the degradation will be slower in natural water bodies, throughout which it will readily dissolve. In water bodies of modest depth (30 cm, 100 cm) the half-lives will range from about half a day in summer to just over a week in autumn, without the influence of factors such as the presence of suspended solids that would have a significant impact on these values.

Materials and Methods

Test Material:
Asulam, purity not relevant, Batch no.: not relevant.

Test Design:
The environmental half-life of asulam in natural aquatic systems was estimated according to the methods that permit calculation of a pseudo first-order rate constant for direct phototransformation from:

- The experimentally determined quantum yield
- The light absorption spectrum of the compound in aqueous solution at above 290 nm
- The solar light intensity in the spectral range which coincides with the light absorption spectrum of the compound

The GCSOLAR program of Zepp and Cline was used to generate data for latitudes 40°, 50° and 60° North, which cover most of Europe. These results are produced by season. This program also has the capability to determine real half-lives of compounds according to the properties of the water (refractive index, light absorption spectrum and depth). The program does not account for cloud cover, concentration of pollutants in the air or suspended solids and vegetation cover in the water. Half-lives in natural water bodies of 30 cm depth (standard EU ditch and stream depth according to FOCUS) and of 100 cm (standard EU pond depth according to FOCUS) were calculated. Asulam is a weak acid and therefore partially ionises in the relevant pH range (4 to 9). The calculations were, therefore, carried out with data from experiments carried out at those pH values.

Results and discussion:
The photolytic half-life of asulam in the top few millimetres of aquatic systems at pH 4 was shown to range from 0.183 days in Summer at latitude 40° N to 5.02 days in Winter at latitude 60° N and that of asulam at pH 9 to range from 0.250 days in Summer at latitude 40° N to 5.13 days in Winter at latitude 60° N. Considering the use pattern and degradation rates of asulam the relevant results (presented in Table B.8. 128 below) are for Spring, Summer and Autumn and principally those for Summer and Autumn.

Table B.8. 128 Photolytic half-lives of asulam sodium at pH 4 and pH 9 in water bodies of 30 cm depth (at latitudes 40°, 50° & 60 North)

Photolytic half-life of Asulam (in days) when dissolved in the top millimetres of natural aquatic systems (Central European latitudes 40°, 50° & 60° north)						
Season	Asulam (at pH 4)			Asulam (at pH 9)		
	40°N	50° N	60° N	40° N	50° N	60° N
Spring	0.242	0.303	0.392	0.342	0.345	0.414
Summer	0.183	0.206	0.238	0.250	0.263	0.283
Autumn	0.422	0.700	1.400	0.501	0.745	1.380

The results for latitude 50° N are very similar to those obtained from the Frank & Köpffler calculation (Lowden, 2007), which are for 52° N.

The corresponding results for natural aquatic bodies of 30 cm and 100 cm depth are presented in Table B.8. 129 and Table B.8. 130.

Table B.8. 129 Photolytic half-lives of asulam sodium at pH 4 and pH 9 in water bodies of 30 cm depth (at latitudes 40°, 50° & 60 North)

Photolytic half-life of Asulam (in days) when dissolved in natural aquatic water bodies of 30 cm depth (European latitudes 40°, 50° and 60° north)						
Season	Asulam at pH 4			Asulam at pH 9		
	40° N	50° N	60° N	40° N	50° N	60° N
Spring	0.652	0.745	0.919	0.499	0.516	0.667
Summer	0.507	0.547	0.607	0.419	0.435	0.464
Autumn	1.100	1.690	3.110	0.829	1.210	2.210

Table B.8. 130 Photolytic half-lives of asulam sodium at pH 4 and pH 9 in water bodies of 100 cm depth (at latitudes 40°, 50° & 60 North)

Photolytic half-life of Asulam (in days) when dissolved in natural aquatic water bodies of 100 cm depth (European latitudes 40°, 50° and 60° north)						
Season	Asulam at pH 4			Asulam at pH 9		
	40° N	50° N	60° N	40° N	50° N	60° N
Spring	1.67	1.97	2.44	0.888	1.000	1.200
Summer	1.35	1.45	1.62	0.745	0.776	0.830
Autumn	2.95	4.50	8.23	1.410	2.180	4.010

The results for the water body of 30 cm depth are about 1½ to 3 times the results for the relevant surface layer and those for the water body of 100 cm depth are about 3 to 6 times the corresponding surface layer result.

The results indicate that asulam in the top few millimetres of aquatic systems will undergo degradation by direct photolytic processes under the conditions prevailing in Central Europe, with half-lives of <0.5 day in Summer, regardless of latitude or pH. The half-lives in Spring will be < 0.5 days and those in Autumn < 1.0 days except for at latitude 60° N at which they may increase to just under 1.5 days.

Real half-lives in natural aquatic systems will vary according to the depth of the water body. For water bodies of 30 cm depth the half-life will be up to 0.6 days in Summer and up to 1 day in Spring. Autumn half-lives would vary from about a day up to about 3 days, depending on latitude. For water bodies with a depth of 100 cm the half-life will be up to 2 days in Summer and up to 2½ days in Spring. Autumn half-lives would vary from about 1½ days up to just over a week, depending on latitude and pH.

Conclusions:
It can be concluded that, although asulam will be rapidly degraded by light in the top few millimetres of an aquatic system, the degradation will be slower in natural water bodies, throughout which it will readily dissolve. In water bodies of modest depth (30 cm, 100 cm) the half-lives will range from about half a day in Summer to just over a week in Autumn.

B.8.4.3 Ready biodegradation

This study has previously been evaluated at EU level. The conclusions are consistent with the EFSA conclusions report and no concerns were raised at EU level.

Report:

C. Mead (1999a) Asulam: Assessment of Ready Biodegradability: CO_2 Evolution Test Asulam (RPA 096125). SafePharm Laboratories Limited, Derby, Derbyshire, England. Unpublished report No. 238/047.

Guidelines:

EU 92/69 (Annex V of 67/548/EEC), EPA OPPTS 835.3110 (M)

Deviations: None

GLP:

Yes

Ready biodegradation of asulam was studied according to OECD guidelines (No. 301/B, 1992) and GLP (OECD 1997).

UK activated sewage sludge (30 mg suspended solids/L) was added to a standard mineral solution and this inoculum was preincubated for 24 hours at 21°C. Duplicate samples of inoculum (3 litres) were then treated with unlabelled asulam (24 mg/L; equivalent to 10 mg ThOC/L), sodium benzoate (17.1 mg/L equivalent to 10 DOC/L) or both. Further samples of untreated inoculum were also prepared. All samples were then incubated at 21°C for 29 days in flasks fitted with traps for CO_2 (Sodium hydroxide)

For all treatments, evolved CO_2 was determined by carbon analysis at day 0 and sixteen further sampling intervals. At study termination, 74% conversion of sodium benzoate to CO_2 was observed, confirming the suitability of the inoculum and test conditions. Evolved CO_2 from the asulam samples showed 51% degradation. Hence, asulam was considered by the study authors as being classified as 'not readily biodegradable'.

(Mead 1999a)

This study has not previously been evaluated at EU level. It has been evaluated at member-state level. The conclusions presented are consistent with those of the previously submitted report and the EFSA conclusions report.

Report:
N Feil (2008). Ready Biodegradability of Asulam in a Manometric Respirometry Test. Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany for AgriChem BV, Unpublished report No.: 39851163
Guidelines:
EU 92/69 (Annex V of 67/548/EEC), EPA OPPTS 835.3110 (M)
Deviations: None

GLP:
Yes

RMS opinion on study: fully acceptable. Please note the summary below is the applicant's. It is an accurate account of the report and considered acceptable by the RMS. The RMS has provided comments at the end of the study summary.

Executive Summary:
A ready biodegradability on asulam technical was conducted in a manometric respirometry Test (OECD Guideline for Testing of Chemicals No. 301 F: "Ready Biodegradability: Manometric Respirometry Test"). Aerobic activated sludge (micro-organisms from a domestic waste-water treatment plant) was supplied by the sewage works of Darmstadt, Germany. Asulam technical was investigated for its ready biodegradability in a manometric respirometry test over a period of 28 days. The biodegradation was followed by the oxygen uptake of the micro-organisms during exposure. As a reference item sodium benzoate was tested simultaneously under the same conditions as the test item, and functioned as a procedure control. The degradation rate of asulam was calculated by the oxygen consumption of the aerobic activated sludge microorganisms after 28 days of incubation.
The concentration of asulam was 105 mg/L corresponding to an oxygen demand of about 124 mg/L ($ThOD_{NH4}$) and 182 mg/L ($ThOD_{NO3}$). The concentration of sodium benzoate was 103 mg/L corresponding to an oxygen demand of about 172 mg/L ($ThOD_{NH4}$) for the procedure control. The study was carried out at 22°C, in the dark. The test item asulam technical contains nitrogen, therefore the evaluation of biodegradation had to be based on the assumption that nitrification occurred, in the following expressed as $ThOD_{NO3}$. The criterion for ready biodegradability under the conditions of a manometric respirometry test is the 10-day window, describing the period between reaching at least 10% degradation and 60% degradation. This period should not exceed 10 days.
After 28 days of incubation the mean biodegradation of asulam technical was 21% ($ThOD_{NO3}$); the 10 day window failed. The nitrate concentration in the controls after 28 days of incubation was 11.9 mg/L (mean). The nitrate concentration in the test item treated vessels was 19.5 mg/L (mean). Therefore, a partial nitrification occurred. The degradation rate of Asulam Technical under consideration of nitrification did not reach 60% within the 10-day window and after 28 days of incubation, therefore, asulam technical is considered not to be readily biodegradable, but an inherent biodegradation potential was found. The reference item sodium benzoate was sufficiently degraded to a mean of 104% after 14 days and to a mean of 110% after 28 days of incubation, thus confirming the suitability of the aerobic activated sludge inoculum used. In the toxicity control containing both, the test item and the reference item sodium benzoate, a mean of 47% biodegradation was noted within 14 days and 50% biodegradation after 28 days of incubation (based on $ThOD_{NH4}$). Thus, the test item can be assumed to be not inhibitory to the aerobic activated sludge micro organisms.

Materials and Methods
Test Material:
Asulam, purity 974.5 g/kg, Batch no.: 071105-I

Test Design:
Aerobic activated sludge, micro organisms from a domestic waste water treatment plant, was supplied by the sewage plant Darmstadt, Germany. This was washed by centrifugation and the supernatant liquid phase was decanted. The solid material was re-suspended in tap water and centrifuged again. This procedure was done three times. The sediment was re-suspended in test water and aerated overnight. An aliquot of the final sludge suspension was weighed, dried and the ratio of wet sludge to its dry weight was determined. Based on this ratio, calculated aliquots of washed sludge suspension, corresponding to 1.5 g dry material per litre were mixed with test water. This suspension was used for the experiment.
The test flasks were incubated at 20 ± 2 C. The pressure decrease in the reaction vessels was measured over complete experimental phase of 28 days using the BSB Sensomat system, Aqualytic Langen. The test flasks were closed gas-tight by a measuring head. Potassium hydroxide solution (45%) was used for trapping the produced carbon dioxide. The amount of O_2 consumed by the activated sludge was calculated from the decrease of pressure in the reaction vessel.

The composition of the flasks is shown below.

Treatment	Flask	Test Item (mg)	Reference Item (mg)	$HgCl_2$ (mL)	Activated Sludge (mL)	Test Water (mL)	Final Volume (mL)
Asulam Technical	1	25.6	—	—	5	239	244
	2	25.4	—	—	5	239	244
Inoculum Control	3	—	—	—	5	239	244
	4	—	—	—	5	239	244
Procedure Control	5	—	25.3	—	5	239	244
Abiotic Control	6	24.9	—	5	—	239	244
Toxicity Control	7	25.0	24.8	—	5	239	244

The amounts of test item and reference item were directly weighed into the test flasks. No emulsifiers or solvents were used, but the solutions were dispersed by stirring to achieve a homogeneous solution of the test item. The closed test flasks were incubated in a climatised room under continuous stirring. The consumption of oxygen was determined by measuring the change of pressure in the flasks. Evolved carbon dioxide was absorbed in an aqueous solution (45%) of potassium hydroxide. The change of pressure in the test flasks was measured by means of a manometric method (BSB/BOD-Sensor-System, Aqualytic, 63231 Neu Isenburg, Germany) each day. Temperature was measured each working day in the temperature-controlled room. pH-values were measured in control and a separately prepared test flask with test item at test start (to prevent loss of test item in the test flasks) and in all flasks at the end of the test using a pH-electrode WTW pH 340i.
The biodegradability (% BOD = mg O_2 per mg test item) exerted after each period was calculated as:

mg O_2 uptake of test item-mg O_2 uptake of inoculum control

mg test item in flask

For the toxicity control, the BOD of the treatment is the mg O_2 uptake of the test item combined with the reference item minus the mg O_2 uptake of the inoculum control divided by the sum of the mg test item and reference item in the flask.
The percentage biodegradation of the test item and of the reference item Sodium Benzoate was calculated as:

$$\% \text{ degradation} = \frac{\text{BOD (mg } O_2\text{/mg test item or reference item)}}{\text{ThOD}_{NH4} \text{ (mg } O_2\text{/mg test item or reference item)}} \times 100$$

or in case of nitrification of the test item:

$$\% \text{ degradation} = \frac{\text{BOD (mg } O_2\text{/mg test item or reference item)}}{\text{ThOD}_{NO3} \text{ (mg } O_2\text{/mg test item or reference item)}} \times 100$$

For N-containing test items as asulam technical, a correction for a potential uptake of oxygen by nitrification was made. For this reason, on exposure days 0 and 28 a sufficient aliquot was withdrawn from the bottles containing the test item and inoculum, and from the inoculum control (after measurement of oxygen concentration) for analysis of nitrate and nitrite using Ion Chromatography. Due to an increase in the concentration of nitrate and nitrite in the test bottles, the oxygen uptake by nitrification was calculated as described in Annex V of the test guidelines. The equipment for nitrogen determination was a Dionex DX 120 ion chromatography system (UV detector UVD 340s and AS 50 Autosampler):

Parameter	NO_2-N + NO_3-N
Wavelength	225 nm
Column (4 mm x 250 mm)	IonPac AS4 A-SC
Guard column (4 mm x 50 mm)	IonPac AG4 A-SC
Eluent (in deionised water)	15 mmol/L KCl
Eluent flow	1.5 mL/min
Temperature	10-30°C

For nitrate and nitrite determination the limits of quantification (LOQ) were calculated from the results of the chromatographic run. The lowest standard of the calibration was used as LOQ

Results and discussion:
The reference item sodium benzoate was sufficiently degraded to 104% after 14 days and to 110% after 28 days of incubation. The percentage biodegradation of the reference item confirms the suitability of the used aerobic activated sludge inoculum.

In the toxicity control containing both the test and the reference item 47% biodegradation was noted within 14 days and 50% biodegradation was determined after 28 days of incubation (based on $ThOD_{NH4}$). Since nitrification occurs, the biodegradation of the toxicity control was determined to be 50% at the end of the test. According to the test guidelines the test item can be assumed to be not inhibitory on the aerobic activated sludge micro organisms because degradation was >25% within 14 days.
The oxygen demand in the abiotic control was zero. No correction of the test item degradation rates had to be done. The oxygen demand of the inoculum control (medium and inoculum) was 25 mg O_2/L and thus not greater than 60 mg O_2/L within 28 days as required by the test guideline. The pH-value of the test item flasks at the end of the test was within the range of pH 6.0 to 8.5 as required by the test guideline. Nitrogen is part of the molecular structure of the test item; therefore nitrification ($ThOD_{NO3}$) was considered for the evaluation of results. The 10-day window was not reached. After correction for the mean biochemical oxygen demand of the inoculum controls the mean biodegradation percentage based on $ThOD_{NH4}$ at the end of the incubation period of 28 days was 31% without consideration of nitrification. In this case the ThOD has to be corrected. Respirometric methods with oxygen uptake as the analytical procedure may be influenced significantly by the oxygen uptake resulting from ammonium oxidation. In the case of a partial nitrification of Asulam Technical, or transformation of ammonium to nitrate, the degradation was 13% after 28 days. The concentration of nitrite in the test solutions was below the quantification limit (0.05 mg/L). The nitrate concentration in the controls after 28 days of incubation was 11.9 mg/L (mean). The nitrate concentration in the test item treated vessels was 19.5 mg/L (mean). Therefore, a partial nitrification occurred.

Conclusions:
The degradation rate of asulam technical did not reach 60% within the 10-day window and after 28 days of incubation. Therefore, asulam technical is considered not to be readily biodegradable, but an inherent biodegradation potential was found.

B.8.4.4 Water/sediment studies

This study has previously been evaluated at EU level. The conclusions are consistent with the EFSA conclusions report and no concerns were raised at EU level.

a) An aerobic water/sediment study was conducted according to BBA IV: 5-1 (1990) guidelines and to GLP. The study was considered acceptable by the Rapporteur.

Samples of two natural water/sediment systems were equilibrated in borosilicate glass cylinders for 82 days at 20 ± 2°C in the dark. Sediment (2mm sieved) was added to each replicate unit to a 2.5 cm depth and surface water was added to achieve a 6 cm depth. The water phase was agitated slightly on an orbital shaker and moistened air was drawn over the surface. Water and sediment characteristics are presented in Table B.8.52. Following equilibration ^{14}C-asulam (radiochemical purity 98.7%, specific activity 73.6µCi/mg), dissolved in sodium hydroxide solution (0.025 M) to form the sodium salt, was added at 142.3 µg, 10.47µCi, (equivalent to an application rate of

4.4 kg a.i./ha). Treated flasks were purged with CO_2 free air and fitted with traps for polar organic volatiles (ethanediol) and for CO_2 (2M NaOH). All units were then incubated at 20±2°C in the dark for up to 153 days. Measurement of the redox potential and dissolved oxygen indicated that the water phase remained aerobic and the sediment phase remained anaerobic throughout.

Duplicate units were analysed at day 0 and at 1, 3, 7, 14, 30, 62, 104 and 153 days after application. The zero hour samples were given a time allocation of 10 minutes (the time to remove the water from the sediment and start HPLC analysis). Radioactivity in the water was quantified by LSC and HPLC. Confirmatory analysis for asulam and its metabolites was by LC/MS. Sediment was extracted with acetone:water (50:50 v/v) and radioactivity in extracts quantified by HPLC. One extracted sediment sample from each aquatic system at 62 and 104 days was re-extracted with sodium hydroxide solution (0.5 M) at 50°C followed by centrifugation.

Table B.8. 131 Characterisation of sediment and associated water

Name	System 1 Mill Stream Pond	System 2 Emperor Lake
Sediment		
% Sand (63 µm to 2 mm)	37.10	29.68
% Silt (2 µm to 63 µm)	39.37	53.94
% Clay (< 2 µm)	23.53	16.08
BBA Classification	Medium sandy loam	Medium clay silt
ADAS (UK) Classification	Clay loam	Sandy silt loam
Dry mass (%)	24.7	42.2
% Organic carbon	5.8	3.4
% Organic matter	10.0	5.8
pH: H_2O	7.8	5.8
pH: KCl	7.5	4.7
Maximum water holding capacity (%)	124.4	86.8
Cation exchange capacity (me/100 g)	33.7	26.9
Total nitrogen (mg/kg)	5817	3038
Total phosphorus (%)	1761	695
Biomass Start	2148.8	293.5
Biomass End	818.1	481.3
Water		
At collection:		
pH	8.29	6.01
Temperature (ºC)	15.3	11.6
% Oxygen content (just below surface)	122	94
% Oxygen content (5 cm above sediment)	122	90
Laboratory measurements:		
% Organic carbon	35.0	33.7
Hardness (CaCO3, mg/L)	204	49.0
Total nitrogen (mg/kg)	2.8	<0.05
Total phosphorus (mg/kg)	0.2	0.1

Radioactivity in the extract and residue (humin fraction) were determined by LSC. The sodium hydroxide extract was acidified (pH2) and radioactivity in the supernatant (fulvic acid fraction) was determined following centrifugation and neutralisation. The solid residue was reconstituted in sodium hydroxide (0.5M) and again the radioactivity determined (fulvic acid fraction). One extracted soil sample from each aquatic system at 153 days were re-Soxhlet extracted with acetonitrile:water (80:20 v/v). Radioactivity in the volatile traps was determined by LSC. A summary of the recovery and characterisation of radioactivity in water, sediment and total system is presented in Table B.8. 132 to Table B.8. 136.

Mean recoveries were acceptable for both systems with an overall mean recovery 96.6% AR. The pattern of degradation of asulam was comparable in both water/sediment systems (see Table B.8. 133 to Table B.8. 136). Soxhlet extraction performed on single replicates for the 153 day timepoint sediments only liberated a further 5 to 6% of AR. Bound residue fractionation performed on 62 day and 104 day samples showed fairly even distribution of radioactivity between fulvic acid, humic acid and humin fractions (see Table B.8. 137). It was suggested by the Notifier that radioactivity associated with the sediment fractions may be tightly bound asulam or its degradates or incorporated breakdown products (including carbon dioxide). Up to 14% and 3% AR was recovered from sodium hydroxide traps in Emperor Lake and Mill Stream Pond systems respectively. Only negligible amounts of AR (<0.1%) were retained in the ethanediol traps.

Asulam was a major component in all surface water and sediment samples analysed. Summation of the separate phases to consider the aquatic system as a whole showed a steady decline in the amount of asulam present down to 26 and 17% AR after 153 days. Four degradation products were identified by co-chromatography with analytical standards, acetyl asulam, sulphanilic acid, sulphanilamide and acetyl sulphanilamide (none greater than 3.8%). Seven other unknown metabolites also occurred. No unidentified metabolite exceeded 2.9% AR.

Table B.8. 132 Percent recovery of applied radioactivity from a water/sediment study conducted with ^{14}C-asulam on two natural systems (results are means of duplicate samples) (Purser 1998a)

Time (days)	Water	Extract of Sediment	Sediment Residue	Sodium Hydroxide	Total
System 1: Mill Stream Pond					
0.007	87.9	9.3	0.3	na	97.5
1	83.7	13.1	1.5	0.1	98.4
3	74.5	19.6	4.0	0.2	98.3
7	67.7	24.0	6.0	0.3	98.0
14	55.8	28.0	12.1	0.4	96.4
30	48.0	26.3	19.9	0.8	95.0
62	42.3	22.3	28.5	1.2	94.3
104	26.2	16.4	51.0	1.9	95.5
153	21.5	12.0	56.4	3.0	93.0
System 2: Emperor Lake					

0.007	90.6	5.5	0.3	na	96.3
1	83.3	12.9	1.5	<0.1	97.8
3	73.3	18.7	5.3	0.1	97.5
7	68.7	22.0	6.9	0.4	98.0
14	55.6	25.5	15.2	0.5	96.7
30	49.5	24.3	21.4	2.2	97.3
62	42.7	18.2	30.4	4.3	95.9
104	27.9	10.9	45.3	11.0	95.2
153	18.2	7.9	57.9	13.9	97.9

Table B.8. 133 Percent of applied radioactivity present as asulam and its degradation products (determined by HPLC) extracted from Mill Stream Pond surface water following ^{14}C-asulam application (means of duplicate samples)

Time (days)	Asulam	Acetyl asulam	Sulfanil -amide	Individual Unk 2	Group of Unknowns	Bkg	Total allocated
0.007	87.3	nd	nd	nd	nd	0.6	87.9
1	82.5	nd	nd	nd	nd	0.4	82.9
3	71.5	nd	0.5	0.4	nd	0.4	72.8
7	65.7	nd	nd	nd	nd	0.3	66.0
14	54.9	nd	0.5	0.3	nd	0.4	56.0
30	46.6	0.7	nd	nd	nd	0.5	47.8
62	38.8	nd	0.2	0.2	nd	0.2	40.6
104	24.2	nd	0.6	nd	nd	0.2	25.9
153	18.2	nd	1.2	0.8	nd	0.1	20.3

nd = not detected

Table B.8. 134 Percent of applied radioactivity present as asulam and its degradation products (determined by HPLC) extracted from Emperor Lake surface water following ^{14}C-asulam application (means of duplicate samples)

Time (days)	Asulam	Sulfanil -amide	Individual Unk 2	Group of Unknowns	Bkg	Total
0.007	93.7	nd	nd	nd	0.1	93.9
1	83.9	nd	nd	nd	0.6	84.5
3	71.5	0.4	nd	nd	0.5	72.4
7	67.8	0.30	nd	nd	0.4	68.4
14	54.2	1.2	nd	nd	0.2	55.5
30	47.1	1.0	0.5	nd	0.4	48.9
62	39.5	1.8	nd	nd	0.3	41.9
104	24.8	2.0	0.3	0.9	0.2	28.0
153	12.7	2.3	0.6	nd	0.1	17.3

nd = not detected

Table B.8. 135 Percent of applied radioactivity present as asulam and its degradation products (determined by HPLC) extracted from the sediment of two natural water/sediment systems following ^{14}C-asulam application (means of duplicate samples)

Time (days)	Asulam	Acetyl asulam	Sulfanilic acid	Sulfanil -amide	Acetyl Sulfanil -amide	Total Unknowns	Bkg	Total allocated
System 1: Mill Stream Pond								
0.007	8.2	nd	nd	0.2	nd	nd	0.1	8.5
1	11.0	nd	nd	0.5	nd	nd	0.1	11.5
3	18.7	nd	nd	0.3	nd	nd	0.1	19.2
7	20.5	0.2	nd	0.7	nd	1.8	0.2	23.4
14	20.1	1.3	nd	0.3	nd	3.8	0.2	25.6
30	21.7	0.2	0.2	0.3	0.1	1.1	0.4	24.0
62	15.7	0.1	nd	0.8	nd	3.5	0.2	20.4
104	13.4	nd	nd	0.3	0.2	0.3	<0.1	14.2
153	8.1	0.1	0.1	0.4	0.3	0.7	0.1	9.7
System 2: Emperor Lake								
0.007	3.3	nd	nd	nd	nd	nd	< 0.1	3.3
1	12.2	nd	nd	0.5	nd	0.5	< 0.1	13.1
3	15.4	0.4	nd	0.4	nd	0.5	<0.1	16.6
7	18.4	0.3	nd	0.5	nd	2.2	0.2	21.6
14	17.2	0.4	0.1	0.9	nd	4.1	0.1	22.8
30	18.3	0.3	0.1	0.9	0.2	2.5	0.3	22.5
62	13.6	0.2	nd	1.5	nd	2.5	0.1	17.9
104	7.1	nd	nd	1.0	0.2	1.4	0.1	9.8
153	4.0	0.1	nd	1.0	0.2	1.0	< 0.1	6.3

nd = not detected

Table B.8. 136 Percent of applied radioactivity present as asulam and its degradation products (determined by HPLC) extracted from two natural water/sediment systems (total system) following ^{14}C-asulam application (means of duplicate samples)

Time (days)	Asulam	Acetyl asulam	Sulfanilic acid	Sulfanil -amide	Acetyl Sulfanil -amide	Total Unknowns	Bkg	Total allocated
System 1: Mill Stream Pond								
0.007	95.5	nd	nd	0.2	nd	nd	0.7	96.3
1	93.5	nd	nd	0.5	nd	nd	0.5	94.4
3	90.2	nd	nd	0.8	nd	0.4	0.5	92.0
7	86.2	0.2	nd	0.7	nd	1.8	0.5	89.3
14	75.0	1.3	nd	0.8	nd	4.1	0.5	81.6
30	68.4	1.0	0.2	0.3	0.1	1.1	0.8	71.8
62	54.5	0.1	nd	1.0	nd	5.0	0.4	60.9

104	37.5	nd	nd	0.9	0.2	1.3	0.2	40.1
153	26.3	0.1	0.1	1.5	0.3	2.5	0.1	30.9
System 2: Emperor Lake								
0.007	97.0	nd	nd	nd	nd	nd	0.2	97.2
1	96.1	nd	nd	0.5	nd	0.5	0.6	97.6
3	86.9	0.4	nd	0.8	nd	0.5	0.6	89.1
7	86.2	0.3	nd	0.8	nd	2.2	0.6	90.0
14	71.4	0.4	0.1	2.0	nd	4.1	0.3	78.3
30	65.3	0.3	0.1	1.9	0.2	3.0	0.7	71.4
62	53.1	0.6	nd	3.3	nd	2.5	0.4	59.8
104	31.9	nd	nd	3.0	0,2	2.5	0.3	37.8
153	16.7	0,8	nd	3.4	1.0	1.8	0.2	23.7

nd = not detected

Table B.8. 137 Percentages of applied radioactivity associated with humin, humic acid and fulvic acid soil fractions of the sediment phase of both water/sediment systems at 62 days and 104 days after application of ^{14}C-asulam at a rate equivalent to 4.4 kg a.s./ha

System and Sampling Time	Humin Fraction	Humic Acid Fraction	Fulvic Acid Fraction
Mill Stream Pond, 62 days	14.2	13.1	7.2
Mill Stream Pond, 104 days	22.6	14.0	9.5
Emperor Lake, 62 days	11.8	8.8	8.5
Emperor Lake, 104 days	16.4	12.0	11.7

Data for the water phase and total system was processed by the Notifier using first order kinetics and a DFOP model. Since results for the first order model gave acceptable results, these are presented in Table B.8.59.

Table B.8. 138 Calculated degradation rates of ^{14}C-asulam for the water phase and total system using first order kinetics

Phase	**Total System**			**Water phase***		
System	**DT_{50}**	**DT_{90}**	**r^2**	**DT_{50}**	**DT_{90}**	**r^2**
Mill Stream Pond	82	273	0.99	68	224	0.96
Emperor Lake	63	211	0.99	58	192	0.98

* for the water phase, the DT50 values represent dissipation rates, since they include loss due to partitioning to sediment.

For the sediment phase an enhanced symmetry decay curve with an accumulation phase was fitted from the following equation using Microsoft Excel software. However

this approach was not considered appropriate by the Rapporteur and the results have been excluded.

$$y = \frac{a.k1}{(k2-k1)}\ x\ (e^{\left(-k1.t^{b}\right)} - e^{\left(-k2.t^{c}\right)})$$

(Purser, 1998a)

This study has not previously been evaluated at EU level. It has been evaluated at member-state level. The conclusions presented are in line with those from the previously submitted report and the EFSA conclusions report.

c) **Report:**
H. Willems (1997a) Degradation of asulam in aerobic aquatic environment. Notox 's-Hertogenbosch, The Netherlands for AgriChem BV. Unpublished report No. 187717.

Guidelines:
EU95/36/EC,
Deviations: None

GLP: Yes

RMS opinion on study: fully acceptable. Please note the summary below is the applicant's. It is an accurate account of the report and considered acceptable by the RMS. The RMS has provided comments at the end of the study summary.

Executive Summary:

Radiolabelled ([ring-U-^{14}C]) asulam was aerobically incubated in two different, uncontaminated water/sediment systems in the dark at 20°C ± 2°C. Asulam was applied at a rate of 1.4-1.9 mg a.i./L in the water layer. This is equivalent to an application rate of 4.4 kg/ha and assuming homogeneous distribution in a 30 cm water layer. Activity was fractionated into $^{14}CO_2$, volatile compounds, activity in the water layer, sediment extractable residues (methanol extraction) and sediment unextractable residues. Asulam degraded in both water/sediment systems with a DT_{50} of 59 days in the OVP system and 17 days in the SW system. Degradation did not follow 1st order degradation kinetics and slowed down with time. The estimated DT_{90} values were 652 days in one system (OVP) and 191 days in the other (SW) system. Considering only the water layer, the DT_{50} values were 34 and 10 days in the OVP and SW systems, respectively.
No metabolites occurring at levels above 10 % were observed in water phases or in sediments after 120 days of incubation or at any other time during incubation. The remaining activity at 120 d was for the largest part recovered as asulam in the water layer (6-30%). Complete mineralization and volatilization were not responsible for the degradation of asulam in both systems.

Materials and Methods
Test Material:
Asulam, purity 95.4%, Batch no.: CFQ9686

Test Design:
The study was conducted with [ring-U-^{14}C]-asulam in two different, uncontaminated water/sediment systems in the dark at 20°C ± 2°C.
The radiochemical purity of ^{14}C-labelled asulam was determined by radio-TLC analysis before the start and after finalization of the experiments. A small amount of ^{14}C labelled asulam was dissolved in MeOH and an aliquot (5 µL) was applied to a Si-60 F254 10x10 cm plate. The plate was developed in ethyl acetate/methanol (95:5 v/v) and re-developed in acetic acid/methanol/benzene (20:15:65 v/v/v). The plates were scanned on a Bioscan System 200 Imaging Scanner.
The systems were:

1) Oostvaardersplassen (OVP). "Oostvaardersplassen is an area endiked from the lake Ijssel, situated near Lelystad, about 30 kilometres north east of Amsterdam. The lake has been closed for river Rhine water for approx. 25 years

2) Schoonrewoerdsewiel (SW). Schoonrewoerdsewiel is a pool near Leerdam, approximately 60 kilometres south of Amsterdam.

The properties of the systems are tabulated below.

Water characteristics:

Characteristic	System 1 Water	System 2 Water
Name	OVP	SW
At collection:		
pH	8.0	7.7
Temperature (ºC)	9.1	11.5
Oxygen content (mg/L)	10.1	4.0
Redox potential (mV, 10 cm below surface)	130	-33
Laboratory measurements:		
Organic carbon (DOC, mg/L), beginning/end	123.6/61.0	26.2/22.4
Hardness (CaCO3, mg/L), beginning/end	578/599	305/623
Total nitrogen (mg/L), beginning/end	< 0.05/0.7	< 0.05/3.5
Total phosphorus (mg/L), beginning/end	0.2/0.6	< 0.05/8.1

Sediment characteristics:

Characteristic	System 1 Sediment	System 2 Sediment
Name	OVP	SW
At collection:		
Redox potential (mV)	+17	+93
Laboratory measurements:		
Particle size distribution:		
Sand (63 µm to 2 mm, %)	28.1	21.8
Silt (2 µm to 63 µm, %)	50.8	65.1
Clay (<2 µm, %)	21.1	13.0
BBA Classification		
ADAS (UK) Classification		
Dry mass (%)	37	16
Organic carbon (%)	4.3	11.3
Cation exchange capacity (me/100 g) beginning/end	14.5/19.5	26.2/22.4
Total nitrogen (mg/kg), beginning/end	2660/2121	6734/7966
Total phosphorus (%), beginning/end	735/770	1502/1263
ATP, beginning/end	213.8/54.5	13.3/42.2

The incubation system consisted of 1L dark brown glass metabolism flasks through which C02 free, humidified air was passed at a rate of 30-60 mL/min. The C02 was removed by passing the air through a 2N sodium hydroxide solution and the air was humidified by passage through Milli-Q water. The outgoing air first passed through two traps containing 2N NaOH to trap released CO_2 and then through one trap containing 2-methoxyethanol to trap volatile organic compounds.
Approximately 200 to 230 g of sieved SW sediment and 118 to 122 g of sieved OVP sediment were weighed out in dark brown glass metabolism flasks, within two days of collection. For each sediment, 25 flasks were prepared. Filtered water from OVP and SW were added to the respective sediment-containing metabolism flasks so that the solid content for the SW system was 7.5-8.5 % and 9.0-9.2 % for the OVP system. The sediment layer thicknesses were 2.5 cm and 2.0 cm for the SW and OVP systems, respectively.

The metabolism flasks were connected to the above described incubation system, except that only one trap filled with Milli-Q water was used. A magnetic floater was inserted in each metabolism flasks which was gently agitated to maintain aerobic conditions in the water layer and the top of the sediment during pre-incubation.
The pre-incubation period was 10.5 weeks at 20°C ± 2°C during which 0_2 content, pH and redox potential were weekly measured in the water layer of two metabolism flasks of each water/sediment system. The water level was maintained constant during pre-incubation by adding OVP or SW water when necessary.
The concentration of asulam in the water layer was calculated based on a maximum application rate of 4.4 kg/ha, assuming equal application to the water surface as to the soil surface and homogeneous distribution in a 30 cm water layer. The calculated target concentration of asulam was 1.47 mg/L. Hereto, [ring-U-14C] asulam was dissolved in methanol. This solution contained 121 kBq per 80 µL, which is equivalent to approximately 39.5 µg asulam per 80 µL (S.A. = 3.06 MBq/mg). For each

water/sediment system, 18 metabolism flasks were spiked with 80 µL of this solution. The remaining asulam was added as unlabeled material dissolved in methanol (3.88 mg/mL). The resulting actual starting concentrations were 1.94 mg/L in the OVP water layer and 1.47 mg/L in the SW water layer. In addition, 2 metabolism flasks of each water/sediment system were spiked with only unlabelled asulam at the same concentrations for the purpose of oxygen, pH, and redox potential measurements during incubation.

The treated metabolism flasks were reconnected to the incubation system and incubated in the dark at 20°C + 2°C. A magnetic floater was inserted in each metabolism flask and gently agitated to maintain aerobic conditions. The water level was maintained constant during incubation by adding OVP or SW water when necessary. Oxygen content, pH, and redox potential of the water layer were weekly measured in two metabolism flasks of each water/sediment system. Two metabolism flasks of each water/sediment system were removed from the incubation system after 0, 1, 2, 7, 14, 28, 58, 90, and 120 days of incubation. The water layer was carefully decanted, weighed and radioactivity in a 1 mL aliquot was measured by liquid scintillation counting (LSC). The sediment and decanted water layer were stored at -20°C until further analysis.

Quantitative Analysis

Microbial activity in the sediments was assessed through ATP measurements after the acclimatization period and at the end of the incubation period (treated sediments). The ATP measurements were performed by EPAS, Eco Process Assistance, HC-University Gent, Technologiepark 3 - 9052 Gent (Zwijnaarde).

Water layer. The water layer was concentrated to approximately 100 mL on a rotary evaporator at 60°C. The concentrated water layer was acidified to pH 3 by adding phosphate buffer and then partitioned four times with ethyl acetate (1 x 200 mL, 3 x 100 mL). Radioactivity in a 1 mL aliquot of the aqueous phase was measured by LSC. The ethyl acetate fractions were combined and evaporated to dryness on a rotary evaporator at 30°C. The residues were redissolved in 5 mL methanol and radioactivity in a 100 µL aliquot was measured by LSC. The extracts were stored at -20°C until further analysis.

Sediment. The sediment was extracted three times with 100 ml methanol for 30 minutes on a shaker. The extract was concentrated on a rotary evaporator at 40°C and the remaining aqueous residue was partitioned (for clean up purposes) once with 200 mL ethyl acetate after the pH was adjusted to approximately pH 8.5 by adding IN sodium hydroxide solution. The basic ethyl acetate fraction was discarded and the pH of the remaining aqueous phase was adjusted to pH 3 by adding phosphate buffer. The acidified aqueous phase was then four times partitioned with 100 mL ethyl acetate. The radioactivity in the aqueous phase was measured by LSC of a 1 mL aliquot. The ethyl acetate fractions were combined and evaporated to dryness. The residues were redissolved in 5 ml methanol and radioactivity was measured in a 100 µL aliquot. The extracts were stored at -20°C until further analysis.

The remaining (unextractable) residues were subjected to a more rigorous extraction procedure: overnight soxhlet extraction with 300 ml ethyl acetate. Radioactivity in a 1 mL aliquot of the ethyl acetate-soxhlet extract was measured by LSC.

Measurement of $C0_2$ production. At the above mentioned sampling times, the volume of sodium hydroxide solution in the traps was measured and a 1 mL aliquot was taken for the determination of radioactivity by LSC.

Measurement of volatile organic compounds. At the above mentioned sampling times, the volume of 2-methoxy-ethanol in the trap was measured and a 1 mL aliquot was taken for the determination of radioactivity by LSC.

Measurement of residual activity in the sediment. Approximately 0.5 to 1.0 g air dried sediment, remaining after the final extraction step, was combusted in a Harvey OX-500 oxidizer. The released CO_2 was trapped in an absorbent liquid and radioactivity measured by LSC.

Qualitative analysis
TLC analysis of water and sediment extracts. Thin layer chromatography was performed by applying 10-20 µL of extract on Si-60_{254} 20x20 cm plates. The plates were developed in acetic acid/methanol/benzene (20:15:65 v/v/v). Location and distribution of radioactivity on the plates was determined by scanning the plates on a Bioscan 200 Imaging Scanner. Selected extracts (10-20 µL) were also spotted on RP-18_{254} 20x20 cm plates and developed in milli-Q water/THF/acetic acid (40:55:5 v/v/v).

Results and Discussion:
The radio-purity of ^{14}C-labelled asulam was measured as95.4 % at the start of the study and 95.6 % at the end of the study.
The average dissolved oxygen content in the OVP water layer was 6.8 mg/L (±1.2 sd) and 6.9 mg/L (±0.9 sd) in the SW water layer, indicating aerobic conditions throughout the incubation period.

The results of the pH, dissolved oxygen and redox potential measurements in the water layer of the OVP and SW water/sediment systems during acclimatization and incubation are given in Table B.8. 139. All values are the average of two independent measurements.

The total recoveries *(%* of applied) of radioactivity from the OVP and SW water/sediment systems are given in Table B.8. 140 and Table B.8. 141. All the values presented are averaged values from two replicate metabolism flasks. Mass balances averaged 106.5 % (±2.8 sd) and 100.5 % (±5.4 sd) for the OVP and SW systems, respectively. Individual mass balances were always between 90% and 110 %. Complete mineralization of the aromatic moiety of asulam was minimal in both water/sediment systems. After 120 d of incubation less than 2% of asulam was mineralized in both systems. No volatile organics were formed in both water/sediment systems.

Table B.8. 139 Oxygen, redox potential and pH of water layer of OVP and SW water/sediment systems.

Week	Oostvaardersplassen			Schoonrewoerdsewiel		
	O_2 (mg/L)	Redox potential (mv)	pH	O_2 (mg/L)	Redox potential (mv)	pH
-10	6.5	-206.1	7.9	6.6	-193.5	8.2
-9	6.3	-152.4	8.1	7.1	-117.6	8.1
-8	5.0	-121.2	7.8	7.5	-88.4	8.0
-7	6.0	-47.4	8.0	7.5	-74.7	8.2
-6	5.9	-78.5	7.7	7.9	-61.7	8.3
-5	6.5	-24.7	7.9	5.9	-34.0	7.9
-4	7.5	-40.8	7.9	5.0	-17.9	7.7
-3	7.0	-32.4	7.7	6.1	-12.7	7.6
-1	5.9	-30.8	7.8	6.9	2.2	7.7
0	7.5	3.8	7.8	7.5	9.9	7.3
1	3.7	6.6	7.7	6.7	20.4	7.7
2	5.0	-27.0	7.8	7.3	13.2	7.7
3	5.9	23.8	7.7	6.6	17.7	7.7
4	6.2	17.9	7.5	7.1	25.6	7.7
5	7.1	79.2	7.8	6.1	122.8	7.7
6	7.7	46.7	8.0	5.8	47.1	7.5
7	7.9	145.3	7.6	6.8	195.9	7.3
8	8.1	134.2	8.0	6.4	148.2	7.7
9	5.8	189.4	7.5	7.9	188.0	7.5
10	7.8	161.9	7.7	6.4	185.4	7.0
11	7.7	156.0	7.9	6.7	181.9	7.4
12	8.1	137.9	7.8	4.8	148.1	7.5
13	8.0	165.0	8.0	7.3	140.9	7.8
14	8.0	104.5	7.8	7.4	112.7	7.5
15	8.3	124.3	7.9	7.4	200.8	7.7
16	7.8	133.3	8.3	9.2	112.5	8.1
17	7.2	140.9	8.3	7.5	186.9	8.0

Table B.8. 140 Recovery of radioactivity from Oostvaardersplassen water/sediment system

Days	% of applied radioactivity in:				
	CO_2	Volatiles	Water Layer	Sediment	Total
0	0.0	< 0.01	105.7	0.9	106.6
1	0.1	< 0.01	93.7	15.8	109.6
2	0.1	< 0.1	82.9	26.1	109.1
7	0.3	< 0.01	77.7	29.5	107.5
14	0.5	< 0.01	68.9	34.6	104.0
28	0.7	< 0.01	68.2	40.4	109.3
58	0.7	< 0.01	60.5	45.2	106.4
90	2.0	< 0.01	40.4	59.3	101.7
120	1.7	< 0.01	36.5	65.7	103.9

Table B.8. 141 Recovery of radioactivity from Schoonrewoerdswiel water/sediment system

Days	% of applied radioactivity in:				
	CO_2	Volatiles	Water Layer	Sediment	Total
0	0.0	< 0.01	97.9	6.9	104.8
1	0.1	< 0.01	76.9	23.7	100.7
2	0.2	< 0.1	57.1	36.5	93.8
7	0.3	< 0.01	49.8	50.0	100.1
14	0.4	< 0.01	54.0	51.4	105.8
28	0.7	< 0.01	39.9	65.0	105.6
58	0.7	< 0.01	24.5	80.5	105.7
90	1.5	< 0.01	15.6	78.6	95.7
120	1.7	< 0.01	8.7	82.1	92.5

In both systems, the total amount of radioactivity in the water layer decreased with time to about 8 % in the SW system and about 36 % in the OVP system after 120 days of incubation. This decrease, in the absence of mineralization and volatilization, is primarily the result of the transfer of radioactive residues from the water layer to the sediment. Only a small portion (< 5 %) of the residue in the water layer of both systems is very polar and remains in the aqueous phase after partitioning with ethyl acetate.

The detailed distribution, based on TLC analysis, of radioactivity in the organic phase is shown in Table B.8. 142 (OVP) and Table B.8. 143 (SW). In both systems, almost all the radioactivity found in the water layer was in the form of asulam. Four minor metabolites were found in the OVP water layer, always below 10 % of the total activity applied. In general, results of the TLC analysis of OVP day 28 extracts were not in line with the other OVP measurements (presence of Met D, low amount of asulam). This is probably due to impurities in the extract which interfered with the TLC analysis (OVP 28 days extract was dirty compared to the other OVP extracts). Therefore the OVP 28 day organic phase was analysed by two-dimensional TLC (same mobile phase). Two of the metabolites were tentatively identified as methylbenzenesulphonylcarbamate (Met F) and sulphanilamide (Met D). In the SW water layer, six minor metabolites were detected of which two were tentatively identified as MBSC (Met F) and sulphanilamide (Met D). None of the metabolites in the SW water layer was found at a level > 10 %.

Sediment extractable residues reached a maximum at 7 days and remained fairly level up to day 14 in the OVP sediment and up to day 58 in the SW sediment. After this period, the extractable residues decreased to approximately 7 %. The decrease in extractable residues is the result of increased unextractability which is most likely caused by bound residue formation through oxidative coupling processes of anilinic transformation products. The extractable residues partitioned mainly to the organic phase. The Unextractable residues increased continuously with incubation time and reached 58.0 % and 73.2 % after 120 days of incubation. A more rigorous extraction (soxhlet ethyl acetate) did not release these bound residues to a significant extent (except in the SW system at 14 days).

The detailed composition of the organic phase of the extractable residues in the sediment for OVP and SW are given in

Table B.8. 144 and Table B.8. 145 respectively. From the tables it is clear that the majority of sediment extractable residues was in the form of asulam in both water/sediment systems. Seven minor metabolites (always < 10 %) were detected in OVP and SW extracts. One of the metabolites was identified as MBSC (Met F). Met D was tentatively identified as sulphanilamide.

Table B.8. 142 Composition of radioactivity in the Oostvaardersplassen water layer (organic partition)

Days	% applied radioactivity as:							
	Met A	Met B	Met C	Met D	Asulam	Met E	Met F	Met G
0	nd	nd	nd	nd	96.7	nd	4.9	nd
1	nd	nd	nd	nd	90.9	nd	nd	nd
2	nd	nd	nd	nd	81.0	nd	nd	nd
7	nd	nd	nd	nd	70.5	4.4	nd	nd
14	nd	nd	nd	nd	63.3	nd	1.7	nd
28	nd	nd	nd	9.3	45.6	8.1	2.0	nd
58	nd	nd	nd	nd	53.7	2.1	0.7	nd
90	nd	nd	nd	nd	34.3	nd	0.7	0.4
120	nd	nd	nd	nd	29.8	nd	1.9	nd

Table B.8. 143 Composition of radioactivity in the Schoonrewoerdswiel water layer (organic partition)

Days	% applied radioactivity as:							
	Met A	Met B	Met C	Met D	Asulam	Met E	Met F	Met G
0	nd	nd	nd	nd	91.9	8.3	nd	nd
1	nd	nd	nd	nd	70.9	4.7	nd	nd
2	nd	nd	nd	1.4	43.6	5.6	0.9	4.3
7	nd	nd	nd	nd	45.9	2.7	nd	nd
14	0.2	nd	nd	8.4	31.5	3.8	8.2	nd
28	nd	nd	1.0	2.5	27.2	7.0	0.6	nd
58	0.1	nd	nd	nd	18.3	4.3	nd	nd
90	< 0.1	nd	nd	nd	11.9	1.1	0.5	0.3
120	nd	nd	nd	nd	6.0	nd	nd	nd

Table B.8. 144 Composition of radioactivity in the Oostvaardersplassen sediment (organic extract)

Days	% applied radioactivity as:							
	Met A	Met B	Met C	Met D	Asulam	Met E	Met F	Met G
0	nd	nd	nd	nd	nd	nd	< 0.1	nd
1	< 0.1	0.1	nd	0.52	5.8	0.9	nd	0.6
2	nd	0.5	nd	0.7	6.3	2.1	1.3	1.6
7	0.3	0.2	nd	nd	15.1	1.2	1.2	nd
14	0.1	nd	nd	0.6	16.3	1.7	nd	nd
28	0.1	nd	nd	0.4	10.1	1.5	0.3	0.8
58	0.1	nd	0.1	nd	7.1	2.3	0.2	0.9
90	nd	nd	nd	nd	7.3	1.0	1.9	0.5
120	nd	nd	nd	nd	6.9	nd	nd	nd

Table B.8. 145 Composition of radioactivity in the Schoonrewoerdswiel sediment (organic extract)

Days	% applied radioactivity as:							
	Met A	Met B	Met C	Met D	Asulam	Met E	Met F	Met G
0	nd	nd	1.4	0.5	0.3	nd	1.0	nd
1	0.1	nd	1.1	2.3	4.5	2.0	4.0	nd
2	nd	nd	nd	nd	5.4	3.9	9.4	nd
7	0.3	nd	nd	nd	10.9	6.8	2.8	8.4
14	1.0	nd	nd	1.0	18.8	1.1	nd	0.4
28	nd	nd	nd	0.5	19.9	2.6	nd	1.8
58	0.3	nd	nd	nd	16.8	3.8	0.5	4.1
90	0.1	1.5	nd	nd	5.7	nd	2.9	0.3
120	0.3	nd	nd	0.4	4.6	1.2	0.9	nd

Table B.8. 146 Composition of radioactivity in the Oostvaardersplassen total system (water layer plus sediment)

Days	% applied radioactivity as:								
	Polar	Met A	Met B	Met C	Met D	Asulam	Met E	Met F	Met G
0	4.1	nd	nd	nd	nd	96.7	nd	4.9	nd
1	2.9	< 0.1	0.1	nd	0.5	96.7	0.9	nd	0.6
2	2.2	nd	0.5	nd	0.7	87.3	2.1	1.3	1.6
7	3.0	0.3	0.2	nd	nd	85.4	5.6	1.2	nd
14	4.1	0.1	nd	nd	0.6	79.6	1.7	1.7	nd
28	3.5	0.1	nd	nd	9.7	55.7	9.6	2.3	0.8
58	4.3	0.1	nd	0.1	nd	60.8	4.4	0.9	0.9
90	5.6	nd	nd	nd	nd	41.6	1.0	2.6	0.9
120	5.6	nd	nd	nd	nd	36.8	nd	1.9	nd

Table B.8. 147 Composition of radioactivity in the Schoonrewoerdswiel total system (water layer plus sediment)

Days	% applied radioactivity as:								
	Polar	Met A	Met B	Met C	Met D	Asulam	Met E	Met F	Met G
0	2.1	nd	nd	1.4	0.5	92.2	8.3	1.0	nd
1	1.5	0.1	nd	1.1	2.3		6.7	4.0	nd
2	1.5	nd	nd	nd	1.4	49.0	9.5	10.3	4.3
7	2.0	0.3	nd	nd	nd	56.8	9.5	2.8	8.4
14	2.1	1.2	nd	nd	9.4	50.3	4.9	8.2	0.4
28	1.8	nd	nd	1.0	3.0	47.1	9.6	0.6	1.8
58	2.5	0.4	nd	nd	nd	35.1	8.1	0.5	4.1
90	2.4	0.2	1.5	nd	nd	17.6	1.1	3.4	0.6
120	4.9	0.3	nd	nd	0.4	10.6	1.2	0.9	nd

First order, square root first order and square root 1.5 order kinetics were fitted to the asulam degradation patterns in the total system (water + sediment) and in the water layer alone. The resulting kinetics (best fit) and DT_{50}/DT_{90} values are given in Table B.8. 148.

Table B.8. 148 DT_{50} and DT_{90} values for asulam aerobic degradation in OVP and SW water/sediment systems

System	Best Fit	r-squared	DT_{50} (d)	DT_{90} (d)
OVP water	sq root 1.5th order	0.95	34.2	932
OVP total	sq root 1.5th order	0.94	59.2	652
SW water	sq root 1st order	0.93	10.2	113
SW total	sq root 1st order	0.87	17.3	191

The DT50 values calculated in Table B.8. 148 above are not considered appropriate by the RMS. The DT50 values have been re-calculated in accordance with currentFOCUS kinetics (2006) in a kinetic re-evalution by Hardy (2011a).

Asulam degraded in both water/sediment systems with a DT_{50} of 59 days in the OVP system and 17 days in the SW system. Considering only the water layer, the DT_{50} values were 34 and 10 days in the OVP and SW systems, respectively.
No metabolites occurring at levels above 10 % were observed after 120 days of incubation or at any other time during incubation, except after 2 days in the total SW system where one metabolite (methylbenzenesulphonylcarbamate) was observed at 10.3 % (water + sediment). In the opinion of the applicant, the results for the SW day 2 sample was an outlier as it did not fit in the observed asulam degradation pattern. The RMS disagrees with this (see comments below).

RMS comments
Overall recoveries are acceptable being in the range 90 – 110%. No pH data is provided for the sediments although it is provided for the water characteristics. The RMS notes that a number of metabolites were detected. Met A, B and C are minor metabolites (max 1.4%). Met D is tentatively assigned as sulphanilamide reaching a maximum of 9.7% AR (day 28) in the Oostvaardersplassen total system. Met E reached a maximum of 9.6% AR and is unidentified. Although this metabolite remains below 10% AR, which was in accordance with the guidance at the time of the study, under modern guidance Met E exceeds 5% on 2 or more consecutive timepoints and should have been identified and given further consideration. Met F is tentatively identified as methylbenzenesulphonylcarbamate (MBSC) reached a maximum of 10.3% AR at day 2 in the Schoonrewoerdswiel total system. The applicant considers this an outlier as it 'does not fit the observed asulam degradation pattern'. The RMS does not agree with this and considers that further evidence should have been provided to support this statement or, since the metabolite exceeds 10% AR it should be considered as a major metabolite. The RMS requested further data from which has been provided by the applicant which includes surface water exposure modelling.

(Willems, 1997a)

This study has previously been evaluated at EU level. The conclusions are consistent with the EFSA conclusions report and no concerns were raised at EU level.

Report:
I.A.J. Hardy (2008c) Asulam: Kinetic Modelling Analysis of Data from Water/Sediment Study. Battelle UK Ltd, Ongar, Essex, England for United Phosphorus limited
Unpublished report No.: NZ/08/001A
Guidelines:
EU95/36/EC, FOCUS recommended procedures
Deviations: None
GLP:
Not relevant

A kinetic evaluation of the water/sediment study reported in the original DAR (Purser 1998a) was performed by the notifier. A full summary of the Purser 1998 study is presented in section B.8.4.4a of the DAR. The study was conducted in accordance with EU/95/36EC, FOCUS recommended procedures. Since the study was modelling it was not a GLP study but was performed to Good Modelling Practice and considered acceptable by the RMS.

The kinetic re-evaluation of the water sediment study was performed by the notifier using the computer program KinGUI in accordance with FOCUS kinetics guidance (FOCUS 2006) for derivation of modelling endpoints for use in the exposure assessment. The DT50 values for asulam in the two water sediment systems, Mill Stream Pond and Emperor Lake were re-calculated based on SFO kinetics. For both systems the Day 0 values for the total system were set to the total recovered radioactivity (see Table B.8. 149).

Table B.8. 149 Percent recovery of applied radioactivity from a water/sediment study conducted with ^{14}C-asulam on two natural systems (results are means of duplicate samples) (Purser 1998a)

Time (days)	Water	Extract of Sediment	Sediment Residue	Sodium Hydroxide	Total
System 1: Mill Stream Pond					
0.007	87.9	9.3	0.3	na	97.5
1	83.7	13.1	1.5	0.1	98.4
3	74.5	19.6	4.0	0.2	98.3
7	67.7	24.0	6.0	0.3	98.0
14	55.8	28.0	12.1	0.4	96.4
30	48.0	26.3	19.9	0.8	95.0
62	42.3	22.3	28.5	1.2	94.3
104	26.2	16.4	51.0	1.9	95.5
153	21.5	12.0	56.4	3.0	93.0
System 2: Emperor Lake					
0.007	90.6	5.5	0.3	na	96.3
1	83.3	12.9	1.5	<0.1	97.8
3	73.3	18.7	5.3	0.1	97.5
7	68.7	22.0	6.9	0.4	98.0
14	55.6	25.5	15.2	0.5	96.7
30	49.5	24.3	21.4	2.2	97.3
62	42.7	18.2	30.4	4.3	95.9
104	27.9	10.9	45.3	11.0	95.2
153	18.2	7.9	57.9	13.9	97.9

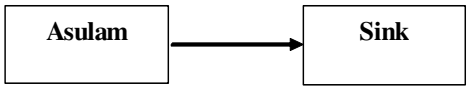
The percentage of applied radioactivity extracted as asulam from the water phase and sediment phase of the two water sediment systems and the total system is presented in Table B.8. 150 below.

Table B.8. 150 Percent of applied radioactivity present as asulam (determined by HPLC) extracted from two natural water/sediment systems following ^{14}C-asulam application (means of duplicate samples)

Time (days)	Water phase	Sediment phase	Total system
System 1: Mill Stream Pond			
0.007	87.27	8.24	95.52
1	82.49	10.96	93.45
3	71.53	18.68	90.21
7	65.70	20.49	85.69
14	54.87	20.14	75.01
30	46.64	21.71	68.38
62	38.79	15.73	54.51
104	24.16	13.37	37.54
153	18.20	8.10	26.30
System 2: Emperor Lake			
0.007	93.71	6.59	82.00
1	83.92	12.21	96.12
3	71.51	15.38	86.9
7	67.81	18.37	86.2
14	54.18	17.22	71.4
30	47.07	18.26	65.3
62	39.49	13.56	53.1
104	24.75	7.13	31.9
153	12.70	3.95	16.7

The values for the total system were entered into KinGUI by the notifier into the SFO kinetic model below for the whole system and optimisations carried out for M0 and rate constant k using the kinetic modelling scheme presented in Figure B.8. 38. The goodness of fit was assessed visually and by Chi^2 (χ^2) error <15% and t-test >99%. The parameters were validated by the RMS using Microsoft Excel and considered acceptable.

Figure B.8. 38 Kinetic modelling scheme in KinGUI for whole sytem



The total system DegT50 and DegT90 calculated using single first order kinetics are summarised below. The geometric mean (whole system) DT50 71.9 d was considered acceptable by the RMS for use in the exposure assessment. The visual fits are presented in Figure B.8. 39 and Figure B.8. 40. The calculated DT50 and DT90 values are summarised in Table B.8. 151.

Figure B.8. 39 SFO model fit for Mill Stream Pond Total System

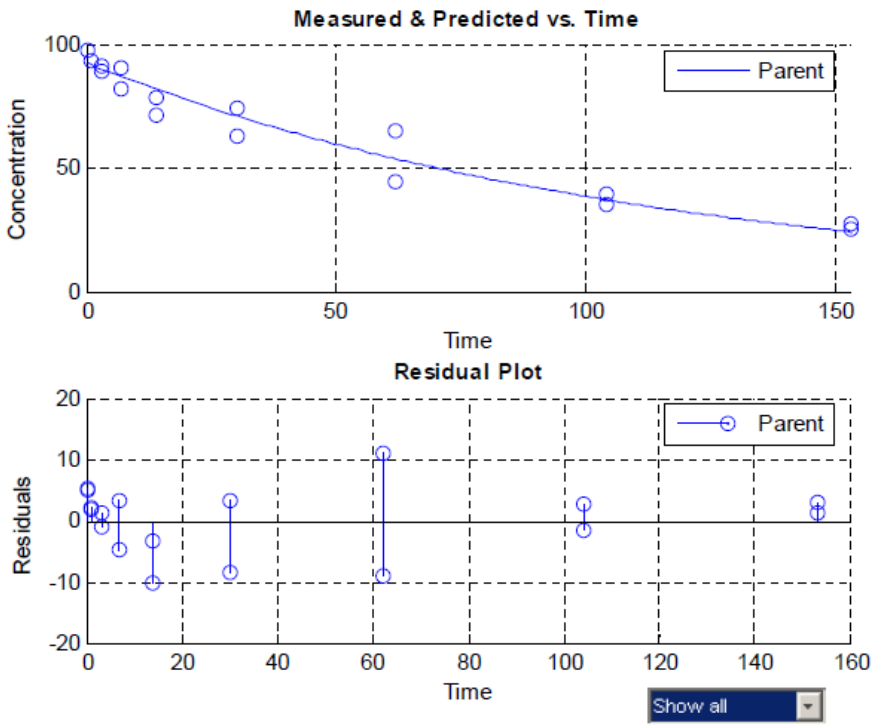


Figure B.8. 40 SFO model fit for Emperor Lake Total System

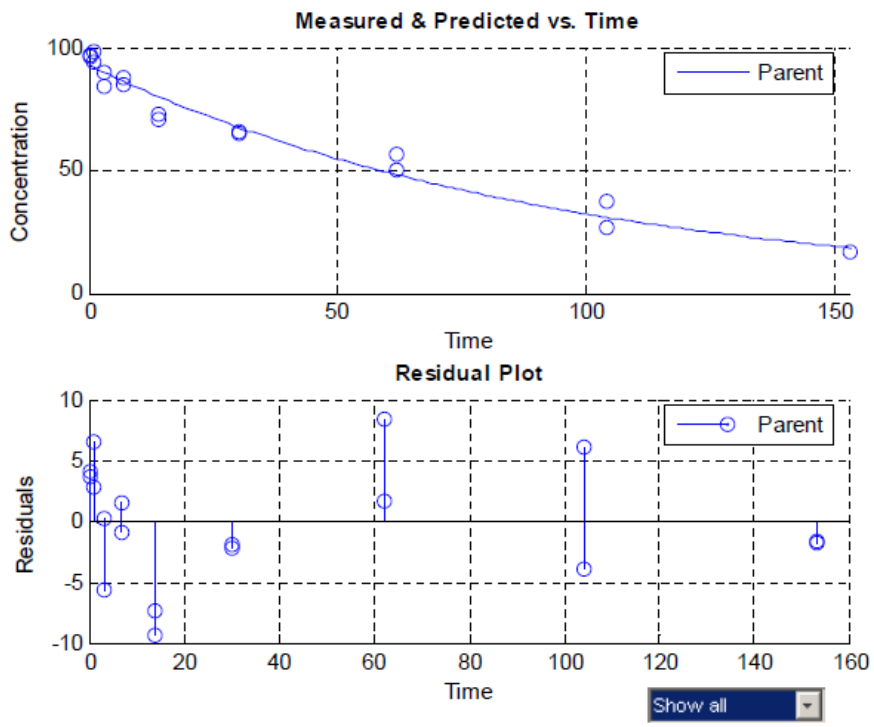


Table B.8. 151 Asulam parameter optimisation results (SFO) for the Mill Stream Pond and Emperor Lake systems

System	M0 (%)	k (d^{-1})	$DegT_{50}$ (days)	$DegT_{90}$ (days)	Min χ^2 error	t-test
Mill Stream Pond	92.38	0.0088	78.8	261.9	3.6	3.8E-10, > 99%
Emperor lake	92.48	0.0106	65.6	217.9	4.8	3.7E-11, > 99%
Geometric mean			**71.9**	**238.9**		

Hardy and Patel, (2008c)

This study has not previously been evaluated at EU level. It has been evaluated at member-state level. The conclusions are similar to those from the previously submitted report and the EFSA conclusions report.

Report:
I.A.J. Hardy. (2011a) Kinetic Modelling Analysis of Asulam and Sulphanilamide Rate of Degradation and Asulam Water / Sediment Studies to Derive Modelling Endpoints Battelle UK Ltd, Ongar, Essex, England for Agrichem BV
Unpublished report No.: OU/10/001A

Guidelines:
EU 91/414/EEC, Section 5, Point 7.1.1.
Deviations: None

GLP:
No, conducted according to Good Modelling Practice recommendations

The applicant has performed a kinetic re-evaluation of the data from the water/sediment study of Willems (1997a) based on FOCUS guidance (2006) using KinGUI versions 1.0 and 1.1. A full summary of the Willems (1997a) study is presented in section B.8.4.4c.

Two systems were investigated, Oostvaardersplassen (a lake) and Schoonrewoerdsewiel (a pool). The residue data from the water phases is presented in Table B.8. 142 and Table B.8. 143 and the total systems in

Table B.8. 146 and Table B.8. 147.

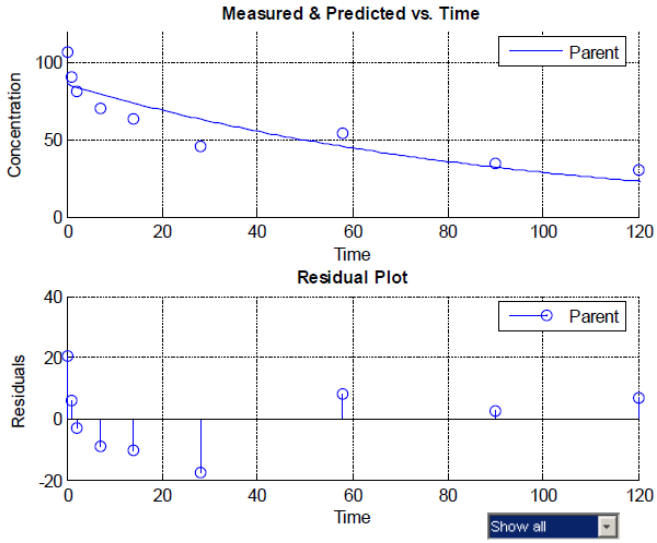
The RMS has validated the applicant's kinetic evaluation using KinGUI version 2.1.

Optimisations using SFO kinetics did not provide acceptable fits for the water phases of either system. Since the DT90 was not reached in the Oostvaardersplassen water phase during the study period, FOMC kinetics were not considered. DFOP and HS kinetics both provided excellent fits. DFOP was considered the best fit both visiually and statistically by the RMS. The fits for Oostvaardersplassen water phase are summarised in Table B.8. 152.

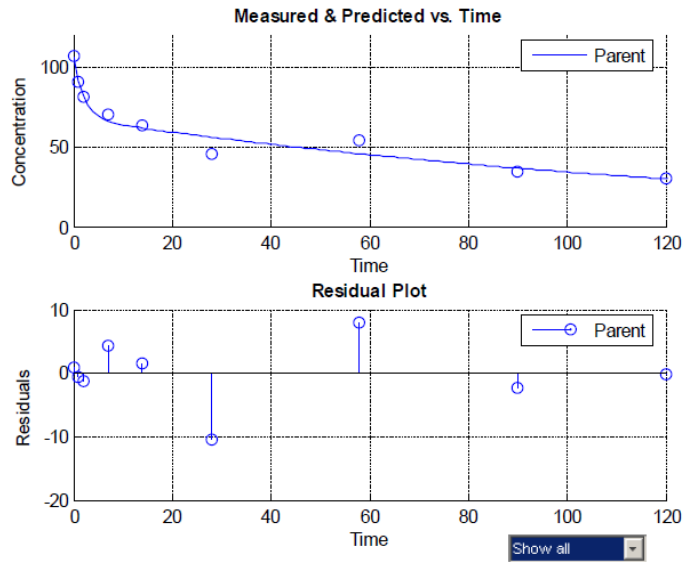
Table B.8. 152 **parameter optimisation results for Oostvaardersplassen water phase – free optimisation**

Oostvaardersplassen water phase	DT_{50} (days)	DT_{90} (days)	Min Chi^2 error (%)	Parameter	t-test (-)	Visual assessment
SFO	63.1	210	13.8	k: 0.0110	0.0026 >99%	Poor
DFOP	36.6	272	6.7	k_1 0.4531 k_2 0.0068 g: 0.3583	k_1 0.0535 >94% k_2 0.0046 >99% g: 0.0011 >99%	Excellent
HS	37.6	263	6.9	k_1 0.1394 k_2 0.0071 tb: 3.2132	k_1 0.0201 >97% k_2 0.0034 >99%	Excellent

Oostvaardersplassen water phase, SFO fit, Chi2 = 13.8%



Oostvaardersplassen water phase, DFOP fit, Chi2 = 6.7%



Oostvaardersplassen water phase, HS fit, Chi2 = 5.9%

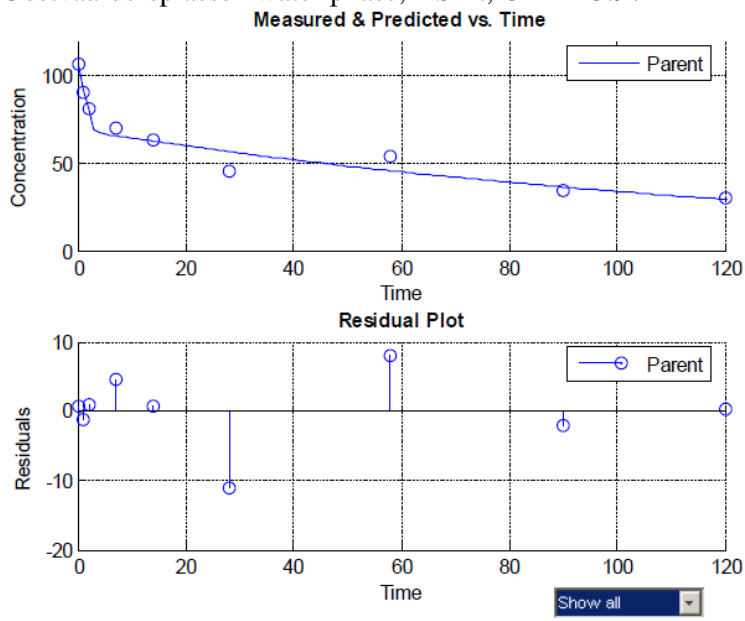
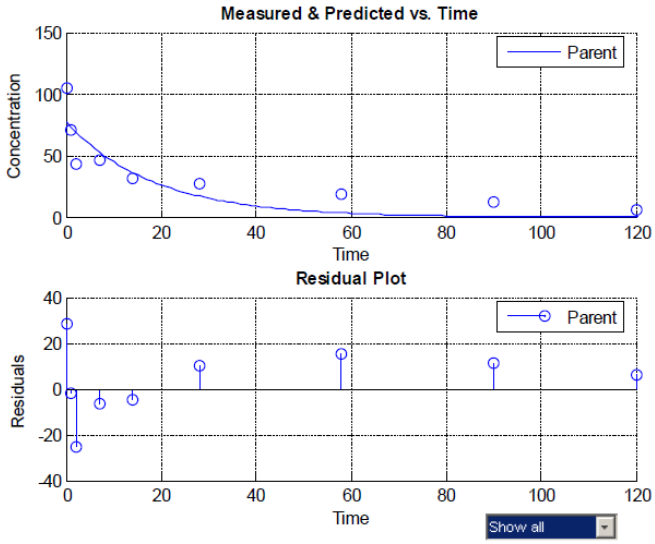


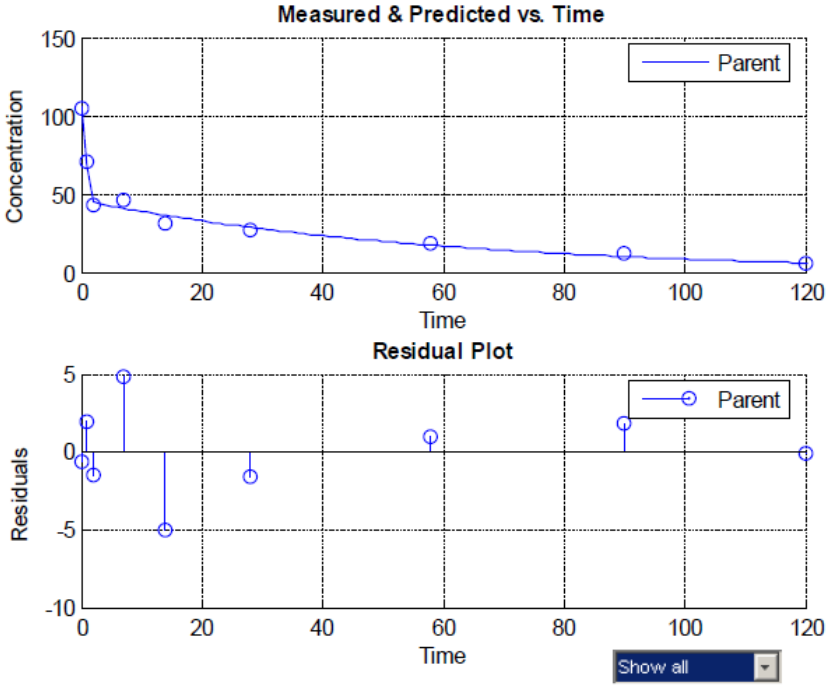
Table B.8. 153 **parameter optimisation results for Schoonrewoerdsewiel water phase – free optimisation**

Schoonrewoerdsewiel water phase	DT_{50} (days)	DT_{90} (days)	Min Chi^2 error (%)	Parameter	t-test (-)	Visual assessment
SFO	12.9	42.9	29.7	k: 0.0110	0.0026 >99%	Poor
FOMC	2.4	261	12.7			Poor
DFOP	1.8	91.1	8.8	k_1 1.0002 k_2 0.0156 g: 5859	k_1 0.0062 >99% k_2 0.0049 >99%	Excellent
HS	**1.6**	**87.6**	**5.9**	**k_1 0.4243** **k_2 0.0169** **tb: 2.0209**	**k_1 5.0E-5 >99%** **k_2 7.5E-4 >99%**	**Excellent**

Schoonrewoerdsewiel water phase SFO fit, Chi2 = 29.7%



Schoonrewoerdsewiel water phase HS fit, Chi2 = 5.9%

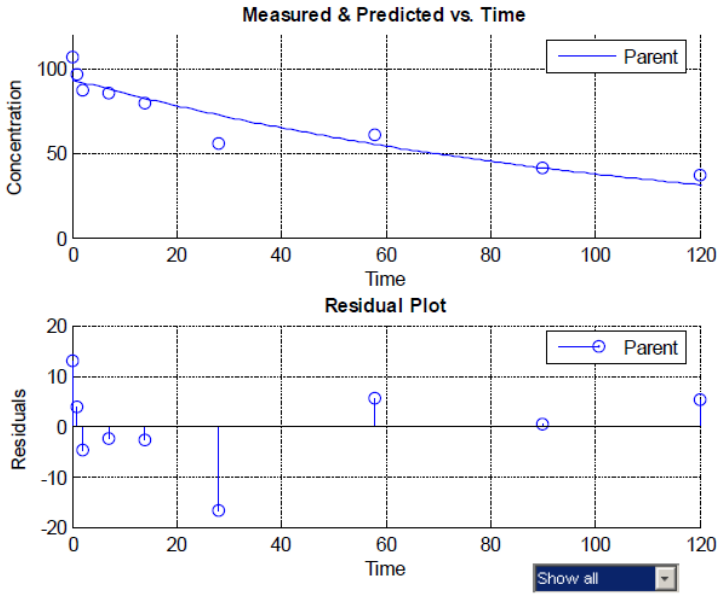


An acceptable SFO visual and statistical fit was only provided for the Oostvaardersplassen total system. The fits for Oostvaardersplassen total system are summarised in

Table B.8. 154.

Table B.8. 154 **parameter optimisation results for Oostvaardersplassen total system – free optimisation**

Oostvaardersplassen total system	DT_{50} (days)	DT_{90} (days)	Min Chi^2 error (%)	Parameter	t-test (-)	Visual assessment
SFO	**76.2**	**253**	**8.8**	**k: 0.0016**	**3.4E-4 >99%**	**Good**



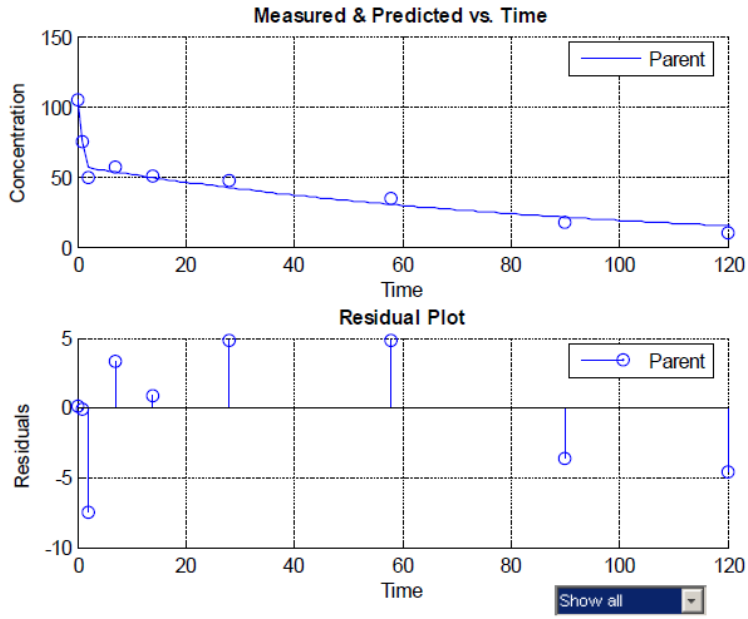
For the Schoonrewoerdsewiel total system, SFO kinetics did not provided an acceptable visual or statistical fit (Chi2 21.9%). DFOP and HS both provided excellent visual and statistical fits. HS kinetics provided the best visual and statistical fit. The kinetic fits are presented in Table B.8. 155**.**

Table B.8. 155 **parameter optimisation results for Schoonrewoerdsewiel total system – free optimisation**

Schoonrewoerdsewiel total system	DT_{50} (days)	DT_{90} (days)	Min Chi^2 error (%)	Parameter	t-test (-)	Visual assessment
SFO	41.7	139	21.9	k: 0.0059	0.0128 >98%	Poor
DFOP	9.5	150	9.4	k_1 1.0002 k_2 0.0156 g: 5859	k_1 0.0537 >94% k_2 0.0042 >99%	Excellent
HS	**8.9**	**153**	**7.4**	**k_1 0.4243** **k_2 0.0169** **tb: 2.0209**	**k_1 0.0071 >99%** **k_2 0.0012 >99%**	**Excellent**

The HS visual fit is presented in Figure B.8. 41 below.

Figure B.8. 41 **Asulam HS model for the Schoonrewoersewiel total system**



The water phase modelling endpoints are presented in Table B.8. 156**.** The SFO DT50 value for Oostvaardersplassen has been calculated from the DFOP slow phase rate constants [ln(2)/k2]. Oostvaardersplassen system did not reach 10% of the initial concentration. The Schoonrewoerdsewiel water phase DT50 was calculated by dividing the HS DT90 by 3.32. The RMS considers this acceptable since <10% of the original material remains at the study end in accordance with FOCUS kinetics.

Table B.8. 156 **Asulam aquatic systems: water phase modelling endpoints**

System	SFO DT_{50} (days)	Origin
Oostvaardersplassen	97.6	LN(2)/DFOP slow-phase k_2 (0.0071)
Schoonrewoerdsewiel	26.4	HS DT90/3.322
Geometric mean	**50.8**	

The total system SFO DT50 values for modelling are presented in Table B.8. 157**.**

Table B.8. 157 **Asulam aquatic systems: total system modelling endpoints**

System	SFO DT_{50} (days)	Origin
Oostvaardersplassen	76.2	SFO
Schoonrewoerdsewiel	61.9	LN(2)/HS slow-phase k_2 (0.0112)
Geometric mean	**68.7**	

(Hardy, 2011a)

The values from the two water/sediment system studies, each with two contrasting systems to give four in total, were combined to allow a mean total system value to be calculated. The DT50 values from both water/sediment studies for the total system, including the geometric mean DT50 for use in exposure modelling is presented in Table B.8. 158**.**

Table B.8. 158 **Proposed Modelling Endpoint for Asulam in Water/Sediment Total System**

Total system	DT_{50} (days)	Derivation
Mill Stream Pond	78.8	SFO
Emperor Lake	65.6	SFO
Oostvaardersplassen	76.2	SFO
Schoonrewoerdswiel	61.9	HS k_2 *
Geometric mean	**70.3**	

* k_2 = slow phase rate constant

A maximum occurrence of 9.7% sulphanilamide was detected in the asulam water sediment study [Willems 1997] and no degradation parameters could be determined. SFO kinetics provided an acceptable fit (both visually and statistically for the Mill Stream Pond, Emperor Lake and Oostvaardersplassen total systems. For the Schoonrewoerdswiel total system, the SFO kinetic fit was not acceptable, biphasic kinetics were more appropriate, the best fit being afforded by Hockey Stick. For modelling, the DT50 was calculated from the slow phase rate constant (ln(2)/k2).

As a conservative estimate, a DT50 value of 1000 days was selected for sulphanilamide.

B.8.4.5 Aquatic dissipation in the field

B.8.4.6 Summary & assessment – fate and behaviour in water

Asulam was stable to hydrolysis at all pH values (pH 5, 7, and 9) over 31 days. There was insufficient degradation to calculate degradation half lives.

An aqueous photolysis study was performed with asulam sodium in sterile buffer solutions at pH 4 and pH9 at 25°C. DT_{50} values for asulam were calculated assuming single first order kinetics to be 0.44 days (pH 4) and 0.87 days (pH9) following artificial illumination (equivalent to 0.78 and 1.56 days of 52°N summer sunlight respectively) (see Table B.8.45). Estimated photolytic half life of asulam in natural surface waters, calculated from the quantum yield, ranged from 7 to 119 hours at pH 4 and 8 to 135 hours at pH 9 in central European latitudes (52°N). Photolytic half life of asulam was also calculated from the quantum yield using the GC Solar program of Zepp and Cline to generate data at 40°, 50° and 60°N by season, at the surface and at depths of 30 and 100cm in the water body. A number of photodegradates were produced at both pH's. The major photodegradate at pH 4 was sulphanilic acid which reached a maximum of 55.5% AR after 20 hours. The major photodegradates at pH 9 identified as N-(4-aminophenyl)formamide and (4(4-

methoxycarbonylaminophenyl)aminophenyl)carbamic acid reached maxima of 24.2 and 11.9% respectively. DT50 values were calculated for the metabolites using ModelManager, however the confidence limits were very wide and were considered by the Rapporteur to be unacceptable.

An aqueous photolysis study was performed with asulam sodium in sterile natural water at 25°C to meet Japanese registration requirements. The DT_{50} was calculated using first order kinetics to be 0.84 days (equivalent to 4.21 days spring sunlight at 35°N in Japan, which is comparable to Athens, Southern Europe). It is noted from dark water sediment studies below that the partitioning of asulam from the water phase to sediment was relatively slow (DT_{50} 58 to 68 days) compared to the photolytic half-life suggesting that asulam would be available in the surface water for photolysis to occur. Many minor photodegradates were formed, all <10% AR. DT_{50}'s were not calculated for these metabolites. In natural surface water, none of the major (i.e. >10%) metabolites identified in the photolysis study performed in sterile buffer solutions summarised above were formed in significant amounts. The Notifier has proposed that the results from the natural water study are more relevant than from the sterile buffer study for the purposes of the exposure assessment, since the effects of indirect phototransformation are considered alongside those of direct phototransformation in the natural water study.

Although the Rapporteur notes that these two aqueous photolysis studies were performed in separate studies under contrasting illumination schemes (i.e. equivalent to either Northern Europe or Southern Europe in each study) and in waters of different pH, overall the Rapporteur considered that the Notifier's case regarding the influence that indirect phototransformation may have on the formation of metabolites was plausible. However the Rapporteur also considered that in the absence of further information on the influence of direct and indirect phototransformation under comparable experimental conditions (i.e. identical illumination, pH, incubation conditions etc) it was not possible to conclude that aqueous photolysis metabolites would not be formed in major amounts in the upper layers of some natural surface waters. In the absence of specific aquatic toxicity data on the major photolysis metabolites formed in the sterile buffer study (i.e. sulphanilic acid, N-(4-aminophenyl)formamide and (4(4-methoxycarbonylaminophenyl) aminophenyl) carbamic acid) no calculation of PECsw values has been performed. Further information is required to assess the environmental relevance of these photolysis metabolites.

A further aqueous photolysis study was also performed with asulam in sterile buffer at pH 4 and pH 9 at 25°C. The DT50 values calculated using SFO kinetics were comparable with the original study above, 0.28 days (pH 4) and 0.86 days (pH 9) following artificial illumination equivalent to 0.54 days (pH4) and 1.64 days (pH9) in natural sunlight at 52°N. The calculated quantum yield was 0.168 at pH 4 and 0.0612 at pH 9 from which the half-life of asulam in the top few millimetres ranged from 0.183 days in Summer at latitude 40° N to 5 days in Winter at latitude 60° N and that of asulam at pH 9 to ranged from 0.25 days in Summer at latitude 40° N to 5.1 days in Winter at latitude 60° N. A number of photodegradates were observed in the irradiated samples. Seven of these exceeded 4% of applied radioactivity. The second most abundant photodegradate reached a maximum of 15.4% and was identified as N (4-aminophenyl)formamide. Sulphanilic acid, a known metabolite of asulam, was

confirmed to be present at levels up to 18.9% of applied radioactivity. There was no evidence for further degradation of the observed photodegradates. No significant degradation of asulam was observed in the non-irradiated system.

A supplementary study was performed to clarify quantification of four metabolites found at between 5% and 10% of applied radioactivity in the above study. At pH 4, the only major metabolite (>10% AR) was identified as sulphanilic acid in the previous study and reached a maximum 61.3%. No other photodegradates reached 4%. In particular the degradate of interest, rrt = 0.95, which reached between 5% and 10% in the previous study, was shown to be very minor and so no further identification of this component was attempted. At pH 9, two major photodegradates (i.e. >10% of applied radioactivity) reached maxima of 18.4% (sulphanilic acid) and 10.6% (N-(4-aminophenyl)formamide). A third photodegradate, at rrt = 0.50, reached 6.2% (benzenesulphonic acid). Three degradates of interest, which reached between 5% and 10% in the previous study which in this study reached 6.1%, 4.1% and 7.2% respectively. These three regions of radioactivity were investigated further and calculated to be respectively 2.2%, 1.4% and 3.0% AR. Thus no unidentified component in the pH 9 samples reached 4% and all considered of minor significance by the applicant and no further attempt at identification was made.

The ready biodegradability of asulam was investigated in two studies. Asulam showed 31% and 51% biodegradation over 28 days, based on biochemical oxygen demand asulam was considered not readily biodegradable.

The fate and behaviour of asulam was investigated in 2 studies totalling 4 aerobic laboratory (dark, 20°C) natural water/sediment systems. Asulam was a major component in all surface water and sediment samples analysed. Partitioning of radioactivity between the surface water and sediment was observed in both studies. The quantity of asulam in the surface water declined from initial levels of 87% to 96% initially to 18% and 13% after 153 days in the first study and 36 to 8.7% after 120 days in the second study. In the first study, small quantities of (up to 2.8%) of sulphanilamide and an unknown compound (up to 0.74%) were detected in the surface water of both systems. Acetyl asulam was detected in one system (up to 1%) after 30 days. In the sediment there was a corresponding increase in the percentage of applied radioactivity as asulam from 8% and 7% at the start which increased to a maximum of 22 and 18% after 30 days and then declined to 8% and 4% after 153 days. Small quantities (of up to 1.7, 0.2, 1.7 and 0.4%) of acetyl asulam, sulphanilic acid, sulphanilamide and acetyl sulphanilamide respectively were detected in the sediment of both systems. A number of unknown compounds were detected in total amounts up to 6.8% AR but no individual unknown was greater than 2.5%. Summation of the separate phases to consider the total system showed a steady decline in the amount of asulam present down to 26 and 17% after 153 days. In the degradation pathway proposed by the Notifier, asulam, or its degradates are bound or incorporated into the sediment (up to 58%) or degraded to carbon dioxide (3.0% and 13.9%) or to one of four degradates (acetyl asulam, sulphanilic acid, sulphanilamide and acetyl sulphanilamide), all less than 3.8%.

In the second study, the RMS notes that a number of metabolites were detected. Met A, B and C are minor metabolites (max 1.4%). Met D is tentatively assigned as sulphanilamide reaching a maximum of 9.7% AR (day 28) in the total system. Met E

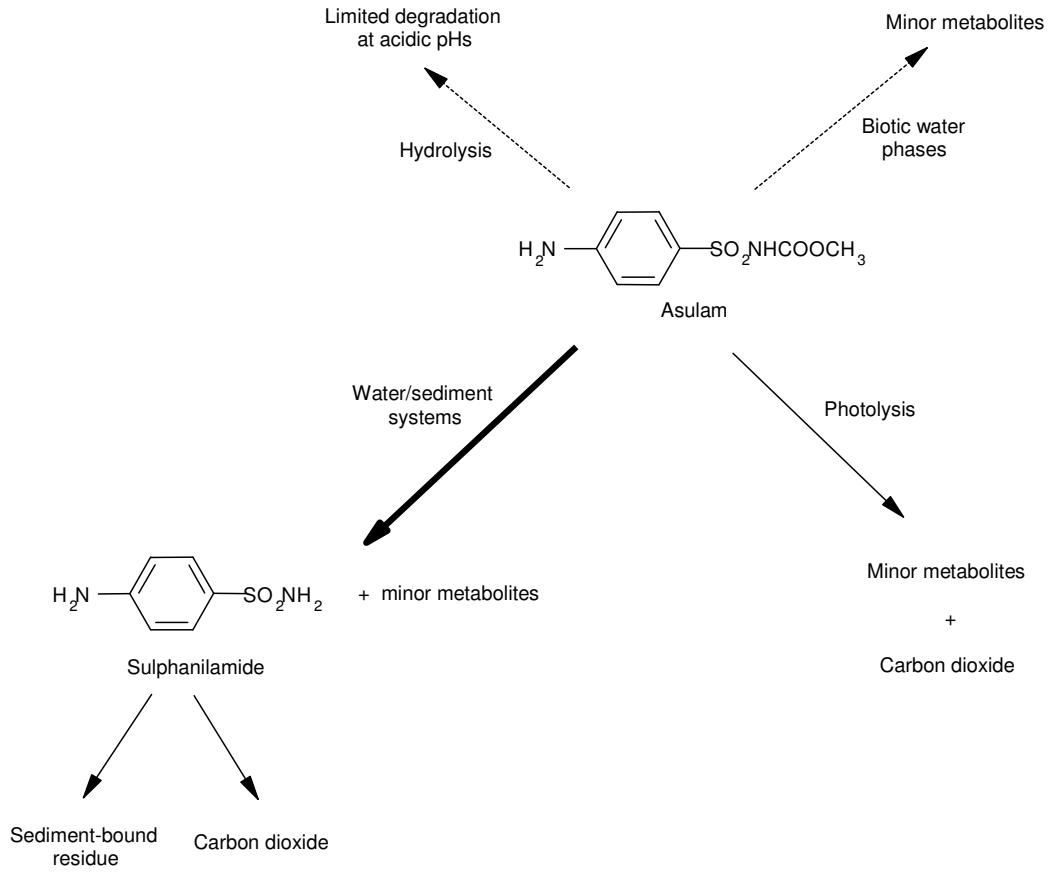
reached a maximum of 9.6% AR and is unidentified. Although this metabolite remains below 10% AR, which was in accordance with the guidance at the time of the study, under modern guidance Met E exceeds 5% on 2 or more consecutive timepoints and should have been identified and given further consideration. Met F is tentatively identified as methylbenzenesulphonylcarbamate (MBSC) reached a maximum of 10.3% AR at day 2 in the total system. The applicant considers this an outlier as it 'does not fit the observed asulam degradation pattern'. The RMS does not agree with this and considers that further evidence should be provided to support this statement or, since the metabolite exceeds 10% AR it is considered by the RMS as a major metabolite and should have been investigated further and included in the surface water exposure modelling. Following a request for further data, the applicant provided soil metabolism and sorption studies and FOCUS sw exposure modelling (Steps 1 – 3).

Water phase dissipation DT_{50} rates for asulam ranged from 26.4 to 97.6 days using a single first order model. Whole system degradation DT_{50} values were 61.9 to 78.8 days (geometric mean 70.3 days).

Degradation in aquatic environments (Points 7.5 to 7.8) Overall Conclusions

A proposed degradation pathway for asulam in aquatic environments is presented in Figure B.8. 42.

Figure B.8. 42 Proposed Degradation Pathway for Asulam Sodium in Aquatic Environments



Hydrolytic degradation of the active substance and metabolites > 10 % ‡	pH 5: Insufficient degradation to calculate degradation half-lives
	pH 7: Insufficient degradation to calculate degradation half-lives
	pH 9: Insufficient degradation to calculate degradation half-lives
Photolytic degradation of active substance and metabolites above 10 % ‡	Buffers pH 9 and pH4 Artificial light equivalent to UK summer sunlight 52°N; Asulam DT_{50}; pH 9: 1.56 days, pH4: 0.78 days pH 9: AP formamide, 24.2%; MCAPAP carbamate, 11.9% pH 4: sulphanilic acid, 55.5% Natural water at 25°C Artificial light equivalent to Japan spring sunlight 35°N comparable with Athens S.Eu. DT50 4.21 d No metabolites >10%
Quantum yield of direct phototransformation in water at Σ > 290 nm	0.157 mol · Einstein $^{-1}$ at pH 9, 0.114 mol · Einstein $^{-1}$ at pH 4 Average half-life in surface waters of Central Europe in June calculated to be 0.5 d at pH 9 and 0.29 d at pH 4.
Readily biodegradable ‡ (yes/no)	Substance considered not readily biodegradable.

Degradation in water / sediment

Parent	Distribution (eg max in water *x* after *n* d. Max. sed x % after *n* d)									
Water / sediment system	pH water phase	pH sed	t. °C	DT_{50} whole sys.	(χ^2)	DT_{50}-DT_{90} water	St. (r^2)	DT_{50}-DT_{90} sed	St. (r^2)	Method of calculation
Mill stream pond	8.29	7.8	20	78.8	3.6					SFO
Emperor lake	6.01	5.8	20	65.6	4.8					SFO
Oostvaardersplassen	8.0	-	20	76.2	8.8					SFO
Schoonrewoerdsewiel	7.7	-	20	61.9	7.4					HS slow phase rate constant
Geometric mean/median				70.3						

Metabolite 1	Distribution (eg max in water *x* after *n* d. Max. sed x % after *n* d)

Water / sediment system	pH water phase	pH sed	t. °C	DT_{50}-DT_{90} whole sys.	St. (r^2)	DT_{50}-DT_{90} water	r^2	DT_{50}-DT_{90} sed	St. (r^2)	Method of calculation
Geometric mean/median										

Mineralization and non extractable residues					
Water / sediment system	pH water phase	pH sed	Mineralization x % after n d. (end of the study).	Non-extractable residues in sed. Max x % after n d	Non-extractable residues in sed. Max x % after n d (end of the study)

Repeat the corresponding rows for as many metabolites as necessary

B.8.5 Impact on water treatment procedures (Annex IIIA 9.2.2)

Not submitted and not required for the representative use of Active X. For potential effects on sewage sludge, see section B.9.10.

B.8.6 Predicted environmental concentrations in surface water and groundwater (PECsw and PECgw) (IIIA 9.2.1, 9.2.3)

Surface Water & Sediment

Report
M Patel, IAJ Hardy and P Lowden (2013) Predicted Environmental Concentrations in Surface Water (PEC_{SW}) and Sediment (PEC_{Sed}) for Asulam and Its Metabolites Following Application to Spinach or Flower-bulbs
Guidelines:
EU 91/414/EEC, Section 5, Point 9.2.3 and Commission Regulation (EC) No 1107/2009
Deviations: None
GLP:
No, conducted according to Good Modelling Practice recommendations

Maximum PEC_{SW} and PEC_{Sed} values for asulam and sulphanilamide were calculated for application at 2.4 kg/ha to spinach or flower-bulbs at FOCUS Steps 1 & 2 and also for asulam at FOCUS Steps 3 & 4 with PEC_{SW} TWAC values as well. For asulam photodegradates conservative maximum PEC_{SW} values have been calculated at Steps 3

& 4. The input parameters were derived from kinetic analyses presented in MII Section 5 and the calculations were conducted to current recommendations.

FOCUS Surface Water Steps 1 and 2

Predicted environmental concentrations in surface water for the active substance following application to spinach and flower bulbs for asulam and major soil metabolite sulphanilamide were calculated following the tiered approach recommended by the FOCUS working group [FOCUS, 2003 (Sanco/4802/2001-rev.2)[3]]. The PEC_{SW} values were calculated using the software package 'Steps 1 – 2 in FOCUS' (Surface water tool for Exposure Predictions – Step 1 and Step 2) version 2.1. In these steps of the exposure assessment, assumptions regarding drift values are based on the existing BBA (2000[4]) values, in addition to a worst-case estimation of the potential loading to surface waters via run-off and drainage. Both tiers of the exposure assessment assume entry to a static water body 30cm deep with a 5cm deep sediment layer with 5% OC and bulk density of 0.8 g/ml. At Step 1 spray drift and runoff/drainage loading are assumed to occur as a single event immediately following the spray application. At Step 2, runoff/drainage loading occurs 4 d after the spray application. The runoff/drainage input enters either the water phase or the sediment phase in proportions determined by the K_{oc}. As a worst case, 0% crop interception was assumed at step 1 and 2.

A summary of the compound related parameters used in the PEC_{SW} calculations is presented in Table B.8. 159. A summary of the worst case scenario data used in Step 1 and Step 2 calculations and the initial or maximum PEC_{SW} values is presented in Table B.8. 159.

Table B.8. 159 Substance related parameters for asulam and sulphanilamide used in FOCUS surface water Step 1 and 2 calculations (based on Hardy et al, 2013)

Parameter	Asulam	Sulphanilamide	Remarks
Molecular weight (g/mol)	230	172	Phys.-chem. properties
Solubility in water (g/L)	962*	1000**	*Phys.-chem. properties **Default for sulphanilamide
Degradation in soil DT_{50} soil (d)	4.6	13.4	Geomean from laboratory aerobic soil degradation studies at 20°C and pF 2
1/n values	0.75	0.71	Arithmetic mean of four soils
Sorption to soil K_{OC} (l/kg)	25.4	166	Mean from soil adsorption studies
Peak formation in soil	-	100 %	Assumed 100% as worst case; Cooper and Hardy (2004b)
Peak formation in water/sediment study	-	9.7 %	Willems (1997)
Degradation in water/sediment DT_{50} whole system	70.3[a]	1000[b]	[a] Arithmetic mean of whole system DT50 assuming DFOP kinetics proposed

[3] FOCUS (2003). FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC. EC Document Reference SANCO/4802/2001-rev.2.
[4] BBA (2000). Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen warden. In: Brundesanzeiger No. 100, p. 9879.

Parameter	Asulam	Sulphanilamide	Remarks
(Step 1) (d) DT_{50} water (Step 2) (d) DT_{50} sediment (Step 2) (d)	 70.3 1000	 1000[b] 1000[b]	by Notifier. Since the whole system DT50 values assuming single first order kinetics gave only a marginally longer mean of 72 d, the value proposed by the Notifier was accepted by the Rapporteur. [b] Worst case assumption for sulphanilamide for all phases

Table B.8. 160 Scenario data used in Step 1 and 2 FOCUS surface water calculations and summary results (based on Hardy, Patel & Lowden 2013)

Crop/ application rate (g/ha)	FOCUS Step 1			Asulam Initial PEC_{SW} (µg/l)	Sulphanilamide Initial PEC_{SW} (µg/l)
	Distance crop-water (m)	Drift (% of application)	Runoff/drainage (% of application)		
Vegetables leafy/bulb (2.4 kg/ha)	1	2.759	10	795.87	86.35
	FOCUS Step 2			Maximum Asulam PEC_{SW} (µg/l)	Maximum Sulphanilamide PEC_{SW} (µg/l)
Vegetables leafy (2.4 kg/ha)	1	2.759	2 (Northern Europe – Mar.-May.)	105.54	15.17 (day 4)
Vegetables leafy (2.4 kg/ha)	1	2.759	4 (Southern Europe – Mar.-May.)	190.15	28.96 (day 4)
Vegetables bulb (2.4 kg/ha)	1	2.759	2 (Northern Europe – Mar.-May.)	96.98	13.80 (day 4)
Vegetables bulb (2.4 kg/ha)	1	2.759	4 (Southern Europe – Mar.-May.)	173.21	26.20 (day 4)

Since the sediment dweller toxicity study was assessed in a water spiked study (see Section B.9.2) it is appropriate to calculate a total load PECsw value for use in the aquatic risk assessment. As there was no chemical analysis performed, and therefore concentrations in water and sediment were not known, this takes into account the total amount of substance entering the aquatic system via drift and runoff/drainage, irrespective of whether the FOCUS models assumes entry direct to the water phase or direct to the sediment phase. The fate and ecotoxicology exposure endpoints are then comparable. At Step 1, the loading via drift was predicted to be 6.6216 mg/m^2 and loading via runoff/drainage was predicted to be 240.0 mg/m^2. Based on a total system loading of 246.62 mg/m^2, the equivalent concentration in a 30cm deep water body

would be 822 µg/L, and this value is appropriate for use in the sediment dweller risk assessment at Step 1 as a worst case. At Step 2 for Southern Europe as a worst case, the loading via drift was predicted to be 6.6216 mg/m^2 and loading via runoff/drainage was predicted to be 46.2804 mg/m^2. Based on a total system loading of 52.90 mg/m^2, the equivalent concentration in a 30cm deep water body would be 176µg/L, and this value is appropriate for use in the sediment dweller risk assessment at Step 2 as a worst case.

Focus Surface Water Step 3

FOCUS Step 3 calculations were undertaken using the SWASH interface, TOXSWA model and MACRO and PRZM models to simulate the worst case early application pattern in leafy vegetables for asulam and sulphanilamide. Application windows in Pesticide Application Timer (PAT) were set to between approximately two weeks prior to leafy vegetable emergence and two weeks post-emergence. For scenarios where it is possible to grow 2 crops per season, the simulation was performed assuming early or late application in two separate model runs. Possible application dates defined by FOCUS are presented in Table B.8. 161 below.

Table B.8. 161 Possible application dates for application of asulam on leafy vegetables

Scenario	Application timing	Possible application dates	
		First	Last
D3	Early	11-Apr*	11-May
D3	Late	22-Jul	21-Aug
D4	Early	26-Apr	26-May
D6	Early	1-Aug	31-Aug
R1	Early	06-Apr	06-May
R1	Late	17-Jul	16-Aug
R2	Early	14-Feb	16-Mar
R2	Late	17-Jul	16-Aug
R3	Early	15-Feb	17-Mar
R3	Late	1-Jun	1-Jul
R4	Early	15-Feb	17-Mar
R4	Late	1-Jun	1-Jul

*actual application date set by model is 10 Apr probably due to weather conditions

The Step 3 results for asulam, showing maximum PEC_{SW} and PEC_{Sed} values are shown in Table B.8. 162 and Table B.8. 163 for spinach and flower-bulbs, respectively. The maximum PEC_{SW} values and the subsequent TWAC values are shown in

Table B.8. 164 and Table B.8. 165.

Table B.8. 162 **Maximum PEC_{SW} and PEC_{Sed} of asulam at Step 3 following application to spinach**

Scenario	1st or 2nd Crop	Application dates	$PEC_{SW, Max}$ (µg L^{-1})	$PEC_{Sed, Max}$ (µg kg^{-1})	Dominant entry route
D3 Vredepeel Ditch	1st	10-Apr	15.204	2.367	Drift
D3 Vredepeel Ditch	2nd	25-Jul	15.253	2.890	Drift
D4 Skousbo Pond	1st	16-May	0.527	0.887	Drift
D4 Skousbo Stream	1st	16-May	12.102	0.465	Drift
D6 Thiva Ditch	1st	04-Aug	15.350	6.784	Drift
R1 Weiherbach Pond	1st	26-Apr	0.525	0.891	Drift
R1 Weiherbach Stream	1st	26-Apr	10.032	1.324	Drift
R1 Weiherbach Pond	2nd	28-Jul	0.525	0.801	Drift
R1 Weiherbach Stream	2nd	28-Jul	9.950	0.669	Drift
R2 Porto Stream	1st	16-Mar	21.850	2.795	Run-off
R2 Porto Stream	2nd	05-Aug	13.492	0.690	Drift
R3 Bologna Stream	1st	03-Mar	36.279	4.421	Run-off
R3 Bologna Stream	2nd	09-Jun	16.007	3.406	Run-off
R4 Roujan Stream	1st	12-Mar	62.969	9.451	Run-off
R4 Roujan Stream	2nd	07-Jun	42.370	6.883	Run-off

Table B.8. 163 **Maximum PEC_{SW} and PEC_{Sed} of asulam at Step 3 following application to flower-bulbs**

Scenario	1st or 2nd Crop	Application dates	$PEC_{sw, max}$ (µg L^{-1})	$PEC_{sed, ma}$ (µg kg^{-1})	Dominant entry route
D3 Vredepeel Ditch	1st	10-Apr	15.199	2.371	Drift
D4 Skousbo Pond	1st	18-Apr	0.527	1.045	Drift
D4 Skousbo Stream	1st	18-Apr-	11.827	0.383	Drift
D6 Thiva Ditch	1st	03-May	15.176	2.281	Drift
D6 Thiva Ditch	2nd	06-Oct	15.350	7.016	Drift
R1 Weiherbach Pond	1st	26-Apr	0.570	1.003	Run-off
R1 Weiherbach Stream	1st	26-Apr	24.052	3.061	Run-off
R2 Porto Stream	1st	06-Mar	21.145	2.716	Run-off
R3 Bologna Stream	1st	19-Feb	36.379	4.410	Run-off
R4 Roujan Stream	1st	27-Feb	61.081	9.243	Run-off

Table B.8. 164 **Maximum PEC_{SW} and TWAC values of asulam at Step 3 following application to spinach**

Scenario	1st or 2nd Crop	$PEC_{SW\ Max}$ (µg/L)	TWACs (µg/L)			
			2-day	7-day	21-day	28-day
D3 Vredepeel Ditch	1st	15.204	7.290	2.135	0.713	0.535
D3 Vredepeel Ditch	2nd	15.253	10.024	3.213	1.074	0.806
D4 Skousbo Pond	1st	0.527	0.516	0.500	0.461	0.445
D4 Skousbo Stream	1st	12.102	0.423	0.130	0.072	0.067
D6 Thiva Ditch	1st	15.350	14.702	12.521	5.713	4.336
R1 Weiherbach Pond	1st	0.525	0.514	0.496	0.465	0.452
R1 Weiherbach Stream	1st	10.032	1.865	0.533	0.271	0.213
R1 Weiherbach Pond	2nd	0.525	0.511	0.489	0.440	0.419
R1 Weiherbach Stream	2nd	9.950	0.788	0.225	0.075	0.056
R2 Porto Stream	1st	21.850	4.615	1.319	0.490	0.368
R2 Porto Stream	2nd	13.492	0.754	0.216	0.072	0.054
R3 Bologna Stream	1st	36.279	9.177	2.627	1.134	0.850
R3 Bologna Stream	2nd	16.007	7.548	2.162	0.949	0.712
R4 Roujan Stream	1st	62.969	22.518	6.437	2.232	1.674
R4 Roujan Stream	2nd	42.370	14.971	4.589	1.612	1.209

Table B.8. 165 **Maximum PEC_{SW} and TWAC values of asulam at Step 3 following application to flower-bulbs**

Scenario	1st or 2nd Crop	$PEC_{SW\ Max}$ (µg/L)	TWACs (µg/L)			
			2-day	7-day	21-day	28-day
D3 Vredepeel Ditch	1st	15.199	7.311	2.141	0.715	0.537
D4 Skousbo Pond	1st	0.527	0.517	0.502	0.467	0.450
D4 Skousbo Stream	1st	11.827	0.345	0.276	0.137	0.117
D6 Thiva Ditch	1st	15.176	2.605	0.930	0.485	0.423
D6 Thiva Ditch	2nd	15.350	14.810	12.814	5.577	4.392
R1 Weiherbach Pond	1st	0.570	0.563	0.545	0.509	0.503
R1 Weiherbach Stream	1st	24.052	5.108	1.460	0.596	0.451
R2 Porto Stream	1st	21.145	4.465	1.276	0.475	0.357
R3 Bologna Stream	1st	36.379	9.143	2.617	1.129	0.847
R4 Roujan Stream	1st	61.081	21.978	6.283	2.206	1.664

Step 4 results for asulam, with no drift buffer and 80% run-off mitigation, showing maximum PEC_{SW} and PEC_{Sed} values are shown in Table B.8. 166 and Table B.8. 167 for spinach and flower-bulbs, respectively. The maximum PEC_{SW} values and the subsequent TWAC values are shown in Table B.8. 168 and Table B.8. 169.

Table B.8. 166 **Maximum PEC_{SW} and PEC_{Sed} of asulam at Step 4 – no drift buffer and 80% run-off mitigation – following application to spinach**

Scenario	1st or 2nd Crop	Application dates	$PEC_{SW, Max}$ (µg/L)	$PEC_{Sed, Max}$ (µg/kg)	Dominant entry route
D3 Vredepeel Ditch	1st	10-Apr	15.204	2.367	Drift
D3 Vredepeel Ditch	2nd	25-Jul	15.253	2.890	Drift
D4 Skousbo Pond	1st	16-May	0.527	0.887	Drift
D4 Skousbo Stream	1st	16-May	12.102	0.465	Drift
D6 Thiva Ditch	1st	04-Aug	15.350	6.784	Drift
R1 Weiherbach Pond	1st	26-Apr	0.525	0.850	Drift
R1 Weiherbach Stream	1st	26-Apr	10.032	0.762	Drift
R1 Weiherbach Pond	2nd	28-Jul	0.525	0.805	Drift
R1 Weiherbach Stream	2nd	28-Jul	9.950	0.669	Drift
R2 Porto Stream	1st	16-Mar	13.157	0.794	Run-off
R2 Porto Stream	2nd	05-Aug	13.492	0.690	Drift
R3 Bologna Stream	1st	03-Mar	14.187	1.464	Run-off
R3 Bologna Stream	2nd	09-Jun	14.144	1.371	Run-off
R4 Roujan Stream	1st	12-Mar	14.946	2.631	Run-off
R4 Roujan Stream	2nd	07-Jun	10.142	1.952	Run-off

Table B.8. 167 **Maximum PEC_{SW} and PEC_{Sed} of asulam at Step 4 – no drift buffer and 80% run-off mitigation – following application to flower-bulbs**

Scenario	1st or 2nd Crop	Application dates	$PEC_{SW, Max}$ (µg L^{-1})	$PEC_{Sed, Ma}$ (µg kg^{-1})	Dominant entry route
D3 Vredepeel Ditch	1st	10-Apr	15.199	2.371	Drift
D4 Skousbo Pond	1st	18-Apr	0.527	1.045	Drift
D4 Skousbo Stream	1st	18-Apr-	11.827	0.383	Drift
D6 Thiva Ditch	1st	03-May	15.176	2.281	Drift
D6 Thiva Ditch	2nd	06-Oct	15.350	7.016	Drift
R1 Weiherbach Pond	1st	26-Apr	0.525	0.870	Run-off
R1 Weiherbach Stream	1st	26-Apr	10.025	0.847	Run-off
R2 Porto Stream	1st	06-Mar	13.151	0.773	Run-off
R3 Bologna Stream	1st	19-Feb	14.185	1.459	Run-off
R4 Roujan Stream	1st	27-Feb	14.514	2.607	Run-off

Table B.8. 168 **Maximum PEC_{SW} and TWAC values of asulam at Step 4 - no drift buffer and 80% run-off mitigation - following application to spinach**

Scenario	1st or 2nd Crop	$PEC_{SW\ Max}$ (µg/L)	TWACs (µg/L)			
			2-day	7-day	21-day	28-day
D3 Vredepeel Ditch	1st	15.204	7.290	2.135	0.713	0.535
D3 Vredepeel Ditch	2nd	15.253	10.024	3.213	1.074	0.806
D4 Skousbo Pond	1st	0.527	0.516	0.500	0.461	0.445
D4 Skousbo Stream	1st	12.102	0.423	0.130	0.072	0.067
D6 Thiva Ditch	1st	15.350	14.702	12.521	5.713	4.336
R1 Weiherbach Pond	1st	0.525	0.514	0.496	0.456	0.438
R1 Weiherbach Stream	1st	10.032	0.981	0.280	0.129	0.099
R1 Weiherbach Pond	2nd	0.525	0.511	0.489	0.440	0.419
R1 Weiherbach Stream	2nd	9.950	0.788	0.225	0.075	0.056
R2 Porto Stream	1st	13.157	1.063	0.304	0.151	0.114
R2 Porto Stream	2nd	13.492	0.754	0.216	0.072	0.054
R3 Bologna Stream	1st	14.187	2.702	0.773	0.461	0.346
R3 Bologna Stream	2nd	14.144	2.389	0.863	0.404	0.303
R4 Roujan Stream	1st	14.946	5.347	1.529	0.578	0.434
R4 Roujan Stream	2nd	10.142	3.618	1.270	0.450	0.337

Table B.8. 169 **Maximum PEC_{SW} and TWAC values of asulam at Step 4 - no drift buffer and 80% run-off mitigation - following application to flower-bulbs**

Scenario	1st or 2nd Crop	$PEC_{SW\ Max}$ (µg/L)	TWACs (µg/L)			
			2-day	7-day	21-day	28-day
D3 Vredepeel Ditch	1st	15.199	7.311	2.141	0.715	0.537
D4 Skousbo Pond	1st	0.527	0.517	0.502	0.467	0.450
D4 Skousbo Stream	1st	11.827	0.345	0.276	0.137	0.117
D6 Thiva Ditch	1st	15.176	2.605	0.930	0.485	0.423
D6 Thiva Ditch	2nd	15.350	14.810	12.814	5.577	4.392
R1 Weiherbach Pond	1st	0.525	0.514	0.496	0.464	0.448
R1 Weiherbach Stream	1st	10.025	1.088	0.311	0.199	0.150
R2 Porto Stream	1st	13.151	1.029	0.294	0.148	0.111
R3 Bologna Stream	1st	14.185	2.686	0.769	0.459	0.345
R4 Roujan Stream	1st	14.514	5.228	1.495	0.603	0.455

Step 4 results for asulam, with a 5 m drift buffer and 80% run-off mitigation, showing maximum PEC_{SW} and PEC_{Sed} values are shown in Table B.8. 170 and Table B.8. 171 for spinach and flower-bulbs, respectively. The maximum PEC_{SW} values and the subsequent TWAC values are shown in Table B.8. 172 and Table B.8. 173.

Table B.8. 170 **Maximum PEC_{SW} and PEC_{Sed} of asulam at Step 4 – 5 m buffer and 80% run-off mitigation – following application to spinach**

Scenario	1st or 2nd Crop	Application dates	$PEC_{SW, Max}$ (µg/L)	$PEC_{Sed, Max}$ (µg/kg)	Dominant entry route
D3 Vredepeel Ditch	1st	10-Apr	4.121	0.739	Drift
D3 Vredepeel Ditch	2nd	25-Jul	4.134	0.903	Drift
D4 Skousbo Pond	1st	16-May	0.457	0.789	Drift
D4 Skousbo Stream	1st	16-May	4.422	0.190	Drift
D6 Thiva Ditch	1st	04-Aug	5.625	2.674	Drainage
R1 Weiherbach Pond	1st	26-Apr	0.454	0.749	Drift
R1 Weiherbach Stream	1st	26-Apr	3.664	0.333	Drift
R1 Weiherbach Pond	2nd	28-Jul	0.454	0.708	Drift
R1 Weiherbach Stream	2nd	28-Jul	3.635	0.267	Drift
R2 Porto Stream	1st	16-Mar	5.057	0.757	Run-off
R2 Porto Stream	2nd	05-Aug	4.928	0.272	Drift
R3 Bologna Stream	1st	03-Mar	8.446	1.234	Run-off
R3 Bologna Stream	2nd	09-Jun	5.166	0.977	Drift
R4 Roujan Stream	1st	12-Mar	14.946	2.587	Run-off
R4 Roujan Stream	2nd	07-Jun	10.142	1.885	Run-off

Table B.8. 171 **Maximum PEC_{SW} and PEC_{Sed} of asulam at Step 4 – 5 m buffer and 80% run-off mitigation – following application to flower-bulbs**

	1st or 2nd Crop	Application dates	$PEC_{SW, Max}$ (µg L^{-1})	$PEC_{Sed, Ma}$ (µg kg^{-1})	Dominant entry route
D3 Vredepeel Ditch	1st	10-Apr	4.119	0.740	Drift
D4 Skousbo Pond	1st	18-Apr	0.457	0.946	Drift
D4 Skousbo Stream	1st	18-Apr-	4.322	0.296	Drift
D6 Thiva Ditch	1st	03-May	4.279	1.374	Drift
D6 Thiva Ditch	2nd	06-Oct	4.160	2.328	Drift
R1 Weiherbach Pond	1st	26-Apr	0.454	0.770	Drift
R1 Weiherbach Stream	1st	26-Apr	5.176	0.784	Run-off
R2 Porto Stream	1st	06-Mar	4.896	0.736	Run-off
R3 Bologna Stream	1st	19-Feb	8.485	1.235	Run-off
R4 Roujan Stream	1st	27-Feb	14.514	2.539	Run-off

Table B.8. 172 **Maximum PEC_{SW} and TWAC values of asulam at Step 4 – 5 m buffer and 80% run-off mitigation - following application to spinach**

Scenario	1st or 2nd Crop	$PEC_{SW\ Max}$ (µg/L)	TWACs (µg/L)			
			2-day	7-day	21-day	28-day
D3 Vredepeel Ditch	1st	4.121	1.972	0.578	0.193	0.145
D3 Vredepeel Ditch	2nd	4.134	2.709	0.870	0.291	0.218
D4 Skousbo Pond	1st	0.457	0.447	0.433	0.399	0.386
D4 Skousbo Stream	1st	4.422	0.180	0.130	0.072	0.056
D6 Thiva Ditch	1st	5.625	3.970	3.372	1.945	1.510
R1 Weiherbach Pond	1st	0.454	0.444	0.429	0.394	0.379
R1 Weiherbach Stream	1st	3.664	0.377	0.108	0.070	0.055
R1 Weiherbach Pond	2nd	0.454	0.442	0.423	0.380	0.362
R1 Weiherbach Stream	2nd	3.635	0.288	0.082	0.027	0.021
R2 Porto Stream	1st	5.057	1.063	0.304	0.120	0.090
R2 Porto Stream	2nd	4.928	0.276	0.079	0.026	0.020
R3 Bologna Stream	1st	8.446	2.127	0.609	0.297	0.223
R3 Bologna Stream	2nd	5.166	1.840	0.527	0.259	0.194
R4 Roujan Stream	1st	14.946	5.347	1.529	0.538	0.404
R4 Roujan Stream	2nd	10.142	3.618	1.120	0.400	0.300

Table B.8. 173 **Maximum PEC_{SW} and TWAC values of asulam at Step 4 – 5 m buffer and 80% run-off mitigation - following application to flower-bulbs**

Scenario	1st or 2nd Crop	$PEC_{SW\ Max}$ (µg/L)	TWACs (µg/L)			
			2-day	7-day	21-day	28-day
D3 Vredepeel Ditch	1st	4.119	1.978	0.580	0.194	0.145
D4 Skousbo Pond	1st	0.457	0.448	0.435	0.405	0.389
D4 Skousbo Stream	1st	4.322	0.345	0.276	0.137	0.117
D6 Thiva Ditch	1st	4.279	0.876	0.434	0.346	0.342
D6 Thiva Ditch	2nd	4.160	3.998	3.451	1.506	1.330
R1 Weiherbach Pond	1st	0.454	0.444	0.429	0.403	0.389
R1 Weiherbach Stream	1st	5.176	1.088	0.311	0.141	0.107
R2 Porto Stream	1st	4.896	1.029	0.294	0.116	0.087
R3 Bologna Stream	1st	8.485	2.126	0.609	0.297	0.223
R4 Roujan Stream	1st	14.514	5.228	1.495	0.538	0.406

B.8.6.1 PECsw photodegradates

Photolysis studies were conducted with asulam for direct photolysis in aqueous buffer solution and indirect photolysis in natural water. In the aqueous photolysis study (Mills and Simmonds 2003a) a number of photodegradates were found which were not formed in the natural water study (Mills and Caine 2004a). The studies are summarised in section B.8.4.5 of the DAR. Three photodegradates >10% AR were

identified as sulphanilic acid (max 55.5%), N-(4-aminophenyl) formamide, max 24.2% (abbreviated to AP formamide) and (4-(4-methoxycarbonylaminophenyl) aminophenyl)carbamic acid max 11.9% (abbreviated to MCAPAP carbamic acid). 4.

The PECsw for asulam in each of the relevant scenarios was assumed to instantly produce the maximum percentage that was detected in the photolysis study.

The calculation is based on the maximum PECsw concentration for asulam taking into account the maximum % occurrence of the photodegradate and correction for the difference in molecular masses using the equation shown below. The units of PECsw for the photodegradates are µg/L.

	Asulam	Sulfanilic acid	AP formamide	MCAPAP carbamic acid
Mol mass	230.24	173.2	136.2	301.3
Max occurence %	x	55.5	24.2	11.9

$$\text{Phot. PECsw} = \text{Asulam PECsw} \times \text{max \% phot. formation} \times \frac{\text{Phot. mol wt.}}{\text{Asulam mol wt.}}$$

where Phot. = Photodegradate
max % phot. formation = maximum %AR of photodegradate found in photolysis study

The results, equivalent to Step 3 for asulam photodegradates, showing maximum PEC_{SW} values are shown in Table B.8. 174 and Table B.8. 175 for spinach and flower-bulbs, respectively.

Table B.8. 174 **PEC_{SW} values of asulam photodegradates at Step 3 following application to spinach**

Scenario	1st or 2nd Crop	PECsw (µg/L)			
		Asulam	Sulphanilic acid	AP formamide	MCAPAP carbamic acid
D3 Vredepeel Ditch	1st	15.204	6.347	2.176	2.368
D3 Vredepeel Ditch	2nd	15.253	6.367	2.183	2.375
D4 Skousbo Pond	1st	0.527	0.220	0.075	0.082
D4 Skousbo Stream	1st	12.102	5.052	1.732	1.885
D6 Thiva Ditch	1st	15.350	6.408	2.197	2.391
R1 Weiherbach Pond	1st	0.525	0.219	0.075	0.082
R1 Weiherbach Stream	1st	10.032	4.188	1.436	1.562
R1 Weiherbach Pond	2nd	0.525	0.219	0.075	0.082
R1 Weiherbach Stream	2nd	9.950	4.154	1.424	1.550
R2 Porto Stream	1st	21.850	9.121	3.127	3.403
R2 Porto Stream	2nd	13.492	5.632	1.931	2.101
R3 Bologna Stream	1st	36.279	15.145	5.191	5.650
R3 Bologna Stream	2nd	16.007	6.682	2.291	2.493
R4 Roujan Stream	1st	62.969	26.287	9.011	9.806
R4 Roujan Stream	2nd	42.370	17.688	6.063	6.598

Table B.8. 175 **PEC_{SW} values of asulam photodegradates at Step 3 following application to flower-bulbs**

Scenario	1st or 2nd Crop	PECsw (µg/L)			
		Asulam	Sulphanilic acid	AP formamide	MCAPAP carbamic acid
D3 Vredepeel Ditch	1st	15.199	6.345	2.175	2.367
D4 Skousbo Pond	1st	0.527	0.220	0.075	0.082
D4 Skousbo Stream	1st	11.827	4.937	1.692	1.842
D6 Thiva Ditch	1st	15.176	6.335	2.172	2.363
D6 Thiva Ditch	2nd	15.350	6.408	2.197	2.391
R1 Weiherbach Pond	1st	0.570	0.238	0.082	0.089
R1 Weiherbach Stream	1st	24.052	10.041	3.442	3.746
R2 Porto Stream	1st	21.145	8.827	3.026	3.293
R3 Bologna Stream	1st	36.379	15.187	5.206	5.665
R4 Roujan Stream	1st	61.081	25.499	8.740	9.512

Results, equivalent to Step 4, for asulam photodegradates, with a 5 m drift buffer and 80% run-off mitigation, showing maximum PEC_{SW} values are shown in Table B.8. 176 and Table B.8. 177 for spinach and flower-bulbs, respectively.

Table B.8. 176 **PEC_{SW} values of asulam photodegradates at Step 4 – 5 m buffer and 80% run-off mitigation - following application to spinach**

Scenario	1st or 2nd Crop	PECsw (µg/L)			
		Asulam	Sulphanilic acid	AP formamide	MCAPAP carbamic acid
D3 Vredepeel Ditch	1st	4.121	1.720	0.590	0.642
D3 Vredepeel Ditch	2nd	4.134	1.726	0.592	0.644
D4 Skousbo Pond	1st	0.457	0.191	0.065	0.071
D4 Skousbo Stream	1st	4.422	1.846	0.633	0.689
D6 Thiva Ditch	1st	5.625	2.348	0.805	0.876
R1 Weiherbach Pond	1st	0.454	0.190	0.065	0.071
R1 Weiherbach Stream	1st	3.664	1.530	0.524	0.571
R1 Weiherbach Pond	2nd	0.454	0.190	0.065	0.071
R1 Weiherbach Stream	2nd	3.635	1.517	0.520	0.566
R2 Porto Stream	1st	5.057	2.111	0.724	0.788
R2 Porto Stream	2nd	4.928	2.057	0.705	0.767
R3 Bologna Stream	1st	8.446	3.526	1.209	1.315
R3 Bologna Stream	2nd	5.166	2.157	0.739	0.805
R4 Roujan Stream	1st	14.946	6.239	2.139	2.328
R4 Roujan Stream	2nd	10.142	4.234	1.451	1.579

Table B.8. 177 **PEC_{SW} values of asulam photodegradates at Step 4 – 5 m buffer and 80% run-off mitigation - following application to flower-bulbs**

Scenario	1st or 2nd Crop	PECsw (µg/L)			
		Asulam	Sulphanilic acid	AP formamide	MCAPAP carbamic acid
D3 Vredepeel Ditch	1st	4.119	1.720	0.589	0.641
D4 Skousbo Pond	1st	0.457	0.191	0.065	0.071
D4 Skousbo Stream	1st	4.322	1.804	0.618	0.673
D6 Thiva Ditch	1st	4.279	1.786	0.612	0.666
D6 Thiva Ditch	2nd	4.160	1.737	0.595	0.648
R1 Weiherbach Pond	1st	0.454	0.190	0.065	0.071
R1 Weiherbach Stream	1st	5.176	2.161	0.741	0.806
R2 Porto Stream	1st	4.896	2.044	0.701	0.762
R3 Bologna Stream	1st	8.485	3.542	1.214	1.321
R4 Roujan Stream	1st	14.514	6.059	2.077	2.260

Conclusions:

Maximum PEC_{SW} and PEC_{Sed} values for asulam and sulphanilamide have been calculated at FOCUS Steps 1 & 2 and also for asulam at FOCUS Steps 3 & 4 with PEC_{SW} TWAC values as well. For asulam photodegradates conservative maximum PEC_{SW} values have been calculated at Steps 3 & 4.

The direct aqueous photolysis study was performed in aqueous buffer solutions at pH 4 and pH 9. These are fairly extreme pH values with regard to natural surface waters in Europe however surface waters approaching pH 9 in the UK are not uncommon. The three photodegradates modelled were present at >10% in the direct photolysis study (sulphanilic acid pH4, AP formamide and MCAPAP carbamate pH 9) but were not formed at significant levels in the natural water study (conducted in a UK reservoir pond, pH 7.8). In the opinion of the RMS, the single natural water study is not necessarily representative of all European surface waters. It is therefore considered that in the real environment, it cannot be excluded that these metabolites might be formed under certain conditions, but they would be likely to be formed at lower levels than under laboratory conditions of the buffer study due to competing routes of degradation (e.g. indirect photolysis in the presence of natural photosensitisers). The metabolite PECsw values listed above are therefore likely to represent a conservative estimate for the purposes of a first tier aquatic risk assessment.

It is noted by the RMS that in the direct photolysis study, a further 6 unidentified metabolites were formed at >5% AR. These metabolites were also not formed in significant amounts in the photolysis study conducted in natural water. Since the metabolites are not formed rapidly and are clearly not above 10% (Guidance Document on Aquatic Ecotoxicology 2002), it is considered that further assessment of these unidentified metabolites is not required.

Surface water metabolite methylbenzenesulphonylcarbamate (MBSC)

The RMS noted that in the water sediment study (Willems, 1997) a number of metabolites were detected. Two of these were tentatively identified. Met D reached 9.7% and tentatively assigned as sulphanilamide. The other metabolite, Met F, tentatively assigned as methylbenzenesulphonylcarbamate (MBSC or desamino asulam) reached a maximum of 10.3% AR at day 2 in the Schoonrewoerdswiel total system. The applicant considered this an outlier as it 'does not fit the observed asulam degradation pattern'. The RMS does not agree with this as it clearly exceeds the trigger of 10% AR. PECsw Modelling was provided by the applicant which is provided below.

FOCUS sw exposure assessments have been conducted (Steps 1 – 3). The input parameters for methylbenzenesulphonylcarbamate (MBSC) are presented in Table B.8. 178 below.

Table B.8. 178 FOCUS Steps 1 – 3 input parameters for MBSC

Parameter	Value	Remarks
PHYSICO-CHEMICAL PROPERTIES		
Molecular weight (g mol^{-1})	215	-
Water solubility (mg L^{-1}) at 20°C; pH 8.9	100000	Assumed value
DEGRADATION IN SOIL		
DT_{50} soil (d) at 20°C and pF2	14.9	Geometric mean Willems [1997]

Max. occur. in soil (%)	6.0	Roohi and Mackenzie [2009]
DEGRADATION IN WATER/ SEDIMENT		
DT_{50} water (d)	1000	Conservative estimate
DT_{50} sediment (d)	1000	Conservative estimate
DT_{50} total system (d)	1000	Conservative estimate
Max. occur. in water/ sediment (%)	10.3	Total system
SORPTION TO SOIL		
K_{foc} (L kg^{-1}) K_{fom} (L kg^{-1})	2.8 1.6	Willems [1998]
1/n	1.0	FOCUS default
MANAGEMENT RELATED PARAMETERS		
Crop interception (%)	0	Conservative default assumption

*The Willems [1997] RoD study on three soils for MBSC reports an average un-normalised DT50 of 12 days. The reported DT50 data have been normalised to pF2 moisture (study conducted at 20°C) and a geometric mean calculated (9.1 days). The RMS re-calculated the geometric mean DT50 of 14.9 days

The average un-normalised DT50 (12 days) for MBSC was reported in Willems (1997) rate of degradation study. The reported DT50s were normalised to pF2 and 20°C (Q10 2.58) by the applicant and a geometric mean DT50 of 9.1 days. The RMS re-calculated the geometric mean DT50 of 14.9 days (see Table B.8. 81).

FOCUSsw Steps 1&2 evaluations conducted for leafy vegetables. No plant interception, March-May application of 1x2.4 kg/ha. The results of the Step 1-2 calculations are presented in Table B.8. 179 below:

Table B.8. 179 PEC_{sw} and PEC_{sed} of MBSC from application of asulam to spinach at Step 1-2

Substance	Application rate (g ha^{-1})	Season	Crop intercept (%)	Leafy vegetables			
				Step 1		Step 2	
				PEC_{sw} (µg L^{-1})	PEC_{sed} (µg kg^{-1})	PEC_{sw} (µg L^{-1})	PEC_{sed} (µg kg^{-1})
MBSC	(1 x 2400)	Mar - May	0	46.8	1.31	8.71	0.24

The critical endpoint for asulam is 270 µg/L (Lemna). No effects data for MBSC is available, so 10x parent toxicity will be assumed resulting in an endpoint of 27 µg/l. For Lemna, a TER of 10 is required resulting in a RAC of 2.7 µg/l for MBSC.

Step 2 passes for equivalent parent toxicity, but not for 10x toxicity, Step 3 is therefore required.

FOCUSsw Step 3 evaluations conducted for leafy vegetables and bulb vegetables (application rates, timings and windows as per asulam and sulphanilamide above).

MBSC simulated as a metabolite formed from asulam in soil and surface water. The maximum occurrence of MBSC in water/sediment systems was at day 2. Due the residence times in the FOCUS waterbodies, evaluation of drift input for MBSC (spray

drift of asulam followed by degradation in the waterbody) in the ditch and pond scenarios is necessary. No drift loading is necessary for the stream scenarios. Drift loadings factored for molecular weight differences and maximum formation in the total system of 10.3%.

All scenarios except R4 2nd crop for spinach (leafy vegetables) pass at Step 3. R4 2nd crop spinach would require refinement with run-off mitigation. The results of Step 3 simualtions for spinach and flower bulbs are presented in Table B.8. 180 and Table B.8. 181 respectively.

Table B.8. 180 Maximum PEC_{sw} and PEC_{sed} of MBSC from application of asulam to spinach at Step 3

Scenario	1st or 2nd Crop	Application dates	$PEC_{SW, Max}$ (µg L^{-1})	$PEC_{Sed, Max}$ (µg kg^{-1})	Dominant entry route
D3 Vredepeel Ditch	1st	10-Apr	1.473	0.100	Drift
D3 Vredepeel Ditch	2nd	25-Jul	1.623	0.233	Drift
D4 Skousbo Pond	1st	16-May	0.214	0.125	Drainage
D4 Skousbo Stream	1st	16-May	0.242	0.083	Drainage
D6 Thiva Ditch	1st	04-Aug	1.620	0.309	Drainage
R1 Weiherbach Pond	1st	26-Apr	0.051	0.021	Drift
R1 Weiherbach Stream	1st	26-Apr	0.150	0.008	Run-off
R1 Weiherbach Pond	2nd	28-Jul	0.051	0.021	Drift
R1 Weiherbach Stream	2nd	28-Jul	0.000	0.000	Run-off
R2 Porto Stream	1st	16-Mar	0.493	0.028	Run-off
R2 Porto Stream	2nd	05-Aug	0.013	0.001	Run-off
R3 Bologna Stream	1st	03-Mar	0.517	0.027	Run-off
R3 Bologna Stream	2nd	09-Jun	1.657	0.138	Run-off
R4 Roujan Stream	1st	12-Mar	1.505	0.111	Run-off
R4 Roujan Stream	2nd	07-Jun	3.714	0.278	Run-off

Table B.8. 181 Maximum PEC_{sw} and PEC_{sed} of MBSC from application of asulam to flower-bulbs at Step 3

Scenario	1st or 2nd Crop	Application dates	$PEC_{SW, Max}$ (µg L^{-1})	$PEC_{Sed, Ma}$ (µg kg^{-1})	Dominant entry route
D3 Vredepeel Ditch	1st	10-Apr	1.476	0.101	Drift
D4 Skousbo Pond	1st	18-Apr	0.455	0.276	Drainage
D4 Skousbo Stream	1st	18-Apr-	1.178	0.233	Drainage
D6 Thiva Ditch	1st	03-May	1.479	0.086	Drift
D6 Thiva Ditch	2nd	06-Oct	1.478	0.293	Drift
R1 Weiherbach Pond	1st	26-Apr	0.051	0.022	Drift
R1 Weiherbach Stream	1st	26-Apr	0.746	0.041	Run-off
R2 Porto Stream	1st	06-Mar	0.462	0.026	Run-off
R3 Bologna Stream	1st	19-Feb	0.525	0.028	Run-off
R4 Roujan Stream	1st	27-Feb	1.518	0.112	Run-off

Parent: Asulam Parameters used in FOCUSsw step 1 and 2	Version control no. of FOCUS calculator: 2.1 Molecular weight (g/mol): 230 Water solubility (mg/L): 962000 K_{OC} (L/kg): 25.4 DT_{50} soil (d): 4.6 days (Lab or field. In accordance with FOCUS SFO) DT_{50} water/sediment system (d): 70.3 (representative worst case from sediment water studies) DT_{50} water (d): 70.3 DT_{50} sediment (d): 1000 Crop interception (%): 0%
Parameters used in FOCUSsw step 3 (if performed)	Version control no.'s of FOCUS software: FOCUS SWASH 3.1 FOCUS MACRO 4.4.2 FOCUS PRZM SW 3.1.1 FOCUS TOXSWA 3.3.1 Vapour pressure: 5×10^{-7} Pa Kom/Koc: 14.7/25.4 1/n: 0.75 (Freundlich exponent general or for soil ,susp. solids or sediment respectively)
Application rate	Crop: vegetables leafy/vegetables bulb Crop interception: calculated by model Number of applications: 1 Interval (d): n/a Application rate(s): 2400 g as/ha Application window: 30 days

FOCUS STEP 1 Scenario	Day after overall maximum	PEC_{SW} (µg/L)		PEC_{SED} (µg/kg)	
		Actual	TWA	Actual	TWA
	0 h	795.87		199.98	
	24 h				
	2 d				
	4 d				
	7 d				
	14 d				
	21 d				
	28 d				
	42 d				

FOCUS STEP 2 Scenario	Day after overall maximum	PEC_{SW} (µg/L)		PEC_{SED} (µg/kg)	
		Actual	TWA	Actual	TWA
Northern EU	0 h	105.54		26.47	
	24 h				
	2 d				
	4 d				
	7 d				
	14 d				
	21 d				
	28 d				
	42 d				
Southern EU	0 h	190.15		47.77	
	24 h				
	2 d				
	4 d				
	7 d				
	14 d				
	21 d				
	28 d				
	42 d				

FOCUS STEP 3:

Maximum PEC_{SW} and PEC_{Sed} of asulam at Step 3 following application to spinach

Scenario	1st or 2nd Crop	Application dates	$PEC_{SW, Max}$ (µg L^{-1})	$PEC_{Sed, Max}$ (µg kg^{-1})	Dominant entry route
D3 Vredepeel Ditch	1st	10-Apr	15.204	2.367	Drift
D3 Vredepeel Ditch	2nd	25-Jul	15.253	2.890	Drift
D4 Skousbo Pond	1st	16-May	0.527	0.887	Drift
D4 Skousbo Stream	1st	16-May	12.102	0.465	Drift
D6 Thiva Ditch	1st	04-Aug	15.350	6.784	Drift
R1 Weiherbach Pond	1st	26-Apr	0.525	0.891	Drift
R1 Weiherbach Stream	1st	26-Apr	10.032	1.324	Drift
R1 Weiherbach Pond	2nd	28-Jul	0.525	0.801	Drift
R1 Weiherbach Stream	2nd	28-Jul	9.950	0.669	Drift
R2 Porto Stream	1st	16-Mar	21.850	2.795	Run-off
R2 Porto Stream	2nd	05-Aug	13.492	0.690	Drift
R3 Bologna Stream	1st	03-Mar	36.279	4.421	Run-off
R3 Bologna Stream	2nd	09-Jun	16.007	3.406	Run-off
R4 Roujan Stream	1st	12-Mar	62.969	9.451	Run-off
R4 Roujan Stream	2nd	07-Jun	42.370	6.883	Run-off

Maximum PEC_{SW} and PEC_{Sed} of asulam at Step 3 following application to flower-bulbs

Scenario	1st or 2nd Crop	Application dates	$PEC_{sw, max}$ (µg L^{-1})	$PEC_{sed, ma}$ (µg kg^{-1})	Dominant entry route
D3 Vredepeel Ditch	1st	10-Apr	15.199	2.371	Drift
D4 Skousbo Pond	1st	18-Apr	0.527	1.045	Drift
D4 Skousbo Stream	1st	18-Apr-	11.827	0.383	Drift
D6 Thiva Ditch	1st	03-May	15.176	2.281	Drift
D6 Thiva Ditch	2nd	06-Oct	15.350	7.016	Drift
R1 Weiherbach Pond	1st	26-Apr	0.570	1.003	Run-off
R1 Weiherbach Stream	1st	26-Apr	24.052	3.061	Run-off
R2 Porto Stream	1st	06-Mar	21.145	2.716	Run-off
R3 Bologna Stream	1st	19-Feb	36.379	4.410	Run-off
R4 Roujan Stream	1st	27-Feb	61.081	9.243	Run-off

Maximum PEC_{SW} and TWAC values of asulam at Step 3 following application to spinach

Scenario	1st or 2nd Crop	$PEC_{SW\ Max}$ (µg/L)	TWACs (µg/L)			
			2-day	7-day	21-day	28-day
D3 Vredepeel Ditch	1st	15.204	7.290	2.135	0.713	0.535
D3 Vredepeel Ditch	2nd	15.253	10.024	3.213	1.074	0.806
D4 Skousbo Pond	1st	0.527	0.516	0.500	0.461	0.445
D4 Skousbo Stream	1st	12.102	0.423	0.130	0.072	0.067
D6 Thiva Ditch	1st	15.350	14.702	12.521	5.713	4.336
R1 Weiherbach Pond	1st	0.525	0.514	0.496	0.465	0.452
R1 Weiherbach Stream	1st	10.032	1.865	0.533	0.271	0.213
R1 Weiherbach Pond	2nd	0.525	0.511	0.489	0.440	0.419
R1 Weiherbach Stream	2nd	9.950	0.788	0.225	0.075	0.056
R2 Porto Stream	1st	21.850	4.615	1.319	0.490	0.368
R2 Porto Stream	2nd	13.492	0.754	0.216	0.072	0.054
R3 Bologna Stream	1st	36.279	9.177	2.627	1.134	0.850
R3 Bologna Stream	2nd	16.007	7.548	2.162	0.949	0.712
R4 Roujan Stream	1st	62.969	22.518	6.437	2.232	1.674
R4 Roujan Stream	2nd	42.370	14.971	4.589	1.612	1.209

Maximum PEC_{SW} and TWAC values of asulam at Step 3 following application to flower-bulbs

Scenario	1st or 2nd Crop	$PEC_{SW\ Max}$ (µg/L)	TWACs (µg/L)			
			2-day	7-day	21-day	28-day
D3 Vredepeel Ditch	1st	15.199	7.311	2.141	0.715	0.537
D4 Skousbo Pond	1st	0.527	0.517	0.502	0.467	0.450
D4 Skousbo Stream	1st	11.827	0.345	0.276	0.137	0.117
D6 Thiva Ditch	1st	15.176	2.605	0.930	0.485	0.423
D6 Thiva Ditch	2nd	15.350	14.810	12.814	5.577	4.392
R1 Weiherbach Pond	1st	0.570	0.563	0.545	0.509	0.503
R1 Weiherbach Stream	1st	24.052	5.108	1.460	0.596	0.451
R2 Porto Stream	1st	21.145	4.465	1.276	0.475	0.357
R3 Bologna Stream	1st	36.379	9.143	2.617	1.129	0.847
R4 Roujan Stream	1st	61.081	21.978	6.283	2.206	1.664

FOCUS STEP 4

Maximum PEC_{SW} and PEC_{Sed} of asulam at Step 4 – no drift buffer and 80% run-off mitigation – following application to spinach

Scenario	1st or 2nd Crop	Application dates	$PEC_{SW, Max}$ (µg/L)	$PEC_{Sed, Max}$ (µg/kg)	Dominant entry route
D3 Vredepeel Ditch	1st	10-Apr	15.204	2.367	Drift
D3 Vredepeel Ditch	2nd	25-Jul	15.253	2.890	Drift
D4 Skousbo Pond	1st	16-May	0.527	0.887	Drift
D4 Skousbo Stream	1st	16-May	12.102	0.465	Drift
D6 Thiva Ditch	1st	04-Aug	15.350	6.784	Drift
R1 Weiherbach Pond	1st	26-Apr	0.525	0.850	Drift
R1 Weiherbach Stream	1st	26-Apr	10.032	0.762	Drift
R1 Weiherbach Pond	2nd	28-Jul	0.525	0.805	Drift
R1 Weiherbach Stream	2nd	28-Jul	9.950	0.669	Drift
R2 Porto Stream	1st	16-Mar	13.157	0.794	Run-off
R2 Porto Stream	2nd	05-Aug	13.492	0.690	Drift
R3 Bologna Stream	1st	03-Mar	14.187	1.464	Run-off
R3 Bologna Stream	2nd	09-Jun	14.144	1.371	Run-off
R4 Roujan Stream	1st	12-Mar	14.946	2.631	Run-off
R4 Roujan Stream	2nd	07-Jun	10.142	1.952	Run-off

Maximum PEC_{SW} and PEC_{Sed} of asulam at Step 4 – no drift buffer and 80% run-off mitigation – following application to flower-bulbs

Scenario	1st or 2nd Crop	Application dates	$PEC_{SW, Max}$ (µg L^{-1})	$PEC_{Sed, Ma}$ (µg kg^{-1})	Dominant entry route
D3 Vredepeel Ditch	1st	10-Apr	15.199	2.371	Drift
D4 Skousbo Pond	1st	18-Apr	0.527	1.045	Drift
D4 Skousbo Stream	1st	18-Apr-	11.827	0.383	Drift
D6 Thiva Ditch	1st	03-May	15.176	2.281	Drift
D6 Thiva Ditch	2nd	06-Oct	15.350	7.016	Drift
R1 Weiherbach Pond	1st	26-Apr	0.525	0.870	Run-off
R1 Weiherbach Stream	1st	26-Apr	10.025	0.847	Run-off
R2 Porto Stream	1st	06-Mar	13.151	0.773	Run-off
R3 Bologna Stream	1st	19-Feb	14.185	1.459	Run-off
R4 Roujan Stream	1st	27-Feb	14.514	2.607	Run-off

Maximum PEC_{SW} and TWAC values of asulam at Step 4 - no drift buffer and 80% run-off mitigation - following application to spinach

Scenario	1st or 2nd Crop	$PEC_{SW\ Max}$ (µg/L)	TWACs (µg/L)			
			2-day	7-day	21-day	28-day
D3 Vredepeel Ditch	1st	15.204	7.290	2.135	0.713	0.535
D3 Vredepeel Ditch	2nd	15.253	10.024	3.213	1.074	0.806
D4 Skousbo Pond	1st	0.527	0.516	0.500	0.461	0.445
D4 Skousbo Stream	1st	12.102	0.423	0.130	0.072	0.067
D6 Thiva Ditch	1st	15.350	14.702	12.521	5.713	4.336
R1 Weiherbach Pond	1st	0.525	0.514	0.496	0.456	0.438
R1 Weiherbach Stream	1st	10.032	0.981	0.280	0.129	0.099
R1 Weiherbach Pond	2nd	0.525	0.511	0.489	0.440	0.419
R1 Weiherbach Stream	2nd	9.950	0.788	0.225	0.075	0.056
R2 Porto Stream	1st	13.157	1.063	0.304	0.151	0.114
R2 Porto Stream	2nd	13.492	0.754	0.216	0.072	0.054
R3 Bologna Stream	1st	14.187	2.702	0.773	0.461	0.346
R3 Bologna Stream	2nd	14.144	2.389	0.863	0.404	0.303
R4 Roujan Stream	1st	14.946	5.347	1.529	0.578	0.434
R4 Roujan Stream	2nd	10.142	3.618	1.270	0.450	0.337

Maximum PEC_{SW} and TWAC values of asulam at Step 4 - no drift buffer and 80% run-off mitigation - following application to flower-bulbs

Scenario	1st or 2nd Crop	$PEC_{SW\ Max}$ (µg/L)	TWACs (µg/L)			
			2-day	7-day	21-day	28-day
D3 Vredepeel Ditch	1st	15.199	7.311	2.141	0.715	0.537
D4 Skousbo Pond	1st	0.527	0.517	0.502	0.467	0.450
D4 Skousbo Stream	1st	11.827	0.345	0.276	0.137	0.117
D6 Thiva Ditch	1st	15.176	2.605	0.930	0.485	0.423
D6 Thiva Ditch	2nd	15.350	14.810	12.814	5.577	4.392
R1 Weiherbach Pond	1st	0.525	0.514	0.496	0.464	0.448
R1 Weiherbach Stream	1st	10.025	1.088	0.311	0.199	0.150
R2 Porto Stream	1st	13.151	1.029	0.294	0.148	0.111
R3 Bologna Stream	1st	14.185	2.686	0.769	0.459	0.345
R4 Roujan Stream	1st	14.514	5.228	1.495	0.603	0.455

Maximum PEC_{SW} and PEC_{Sed} of asulam at Step 4 – 5 m buffer and 80% run-off mitigation – following application to spinach

Scenario	1st or 2nd Crop	Application dates	$PEC_{SW, Max}$ (µg/L)	$PEC_{Sed, Max}$ (µg/kg)	Dominant entry route
D3 Vredepeel Ditch	1st	10-Apr	4.121	0.739	Drift
D3 Vredepeel Ditch	2nd	25-Jul	4.134	0.903	Drift
D4 Skousbo Pond	1st	16-May	0.457	0.789	Drift
D4 Skousbo Stream	1st	16-May	4.422	0.190	Drift
D6 Thiva Ditch	1st	04-Aug	5.625	2.674	Drainage
R1 Weiherbach Pond	1st	26-Apr	0.454	0.749	Drift
R1 Weiherbach Stream	1st	26-Apr	3.664	0.333	Drift
R1 Weiherbach Pond	2nd	28-Jul	0.454	0.708	Drift
R1 Weiherbach Stream	2nd	28-Jul	3.635	0.267	Drift
R2 Porto Stream	1st	16-Mar	5.057	0.757	Run-off
R2 Porto Stream	2nd	05-Aug	4.928	0.272	Drift
R3 Bologna Stream	1st	03-Mar	8.446	1.234	Run-off
R3 Bologna Stream	2nd	09-Jun	5.166	0.977	Drift
R4 Roujan Stream	1st	12-Mar	14.946	2.587	Run-off
R4 Roujan Stream	2nd	07-Jun	10.142	1.885	Run-off

Maximum PEC_{SW} and PEC_{Sed} of asulam at Step 4 – 5 m buffer and 80% run-off mitigation – following application to flower-bulbs

Scenario	1st or 2nd Crop	Application dates	$PEC_{SW, Max}$ (µg L^{-1})	$PEC_{Sed, Ma}$ (µg kg^{-1})	Dominant entry route
D3 Vredepeel Ditch	1st	10-Apr	4.119	0.740	Drift
D4 Skousbo Pond	1st	18-Apr	0.457	0.946	Drift
D4 Skousbo Stream	1st	18-Apr-	4.322	0.296	Drift
D6 Thiva Ditch	1st	03-May	4.279	1.374	Drift
D6 Thiva Ditch	2nd	06-Oct	4.160	2.328	Drift
R1 Weiherbach Pond	1st	26-Apr	0.454	0.770	Drift
R1 Weiherbach Stream	1st	26-Apr	5.176	0.784	Run-off
R2 Porto Stream	1st	06-Mar	4.896	0.736	Run-off
R3 Bologna Stream	1st	19-Feb	8.485	1.235	Run-off
R4 Roujan Stream	1st	27-Feb	14.514	2.539	Run-off

Metabolites: Methylbenzenesulphonylcarbamate (MBSC) Parameters used in FOCUSsw step 1 and 2	Molecular weight: 215 Water solubility (mg/L): 100,000 (20°C) Soil or water metabolite: water Koc/Kom (L/kg): 2.8/1.6 (if necessary, soil metabolites) DT_{50} soil (d): 14.9 days (If necessary, Lab or field. In accordance with FOCUS SFO) DT_{50} water/sediment system (d): (representative worst case from sediment water studies) DT_{50} water (d): 1000 DT_{50} sediment (d): 1000 Crop interception (%): 0 Maximum occurrence observed (% molar basis with respect to the parent) Water: 10.3% Sediment:10.3%
Parameters used in FOCUSsw step 3 (if performed)	Vapour pressure: 0 Koc/Kom (L/kg): 2.8/1.6 1/n: 1.0 (Freundlich exponent general or for soil ,susp. solids or sediment respectively) Metabolite kinetically generated in simulation (yes/no): no Formation fraction in soil (k_{dp}/k_f): (If formation degradation of metabolite is kinetically simulated by PRZM) n/a
Application rate	Crop: spinach/flower bulbs Number of applications: 1 Interval (d): n/a Application rate(s): 2400 g as/ha Depth of water body: 30 cm Application window: 30 days
Main routes of entry	Drift/Runoff

FOCUS STEP 1 Scenario	Day after overall maximum	PEC_{SW} (µg/L)		PEC_{SED} (µg/kg)	
		Actual	TWA	Actual	TWA
Leafy vegetables	0h	46.8		1.31	
	24h				
	2d				
	4d				
	7d				
	14d				
	21d				
	28d				
	42d				

FOCUS STEP 2 Scenario	Day after overall maximum	PEC_{SW} (µg/L)		PEC_{SED} (µg/kg)	
		Actual	TWA	Actual	TWA
Northern EU	0 h	8.71		0.24	
	24 h				
	2 d				
	4 d				
	7 d				
	14 d				
	21 d				
	28 d				
	42 d				
Southern EU	0 h				
	24 h				
	2 d				
	4 d				
	7 d				
	14 d				
	21 d				
	28 d				
	42 d				

FOCUS STEP 3

Maximum PEC_{sw} and PEC_{sed} of MBSC from application of asulam to spinach at Step 3

Scenario	1st or 2nd Crop	Application dates	$PEC_{SW, Max}$ (µg L^{-1})	$PEC_{Sed, Max}$ (µg kg^{-1})	Dominant entry route
D3 Vredepeel Ditch	1st	10-Apr	1.473	0.100	Drift
D3 Vredepeel Ditch	2nd	25-Jul	1.623	0.233	Drift
D4 Skousbo Pond	1st	16-May	0.214	0.125	Drainage
D4 Skousbo Stream	1st	16-May	0.242	0.083	Drainage
D6 Thiva Ditch	1st	04-Aug	1.620	0.309	Drainage
R1 Weiherbach Pond	1st	26-Apr	0.051	0.021	Drift
R1 Weiherbach Stream	1st	26-Apr	0.150	0.008	Run-off
R1 Weiherbach Pond	2nd	28-Jul	0.051	0.021	Drift
R1 Weiherbach Stream	2nd	28-Jul	0.000	0.000	Run-off
R2 Porto Stream	1st	16-Mar	0.493	0.028	Run-off
R2 Porto Stream	2nd	05-Aug	0.013	0.001	Run-off
R3 Bologna Stream	1st	03-Mar	0.517	0.027	Run-off
R3 Bologna Stream	2nd	09-Jun	1.657	0.138	Run-off
R4 Roujan Stream	1st	12-Mar	1.505	0.111	Run-off
R4 Roujan Stream	2nd	07-Jun	3.714	0.278	Run-off

Maximum PEC_{sw} and PEC_{sed} of MBSC from application of asulam to flower-bulbs at Step 3

Scenario	1st or 2nd Crop	Application dates	$PEC_{SW, Max}$ (µg L^{-1})	$PEC_{Sed, Ma}$ (µg kg^{-1})	Dominant entry route
D3 Vredepeel Ditch	1st	10-Apr	1.476	0.101	Drift
D4 Skousbo Pond	1st	18-Apr	0.455	0.276	Drainage
D4 Skousbo Stream	1st	18-Apr-	1.178	0.233	Drainage
D6 Thiva Ditch	1st	03-May	1.479	0.086	Drift
D6 Thiva Ditch	2nd	06-Oct	1.478	0.293	Drift
R1 Weiherbach Pond	1st	26-Apr	0.051	0.022	Drift
R1 Weiherbach Stream	1st	26-Apr	0.746	0.041	Run-off
R2 Porto Stream	1st	06-Mar	0.462	0.026	Run-off
R3 Bologna Stream	1st	19-Feb	0.525	0.028	Run-off
R4 Roujan Stream	1st	27-Feb	1.518	0.112	Run-off

PECsw Photodegradates
Three major photodegradates formed in the direct photolysis study were:

	Sulphanilic acid	AP formamide	MCAPAP carbamic acid
Molecular mass	173	136	301
% formation	55.5	24.2	11.9

Step 3 and Step 4 PECsw values were calculated based on the concentration of asulam and the relative molecular mass and percent formation.

STEP 3

PEC_{SW} values of asulam photodegradates at Step 3 following application to spinach

Scenario	1st or 2nd Crop	PECsw (µg/L)			
		Asulam	Sulphanilic acid	AP formamide	MCAPAP carbamic acid
D3 Vredepeel Ditch	1st	15.204	6.347	2.176	2.368
D3 Vredepeel Ditch	2nd	15.253	6.367	2.183	2.375
D4 Skousbo Pond	1st	0.527	0.220	0.075	0.082
D4 Skousbo Stream	1st	12.102	5.052	1.732	1.885
D6 Thiva Ditch	1st	15.350	6.408	2.197	2.391
R1 Weiherbach Pond	1st	0.525	0.219	0.075	0.082
R1 Weiherbach Stream	1st	10.032	4.188	1.436	1.562
R1 Weiherbach Pond	2nd	0.525	0.219	0.075	0.082
R1 Weiherbach Stream	2nd	9.950	4.154	1.424	1.550
R2 Porto Stream	1st	21.850	9.121	3.127	3.403
R2 Porto Stream	2nd	13.492	5.632	1.931	2.101
R3 Bologna Stream	1st	36.279	15.145	5.191	5.650
R3 Bologna Stream	2nd	16.007	6.682	2.291	2.493
R4 Roujan Stream	1st	62.969	26.287	9.011	9.806
R4 Roujan Stream	2nd	42.370	17.688	6.063	6.598

PEC_{SW} values of asulam photodegradates at Step 3 following application to flower-bulbs

Scenario	1st or 2nd Crop	PECsw (µg/L)			
		Asulam	Sulphanilic acid	AP formamide	MCAPAP carbamic acid
D3 Vredepeel Ditch	1st	15.199	6.345	2.175	2.367
D4 Skousbo Pond	1st	0.527	0.220	0.075	0.082
D4 Skousbo Stream	1st	11.827	4.937	1.692	1.842
D6 Thiva Ditch	1st	15.176	6.335	2.172	2.363
D6 Thiva Ditch	2nd	15.350	6.408	2.197	2.391
R1 Weiherbach Pond	1st	0.570	0.238	0.082	0.089
R1 Weiherbach Stream	1st	24.052	10.041	3.442	3.746
R2 Porto Stream	1st	21.145	8.827	3.026	3.293
R3 Bologna Stream	1st	36.379	15.187	5.206	5.665
R4 Roujan Stream	1st	61.081	25.499	8.740	9.512

STEP 4

PEC_{SW} values of asulam photodegradates at Step 4 – 5 m buffer and 80% run-off mitigation - following application to spinach

Scenario	1st or 2nd Crop	PECsw (µg/L)			
		Asulam	Sulphanilic acid	AP formamide	MCAPAP carbamic acid
D3 Vredepeel Ditch	1st	4.121	1.720	0.590	0.642
D3 Vredepeel Ditch	2nd	4.134	1.726	0.592	0.644
D4 Skousbo Pond	1st	0.457	0.191	0.065	0.071
D4 Skousbo Stream	1st	4.422	1.846	0.633	0.689
D6 Thiva Ditch	1st	5.625	2.348	0.805	0.876
R1 Weiherbach Pond	1st	0.454	0.190	0.065	0.071
R1 Weiherbach Stream	1st	3.664	1.530	0.524	0.571
R1 Weiherbach Pond	2nd	0.454	0.190	0.065	0.071
R1 Weiherbach Stream	2nd	3.635	1.517	0.520	0.566
R2 Porto Stream	1st	5.057	2.111	0.724	0.788
R2 Porto Stream	2nd	4.928	2.057	0.705	0.767
R3 Bologna Stream	1st	8.446	3.526	1.209	1.315
R3 Bologna Stream	2nd	5.166	2.157	0.739	0.805
R4 Roujan Stream	1st	14.946	6.239	2.139	2.328
R4 Roujan Stream	2nd	10.142	4.234	1.451	1.579

PEC_{SW} values of asulam photodegradates at Step 4 – 5 m buffer and 80% run-off mitigation - following application to flower-bulbs

Scenario	1st or 2nd Crop	PECsw (µg/L)			
		Asulam	Sulphanilic acid	AP formamide	MCAPAP carbamic acid
D3 Vredepeel Ditch	1st	4.119	1.720	0.589	0.641
D4 Skousbo Pond	1st	0.457	0.191	0.065	0.071
D4 Skousbo Stream	1st	4.322	1.804	0.618	0.673
D6 Thiva Ditch	1st	4.279	1.786	0.612	0.666
D6 Thiva Ditch	2nd	4.160	1.737	0.595	0.648
R1 Weiherbach Pond	1st	0.454	0.190	0.065	0.071
R1 Weiherbach Stream	1st	5.176	2.161	0.741	0.806
R2 Porto Stream	1st	4.896	2.044	0.701	0.762
R3 Bologna Stream	1st	8.485	3.542	1.214	1.321
R4 Roujan Stream	1st	14.514	6.059	2.077	2.260

Groundwater

B.8.6.2 Groundwater (PECgw)

The calculations of PECgw values were carried out according to recommendations by the FOCUS groundwater scenarios work group (FOCUS, 2000[5], 2002[6]) based on the kinetic re-evaluations of asulam and sulphanilamide in studies Hardy 2011a and Hardy 2013a. The studies are summarised in the previous section, B.8.1.3. The leaching model FOCUS PELMO (Version 3.3.2) and FOCUS PEARL v.3.3.3 were used to investigate the potential for contamination of groundwater by asulam and the soil metabolite sulphanilamide. Degradation was assumed to follow first order kinetics.

The representative GAP in Europe for asulam stipulates one pre- or post-emergence application to spinach and one post-emergence application to glower-bulbs at a maximum rate of 2400 g/ha, for Northern and Southern Europe. A crop interception of 0% was considered for application to spinach and 10% for application to flower-bulbs.

The Good Agricultural Practice (GAP) proposed for the use of asulam is presented in Table B.8. 182 below:

Table B.8. 182 GAP used in groundwater modelling for asulam and soil metabolite sulphanilamide

Crop	Location	Application		Plant Interception (%)
		Growth Stage BBCH	Rate of asulam (g ha^{-1})	
Spinach (Cabbage)	Europe	Pre- or early post-emergence (12) at 2 – 4 true leaf stage	1 x 2400	0
Flower bulbs (Onions)	Europe	Post-emergence Up to 40	1 x 2400	10

Calculations were based on the assumptions presented in

[5] FOCUS (2000). FOCUS groundwater scenarios in the EU review of active substances - The report of the work of the Groundwater Scenarios Workgroup of FOCUS (Forum for the Co-ordination of pesticide fate models and their USe), Version 1 of November 2000. EC Document Reference Sanco/321/2000 rev. 2.
[6] FOCUS (2002). Generic guidance for FOCUS groundwater scenarios. Version 1.1, April 2002.

Table B.8. 183.

Table B.8. 183 **Summary of the input values for asulam and its metabolite sulphanilamide for calculation of PEC_{GW} values**

Input parameter	Selected value
Molecular weight asulam (g mol^{-1})	230.24
Molecular weight sulphanilamide (g mol^{-1})	172.20
Water Solubility asulam (mg L^{-1}, 25°C)	962000
Vapour Pressure asulam (Pa, 20°C)	5×10^{-7}
Water Solubility sulphanilamide (mg L^{-1}, 25°C)	7500
Vapour Pressure sulphanilamide (Pa, 20°C)*	9.7×10^{-4}
K_{Foc} asulam (L kg^{-1})	25.4
K_{Fom} asulam (L kg^{-1})	14.7
Freundlich exponent 1/n asulam	0.75
K_{Foc} sulphanilamide (L kg^{-1})	166.0
K_{Fom} sulphanilamide (L kg^{-1})	96.3
Freundlich exponent 1/n sulphanilamide	0.71
Geomean DT_{50} asulam at 20°C and pF2 (d)	4.6
Formation fraction of asulam to sulphanilamide (%)	100
Mean DT_{50} sulphanilamide at 20°C and pF2 (d)	13.4
Application rate (g ha^{-1})	2400
Crop Intercept (%) for spinach	0
Crop Intercept (%) for flower bulbs	10
Crop uptake	0 (default value)
Molar activation energy (kJ mol^{-1})	65.4
Q_{10}	2.58

The timings of application for each scenario were as reported in the FOCUS Groundwater report for cabbage which are presented in Table B.8. 184 below.

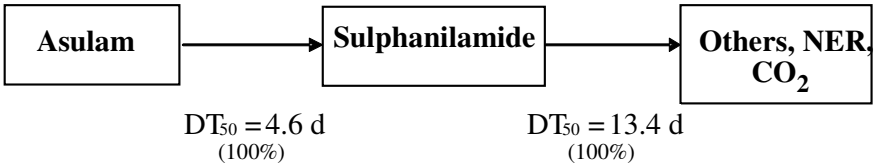
Table B.8. 184 Application timings in the FOCUS groundwater scenarios for cabbage or bulbs

Scenario	Emergence *
Châteaudun	20th Apr + 31st Jul
Hamburg	20th Apr + 31st Jul
Jokioinen	20th May
Kremsmünster	20th Apr + 31st Jul
Porto	28th Feb + 31st Jul
Sevilla	1st Mar + 15th Jun
Thiva	15th Aug

* transplanted from seed bed on dates shown

A worst-case transformation percentage of 100% has been assumed for the formation of sulphanilamide. The formation and degradation of the metabolite sulphanilamide is simulated using the standard metabolite function of the PELMO model as a transformation product from the parent asulam. The degradation scheme proposed by the Notifier which is considered acceptable by the Rapporteur, is presented in Figure B.8. 43 below.

Figure B.8. 43 Degradation scheme for asulam and sulphanilamide used in the FOCUS groundwater evaluation used by the Notifier



The RMS considered that the parameters used in the groundwater modelling were acceptable The results of the groundwater modelling are presented in Table B.8.23. For asulam and sulphanilamide, the 80th percentile annual average in leachates below 1 m were predicted to be less than 0.001 µg/L in seven FOCUS scenarios modelled for both models (PELMO and PEARL).

The RMS notes that although the concentrations in groundwater are predicted to be less than 0.001 µg/L by both models and for all scenarios, monitoring studies have demonstrated the potential for leaching into groundwater in areas of high vulnerability. One study performed in Denmark and two studies in the USA showed concentrations of asulam and sulphanilamide exceeding the EU drinking water limit of 0.1 µg/L. Full details are presented in section B.8.7 of the DAR.

The results following application to spinach are presented in Table B.8. 185 and Table B.8. 186, and those following application to flower-bulbs are presented in Table B.8. 187 and

Table B.8. 188.

Table B.8. 185 **80th percentile concentrations for asulam and sulphanilamide at 1m depth following application of asulam at 1 x 2400 g ha^{-1} to spinach, calculated using FOCUS PEARL**

Scenario	Predicted Environmental Concentrations in groundwater (µg L^{-1})	
	Asulam	Sulphanilamide
Châteaudun	<0.001	<0.001
Hamburg	<0.001	<0.001
Kremsmünster	<0.001	<0.001
Okehampton	<0.001	<0.001
Piacenza	<0.001	<0.001
Porto	<0.001	<0.001

Table B.8. 186 80th percentile concentrations for asulam and sulphanilamide at 1m depth following application of asulam at 1 x 2400 g ha^{-1} to spinach, calculated using FOCUS PELMO

Scenario	Predicted Environmental Concentrations in groundwater (µg L^{-1})	
	Asulam	Sulphanilamide
Châteaudun	<0.001	<0.001
Hamburg	<0.001	<0.001
Kremsmünster	<0.001	<0.001
Okehampton	<0.001	<0.001
Piacenza	<0.001	<0.001
Porto	<0.001	<0.001

Table B.8. 187 80th percentile concentrations for asulam and sulphanilamide at 1m depth following application of asulam at 1 x 2400 g ha^{-1} to flower bulbs, calculated using FOCUS PEARL

Scenario	Predicted Environmental Concentrations in groundwater (µg L^{-1})	
	Asulam	Sulphanilamide
Châteaudun	<0.001	<0.001
Hamburg	<0.001	<0.001
Kremsmünster	<0.001	<0.001
Okehampton	<0.001	<0.001
Piacenza	<0.001	<0.001
Porto	<0.001	<0.001

Table B.8. 188 80th percentile concentrations for asulam and sulphanilamide at 1m depth following application of asulam at 1 x 2400 g ha^{-1} to flower bulbs, calculated using FOCUS PELMO

Scenario	Predicted Environmental Concentrations in groundwater (µg L^{-1})	
	Asulam	Sulphanilamide
Châteaudun	<0.001	<0.001
Hamburg	<0.001	<0.001
Kremsmünster	<0.001	<0.001
Okehampton	<0.001	<0.001
Piacenza	<0.001	<0.001
Porto	<0.001	<0.001

Method of calculation and type of study (*e.g.* modelling, field leaching, lysimeter)	For FOCUS gw modelling, values used – Modelling using FOCUS model(s), with appropriate FOCUSgw scenarios, according to FOCUS guidance. Model(s) used: (with version control no.(s)) FOCUS PEARL v.4.4.4 and FOCUS PELMO v. 4.4.3 Scenarios (list of names): Châteaudun, Hamburg, Jokioinen, Kremsmünster, Porto, Sevilla, Thiva Crop: Cabbage (surrogate for spinach), flower bulbs Geometric mean or median parent DT_{50lab} 4.6 d (normalisation to 10kPa or pF2, 20 °C with Q10 of 2.58). K_{OC}: parent, arithmetic mean 25.4, $^1/_n$= 0.75. Metabolites: Geometric mean sulphanilamide DT50lab 13.4 d (normalisation to 10kPa or pF2, 20°C with Q10 of 2.58). Kfoc: sulphanilamide, arithmetic mean 166, 1/n= 0.71.
Application rate	Application rate: 2400 g/ha. No. of applications: 1 Time of application (month or season): Spring/summer

PEC(gw) - FOCUS modelling results (80th percentile annual average concentration at 1m) Cabbage

	Scenario	Asulam (µg/L)	Sulphanilamide (µg/L)
PELMO & PEARL/Spinach	Châteaudun	<0.001	<0.001
	Hamburg	<0.001	<0.001
	Jokioinen	<0.001	<0.001
	Kremsmünster	<0.001	<0.001
	Porto	<0.001	<0.001
	Sevilla	<0.001	<0.001
	Thiva	<0.001	<0.001

PEC(gw) - FOCUS modelling results (80th percentile annual average concentration at 1m) Flower bulbs

	Scenario	Asulam (µg/L)	Sulphanilamide (µg/L)
PELMO & PEARL/Flower bulbs	Châteaudun	<0.001	<0.001
	Hamburg	<0.001	<0.001
	Jokioinen	<0.001	<0.001
	Kremsmünster	<0.001	<0.001
	Porto	<0.001	<0.001
	Sevilla	<0.001	<0.001
	Thiva	<0.001	<0.001

Note that although asulam is predicted <0.001 µg/L in all scenarios, monitoring studies have shown levels exceeding 0.1 µ/L in areas of high vulnerability that do not necessarily correspond with areas of spinach use (see Monitoring Data below).

B.8.7 Fate and behaviour in air (IIA 7.2.2, IIIA 9.3)

a) A theoretical calculation of the potential for photo-oxidation of asulam in the atmosphere was submitted, using the AOPWIN v1.88 computer package. A rate constant of 28.732×10^{-12} cm³/molecule/second was calculated for reaction with OH radicals. This corresponded to a first order DT_{50} of 0.372 days based on an OH radical concentration of 1.5 x 10^6 cm^{-3} on a 12 h day basis. Based on the short half-life and the low vapour pressure (~10^{-7} Pa) of asulam it is concluded that asulam would be unlikely to be subject to long range transport.

(van der Gaauw, 2001a)

Predicted environmental concentrations in air (PECa) (IIIA 9.3)

Negligible.

Summary & assessment – fate in air

Direct photolysis in air ‡	Not studied - no data requested
Quantum yield of direct phototransformation	0.157 mol · Einstein $^{-1}$ at pH 9, 0.114 mol · Einstein $^{-1}$ at pH 4 Average half-life in surface waters of Central Europe in June calculated to be 0.5 d at pH 9 and 0.29 d at pH 4.
Photochemical oxidative degradation in air ‡	DT_{50} of 0.372 days derived by the Atkinson model (AOPWIN v1.88). OH (12 h) concentration assumed = 1.5 x 10^{-6}
Volatilisation ‡	from plant surfaces (BBA guideline): Not studied - no data requested
	from soil surfaces (BBA guideline): Not studied - no data requested
Metabolites	None

B.8.8 Predicted environmental concentrations in air (PECa) (IIIA 9.3)

Method of calculation	Expert judgement, based on vapour pressure, dimensionless Henry's Law Constant and information on volatilisation from plants and soil.

$PEC_{(a)}$

Maximum concentration	negligible

Residues requiring further assessment

Environmental occurring metabolites requiring further assessment by other disciplines (toxicology and ecotoxicology).	Soil: Asulam, Sulphanilamide Surface Water: Asulam, Sulphanilamide, sulphanilic acid, AP formamide, MCAPAP carbamate Sediment: Asulam, Sulphanilamide Ground water: Asulam, Sulphanilamide Air: Asulam

B.8.9 Monitoring data

a) An investigation was conducted into the concentrations of asulam and its metabolite, sulphanilamide in the 'Feddet Plantation' in Denmark. The study did not follow any guideline and was not conducted to GLP however the study was conducted prior to the requirement for GLP.

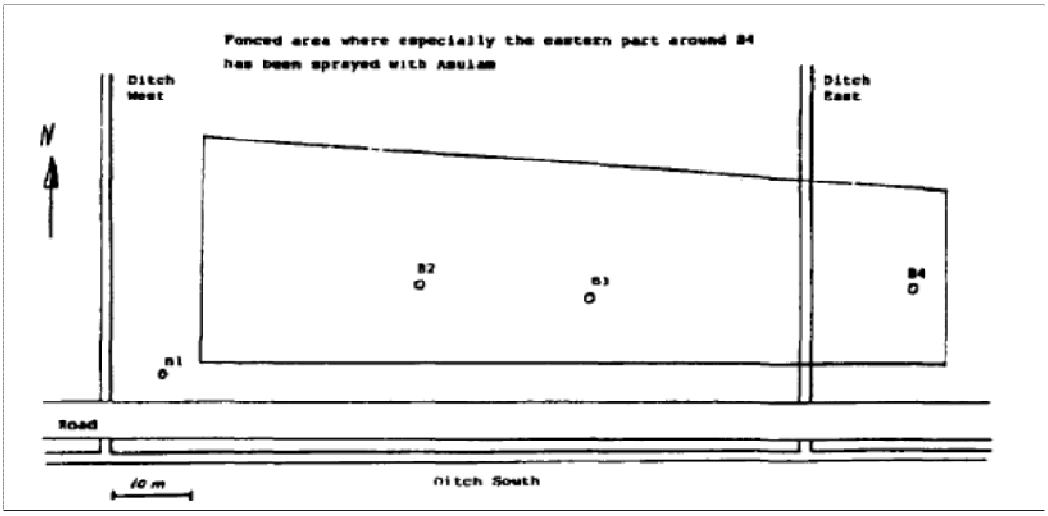
The Feddet Plantation is in a shallow sandy soil area and the groundwater table is less than 1m under the surface. This was considered by the Notifier to be an area extremely vulnerable to pollution from the surface. The area had been cleared of pinewood and sprayed with asulam at an application rate of 2.8 kg/ha (slightly above the GAP dose of 2.4 kg/ha) over the period August 1990 and May 1991 prior to re-forestation with oak. The spraying was undertaken over the eastern area only but the spray was reported to drift beyond this area. Four boreholes were drilled running from west to east numbered B1 to B4 respectively. Five groundwater samples were taken, 1 each from boreholes 1 to 3 and two from borehole number 4, concentrating sampling over the eastern area. A sketch of the sprayed site is shown diagrammatically in

Figure B.8. 44. The results of the sampling are presented in Table B.8. 189. The study authors do not state the method of analysis used. One sample for asulam and for sulphanilamide (at greater depth) exceeded 0.1µg/L. It was concluded by the Notifier that because the area is covered by shallow sandy soil, the seepage of contaminants would reach groundwater very quickly when the autumn and winter downward water movement starts. In addition the conditions in the aquifer were considered by the authors to be anaerobic due to the high organic matter content under which conditions the degradation of asulam is slow. The Notifier suggested that for the use of asulam on spinach, the application rate is lower and treatment is in spring or early summer, there is not normally a downward movement of water and risk of contamination would be less. The Rapporteur notes that the FOCUS groundwater modelling predicted that levels of asulam would be <0.001 µg/L in groundwater.

Table B.8. 189 Concentrations of Asulam and Sulphanilamide in groundwater samples from the 'Feddet Plantation' in Denmark

Date	Drilling	Depth (m)	Asulam (µg/L)	Sulphanilamide (µg/L)
25/03/92	B1a	0.75	0.01	<0.01
25/03/92	B2	1.75	0.04	<0.01
25/03/92	B3	1.25	0.03	<0.01
25/03/92	B4	0.75	0.07	0.06
25/03/92	B4	1.25	0.21	0.13

Figure B.8. 44 Sketch of the sprayed site with borehole locations



(Thorvardason and Kristiansen, 1992a)

b) A small scale prospective groundwater monitoring study with asulam sodium applied to sugar cane and turf was performed following a "Data Call In Notice for Groundwater Monitoring Data on asulam sodium" issued by the United States Environmental Protection Agency since available soil dissipation studies were found to be inadequate by the Agency. The study was not performed in accordance with any guideline however it was conducted to GLP.

Three sites were chosen which had not been treated with asulam during the previous 2 years and characterised as having sandy subsurface soils and having a water table within 3 m of the surface. The three sites were a Florida turf grass site, a Florida sugar cane site and a Louisiana sugar cane site. Asulam sodium was applied at a rate of 2.25 kg/ha in January at the Florida turf site, at 3.98 kg/ha in February in the Florida cane site and at 7.4 kg/ha in May at the Louisiana cane site. At the Louisiana site asulam was tank mixed with sodium bromide at 88.7 kg/ha as a tracer to track the movement of water through the soil to groundwater.

Following application of asulam, soil, groundwater and surface water were sampled for 14 months at the Florida turf site, 13 months at the Florida cane site and 10 months at the Louisiana cane site. Sampling was suspended in agreement with EPA Environmental Branch because no residues of asulam or sulphanilamide were detected in soil samples taken during the last 6 to 10 months and because further information about the residues of asulam in groundwater would not be gained by continuing sampling.

Methods of analysis for asulam and sulphanilamide were developed with an LOD of 1 µg/L for water and 10 µg/L for soil. It is noted by the Rapporteur that the LOD is above the statutory limit (0.1 µg/L) for a pesticide in ground water.

Asulam residues were found in the surface water from each test site soon after application. No residues were found after the 0.5 month post application sampling

interval except at the Florida turf site which was attributed to 'spot spraying' of noxious weeds in the area.

At the Florida turf site, asulam and sulphanilamide were detected sporadically (>1µg/L) in groundwater from one of the two shallow 'in field' wells and from the two 'out field' groundwater well clusters which was observed in these wells prior to asulam application. This was considered to be due to illegal spot spraying of asulam to nearby fields around the test plot. The only detection in groundwater at the Florida cane site was from an 'in field well' taken 1 month post application (8 µg/L) but which dissipated to <1µg/L within 5 months post-application. Contamination of this well, which was in the spraying zone, was suspected.

At the Louisiana cane site sodium bromide was used to track the movement of water through the soil and demonstrate that bromide residues were contained within the top metre of soil during the first 2 months post application during which the asulam degraded. High levels of bromide were observed in the groundwater sampled 9 months post application. No residues of asulam (>1 µg/L) were detected in groundwater samples.

Overall it was concluded by the Notifier that asulam does not persist in soil or surface water but the potential for asulam to be found in surface water soon after application does exist. The source of asulam in surface water was considered to be due to low level drift during application. The source of asulam residues in groundwater is probably due to the vertical and lateral movement of water in soil but also via irrigation through the canals from nearby applications. It is concluded by the Rapporteur that this study was of interest but had limited relevance to application of asulam to spinach in Europe.

(Norris, 1992a)

c) A one year drinking water monitoring study was conducted in USA to assess human exposure to residues of asulam and its metabolite sulphanilamide in high use areas of Florida and Louisiana. The study was not performed in accordance with any guideline but was conducted to GLP.

Monthly sampling was conducted for one year from the surface water used by community water systems in Florida and Louisiana (five in each state). In addition, single samples were collected from several potable wells in the high use areas of both states (28 in Florida and 16 in Louisiana). All samples were analysed for asulam and sulphanilamide by an LC-MS/MS method with LOQ for each of 1µg/L and LOD of 0.2µg/L for asulam and 0.1µg/L for sulphanilamide. The requirement for the sampling locations was that they utilised surface water taken from asulam treatment areas or wells within 1000 feet of asulam treated areas. Sampling of surface water from Louisiana began in June 1991 and from Florida in September 1991. Potable wells were sampled once in both Louisiana and Florida.

The results for Louisiana surface waters showed traces of asulam and sulphanilamide at <1µg/L at four of the five community water systems in the first 2 months of sampling but were not seen in any samples collected from August to April.

The results for Florida surface waters showed residues at <1 µg/L were found during May and June but none were found during other periods of the year.

Samples were collected from 16 potable wells in Louisiana, from which no residues of asulam were detected.

In Florida a total of 28 wells satisfying the sampling criteria were identified. Three wells contained residues of asulam >1µg/L and ten contained traces of asulam <1µg/L.

It was concluded by the Notifier that human exposure to asulam via drinking water was very limited and well below the estimated Maximum Contaminant Level Goal (MCGL) of 350µg/L for asulam (a level set by the US EPA), even in areas of high use and in which use is much greater than in Europe. It was considered by the Rapporteur that detection limits for asulam and sulphanilamide were inadequate and that the levels of asulam found in well water failed to satisfy the 0.1µg/L limit for pesticides in groundwaters in Europe. However estimates of the potential for groundwater contamination following the standard FOCUS modelling approach no concern (section B.8.5).

(Gustafson, 1993a)

d) A joint study was set up between the UK National Rivers Authority, Yorkshire Water and Rhone-Poulenc Agriculture Limited to investigate the presence and persistence of asulam in surface water following aerial applications to bracken. The study was not conducted to any guideline and was not conducted according to GLP however the Rapporteur considers that the conduct of the study was acceptable.

Three sites were treated near Osmotherly (North Yorkshire, UK) on 6th August 1993. This region was chosen because asulam was regularly applied there. The sites chosen were:

Site B: Pamperdale, a sloping moorland upstream of Cod Beck reservoir (a water supply reservoir to Osmotherly water treatment works. (4 sampling points)
Site C: Slape Stones Beck, a portion of Middlesbrough Hill on the northern side of which a stream flows leading to Lower Oakdale reservoir (a river compensation reservoir) (6 sampling points).
Site D: NE part of Thimbleby Moor. An area where several springs emerge (4 sampling points).

Application of asulam was delayed due to heavy rain and wind (approximately 76 mm rainfall 2 days prior to application). Asulam was sprayed by helicopter (application rate equivalent to 3.7 kg a.i./ha). Observers were sent to each site before spraying started to record spray drift. Larger areas than specified were reported to have been sprayed. In Site C, application to the southern side of Middlesbrough Hill could not be watched. At site D the helicopter returned several times due to a technical fault and seemed to re-spray the entire area.

Sampling was spread over a two week period which was considered by the Notifier to cover the degradation of asulam. In addition to a pre-spraying collection of control samples, sampling was performed at day 0, day 1, day 7 and day 13. Analysis was by

HPLC with fluorimetric detection providing limits of detection of 0.01 and 0.05µg/L for asulam and sulphanilamide respectively.

No asulam residues were detected in pre-study samples. The results of the analyses over the study period are summarised in Table B.8. 190.

Table B.8. 190 Results of the analyses for asulam (A) and sulphanilamide (S) in µg/L

Sampling Point	Pre-study		Application Day		1 Day		7 Days		13 Days	
	A	S	A	S	A	S	A	S	A	S
B1	<0.01	<0.05	0.34	<0.05	<0.01	<0.05	0.11	<0.05	0.03	<0.05
B2	<0.01	<0.05	28.4	0.77	1.16	<0.05	0.15	<0.05	0.10	<0.05
B3	<0.01	<0.05	<0.01	<0.05	2.20	0.06	0.15	<0.05	0.12	<0.05
B4	<0.01	<0.05	<0.01	<0.05	<0.01	<0.05	0.14	<0.05	0.20	<0.05
C1	<0.01	<0.05	496	10.1	9.23	0.15	0.15	0.05	-	-
C2	<0.01	<0.05	570	13.5	19.6	0.22	0.83	0.05	0.39	0.05
C3	<0.01	<0.05	464	9.32	93.7	1.26	9.3	0.21	2.31	0.1
C4	<0.01	<0.05	618	13.9	41.7	0.32	2.53	0.06	0.57	0.05
C5	<0.01	<0.05	109	3.14	7.81	0.14	1.10	<0.05	0.38	<0.05
C6	<0.01	<0.05	0.21	<0.05	4.53	0.11	0.59	<0.05	0.20	<0.05
D1	<0.01	<0.05	<0.01	<0.05	5.92	0.13	0.28	<0.05	0.05	<0.05
D2	<0.01	<0.05	0.48	<0.05	61.7	0.88	21.3	0..39	0.55	<0.05
D3	<0.01	<0.05	<0.01	<0.05	0.41	0.05	47.1	0.57	-	-
D4	<0.01	<0.05	<0.01	<0.05	<0.01	<0.05	0.12	<0.05	<0.01	<0.05

The low level of asulam detected at B1 and the higher concentration in B2 were consistent with the reported spray drift.

In site C, a portion of the stream was treated by accident which accounted for the high levels of asulam found at day 0 in samples from this site.

In site D, a delay in spraying due to the helicopter technical fault may have resulted in samples being taken before the application was complete reflected in higher residues being collected on day 1.

Asulam concentrations in surface water dropped several orders of magnitude within 2 weeks of application although the concentrations detected at the two reservoir outlets (B4 and C6) and exceeded the EC Drinking water directive limit of 0.1µg/L.

In the above study the application rate of asulam was up to three times the GAP for its use on spinach. Although the study may be of interest for additional uses at Member State level, it is of limited significance to the application of asulam to spinach considered for approval.

(Boussemart, 1994a)

B.8.9.1 Summary and assessment

Four monitoring studies were performed to investigate the leaching of asulam into groundwater or surface water following application of asulam in the field. A Danish study demonstrated the potential for asulam to leach into groundwater in areas of high vulnerability with shallow sandy soil. Two studies were performed in America. A groundwater monitoring study was investigated with application of asulam sodium to sugar cane and turf. Asulam was found in surface water soon after application and was observed in groundwater but dissipated quickly. A drinking water monitoring study showed residues of asulam at greater than the 0.1 µg/L limit for drinking water although the studies were performed in areas of high application and limits of quantitation were 1 µg/L. A UK study was conducted in which asulam was applied by aerial spray from a helicopter to bracken close to streams and reservoirs. Monitoring of levels of asulam and sulphanilamide demonstrated the importance of adequate no spray zones to avoid spray drift. Levels of asulam in surface water were initially high but decreased rapidly although the concentrations detected at the two reservoir outlets exceeded the EC Drinking water directive limit of 0.1µg/L after 13 days. In the above studies, application rates of asulam were higher (up to 3 times) than the GAP for use on spinach and although may be of interest for additional uses at Member State level are of limited significance to the application to spinach considered for approval.

B.8.10 Definition of the residue (IIA 7.3)

The metabolic pathway of asulam in the environment has been extensively studied and a summary of the pathway proposed by the Notifier is presented in Figure B.8.5

In soil the extractable residues of asulam comprise principally the parent plus sulphanilamide (>10%). Under aerobic conditions, only asulam was the major component (>10%).

In surface water and sediment asulam was the major component in the residue (>10%), sulphanilamide being a minor metabolite.

Monitoring data, if available (Annex IIA, point 7.4)

Soil (indicate location and type of study)	No data provided - none requested
Surface water (indicate location and type of study)	Florida and Lousiana, USA, to assess human exposure to asulam and sulphanilamide in high use areas. Louisiana site: traces of asulam and sulphanilamide <1µg/L I surface water. No residues of asulam detected in potable wells. Florida site: residues <1µg/L May & June only. North Yorkshire, UK, 1993. in steams and receiving rivers in an area where 'active substance' was applied annually to treat bracken in moorland (active substance

	and sulphanilamide). streams: peaks of 28.4 to 618 µg/L on day of application µg/l Reservoirs: peaks of *0.2* µg/l
Ground water (indicate location and type of study)	Feddet Plantation, Denmark, monitoring of a shallow aquifer underlying forested land from 1990-1991 (active substance and Met I). Asulam: Maximum in borehole 0.21 µg/L Sulphanilamide: Maximum in borehole 0.13 µg/l Florida and Louisiana, USA, 1989-1991, groundwater monitoring following application to sugar cane and turf. Florida turf site asulam and sulphanilamide sporadically >1%. Florida cane site: asulam peak of 8 µg/L in field well. Louisiana cane site: asulam not greater than 1 µg/L
Air (indicate location and type of study)	No data available

In soil asulam and sulphanilamide were the major (>10% Applied) components of the residue

In surface water asulam, sulphanilamide and MBSC were the major (>10% Applied) components of the residue

In soil groundwater asulam and sulphanilamide were the major (>10% Applied) components of the residue

In groundwater asulam and sulphanilamide were the major (>10% Applied) components of the residue

The relevant residue for monitoring would therefore be asulam and sulphanilamide in soil, asulam, Sulphanilamide, sulphanilic acid, AP formamide, MCAPAP in surface water, asulam and sulphanilamide in ground water and in sediment.

B.8.11 Literature search

The Applicant has submitted a literature review in support of the new active substance asulam. The applicant searched for published information on the herbicidal active substance asulam sodium and any potential relevant metabolites. For asulam, the search included the terms ‘asulam’, ‘asulam sodium’ and ‘Asulox’. For potential relevant metabolites the code name, CAS number and structures were searched. A further search of the of the chemical name for metabolites acetyl asulam, AP formamide, MCAPAP carbamic acid, malonoyl asulam and asulam glc was also performed. The RMS agrees with the terms used in the search. This search considered studies published since 2004. The remit of this search process was to check for new information that may affect the toxicological and environmental risk assessment. It was considered by the applicant that articles published prior to this stage would not be

considered as new information. This is in line with Article 8(5) of Regulation (EC) No 1107/2009 stating that applicants should include in the dossier the most recent scientific peer-reviewed open literature published during ten years prior to the dossier submission date. The RMS agrees with this.

The databases searched include the following:

STN-Databases:
Anabstr - Analytical abstracts
Biosis
Caplus - chemical abstracts plus
Chemlist
Embase - The Excerpta Medica database
Scisearch
Toxcenter
Medline
Rtecs- Registry of Toxic Effects of Chemical Substances

Other Databases
Pubmed
Science Direct
Wiley online library

A review of the published literature for asulam sodium and potential relevant metabolites revealed one article that was considered to be relevant for the risk assessment of human health, animal health or the environment.

A summary of the results of the literature search for asulam / asulam sodium / asulox is presented in **Error! Reference source not found.**. A summary of the search results for asulam sodium metabolites is presented in Table B.8. 192.

Table B.8. 191 An overview of the results from the defined search terms and databases for asulam / asulam-sodium/asulox

Data requirement(s) captured in the search	Number
Total number of *summary records* retrieved after *all** searches of peer-reviewed literature (excluding duplicates)	344
Number of *summary records* excluded from the search results after rapid assessment for relevance	333
Total number of *full-text* documents assessed in detail*	11
Number of *studies* excluded from further consideration after detailed assessment for relevance	10
Number of *studies* not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	1

*both from bibliographic databases and other sources of peer-reviewed literature

Table B.8. 192 An overview of the results from the defined search terms and databases for asulam sodium metabolites

Data requirement(s) captured in the search	Number
Total number of *summary records* retrieved after *all** searches of peer-reviewed literature (excluding duplicates)	10060

Number of *summary records* excluded from the search results after rapid assessment for relevance	10056
Total number of *full-text* documents assessed in detail*	4
Number of *studies* excluded from further consideration after detailed assessment for relevance	4
Number of *studies* not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	0

*both from bibliographic databases and other sources of peer-reviewed literature

One potentially relevant reference for asulam was found in relation to fate and behaviour in the first tier relevance assessment. The second tier screen for relevance was performed following an assessment of the full article. No publications were found relevant to fate and behaviour and the RMS agrees.

The RMS considers that the search criteria and relevance criteria applied by United Phosphorus for their literature review in the environmental area were acceptable. No reliability assessment was conducted as no studies were considered relevant.

B.8.12 References relied on

In every chapter (B.1, B.2, etc.) in Volume 3 (AS) the reference relied on heading should start with a paragraph indicating how the literature search was carried out and if this is considered acceptable. It should also be indicated if the RMS can agree with the justifications given by the notifier (especially for non-relevant literature).This is not expected to be a detailed study-by-study consideration. Relevant literature would be evaluated and assessed in the normal way within each section.

For (draft) renewal assessment reports the reference lists at the end of each section/chapter (sorted by data requirement) should include the newly submitted data relied upon as well as those original submitted tests and studies that are still considered relevant to support the application for renewal. However these studies should be clearly identified in the reference list as well as in the individual study sections. This could be done by consistent use of a statement for each study:
Previous evaluation: responded "N.A". for NAS, "Submitted for the purpose of renewal", or "In DAR (year)", "In addendum to DAR (year)" or any other appropriate

Active substance

*details to be added during Peer review

Data Point	Author(s)	Year	Title Company Report No. Source (where different from Company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	*Previous evaluation
CA, 7.1.1/01	Yeomans, P.	2000a	[^{14}C]-Asulam sodium: aerobic route and rate of degradation Covance Laboratories Ltd, Harrogate, North Yorkshire, England Covance Report No. 68/171-D2142 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.1.1/02	Yeomans, P., Swales, S.	2003a	[^{14}C]-Asulam sodium: Characterisation and degradation kinetic of a putative soil metabolite Covance Laboratories Ltd, Harrogate, North Yorkshire, England Covance Report No. 1849/20-D2149 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.1.1/03	Roohi, A., Mackenzie, E.	2009a	[^{14}C]-Asulam Sodium: Route and Rate of Degradation in Soil at 20°C Battelle UK Ltd, Ongar, Essex, England Battelle Report No. OU/07/010 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.1.1/04	Völkel, W.	2000a	Degradation of [^{14}C]-Asulam in One Soil Incubated under Aerobic Conditions. RCC Ltd., Itingen, Switzerland RCC Report No. 689940 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	

Data Point	Author(s)	Year	Title Company Report No. Source (where different from Company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	*Previous evaluation
CA, 7.1.1/05	Lowden, P.	2013a	Recalculation of the Distribution of Extractable Radioactivity in An Asulam Soil Study following the Demonstration that One HPLC Peak is an Artefact. Battelle UK Ltd, Ongar, Essex, England Battelle Report No. NZ/10/003A Not GLP, Unpublished	N	N	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.1.1/06	Lowden, P.	2013b	Composition of an 'unknown' soil metabolite in Asulam soil degradation studies. Battelle UK Ltd, Ongar, Essex, England Battelle Report No. NZ/10/003B Not GLP, Unpublished	N	N	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.1.2/01	Yeomans, P.	2003a	[^{14}C]-Asulam sodium: Metabolism in flooded soil Covance Laboratories Ltd, Harrogate, North Yorkshire, England Covance Report No. 1849/21-D2149 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.1.2/02	Morlock, G.	2009a	Degradation of Asulam (^{14}C- labelled) in One Soil under Anaerobic Conditions at 20 °C in the Dark. eurofins-GAB GmbH, Niefern-Oschelbronn, Germany Eurofins Report No. SOS-00327 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.1.3/01	Yeomans ,P., Swales, S.	2000a	[^{14}C]-Asulam sodium: photodegradation on a soil surface Covance Laboratories Ltd, Harrogate, North Yorkshire, England Covance Report No. 68/172-D2142 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	

Data Point	Author(s)	Year	Title Company Report No. Source (where different from Company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	*Previous evaluation
CA, 7.1.3/02	Doble, M., Oddy, A.	2007a	[^{14}C]-Asulam: Soil Photolysis Battelle UK Ltd, Ongar, Essex, England Battelle Report No. OU/07/007 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.2.1/01	Yeomans, P.	2000a	[^{14}C]-Asulam sodium: aerobic route and rate of degradation Covance Laboratories Ltd, Harrogate, North Yorkshire, England Covance Report No. 68/171-D2142 GLP, Unpublished ⇒ CA 7.1.1/01	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.2.1/02	Yeomans, P., Swales, S.	2003a	[^{14}C]-Asulam sodium: Characterisation and degradation kinetic of a putative soil metabolite Covance Laboratories Ltd, Harrogate, North Yorkshire, England Covance Report No. 1849/20-D2149 GLP, Unpublished ⇒ CA 7.1.1/02	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.2.1/03	Roohi, A., Mackenzie, E.	2009a	^{14}C]-Asulam Sodium: Route and Rate of Degradation in Soil at 20°C Battelle UK Ltd, Ongar, Essex, England Battelle Report No. OU/07/010 GLP, Unpublished ⇒ CA 7.1.1/03	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	

Data Point	Author(s)	Year	Title Company Report No. Source (where different from Company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	*Previous evaluation
CA, 7.2.1/04	Völkel, W.	2000a	Degradation of [^{14}C]-Asulam in One Soil Incubated under Aerobic Conditions. RCC Ltd., Itingen, Switzerland RCC Report No. 689940 GLP, Unpublished ⇒ CA 7.1.1/04	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA 7.2.1/05	Hardy, I.A.J.	2013a	Kinetic Modelling Analysis of Asulam and Sulphanilamide Rate of Degradation Studies to Derive Modelling Endpoints. Battelle UK Ltd, Ongar, Essex, England Battelle Report No. NZ/10/003C Not GLP, Unpublished	N	N	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA 7.2.1/06	Hardy, I.A.J.	2011a	Kinetic Modelling Analysis of Asulam and Sulphanilamide Rate of Degradation and Asulam Water / Sediment Studies to Derive Modelling Endpoints. Battelle UK Ltd, Ongar, Essex, England Battelle Report No. OU/10/001 Not GLP, Unpublished	N	N	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.2.2/01	Yeomans, P.	2000a	[^{14}C]-Asulam sodium: aerobic route and rate of degradation Covance Laboratories Ltd, Harrogate, North Yorkshire, England Covance Report No. 68/171-D2142 GLP, Unpublished ⇒ CA 7.1.1/01	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	

Data Point	Author(s)	Year	Title Company Report No. Source (where different from Company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	*Previous evaluation
CA, 7.2.2/02	Roohi, A. Mackenzie, E.	2009a	[^{14}C]-Asulam Sodium: Route and Rate of Degradation in Soil at 20°C Battelle UK Ltd, Ongar, Essex, England Battelle Report No. OU/07/010 GLP, Unpublished ⇒ CA 7.1.1/03	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.2.3/01	Oddy, A.M.	2003a	[^{14}C]-Sulfanilamide: Rate of degradation in three soils at 20 degrees Celsius Battelle AgriFood Ltd., Ongar, Essex, England Battelle Report No. CX/02/023 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.2.3/02	Mackenzie, E., Roohi, A.	2008a	[^{14}C]-Sulfanilamide: Rate of Degradation in Three Soils at 20°C. Battelle AgriFood Ltd, Ongar, Essex, England Battelle Report No. OU/07/011 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.2.3/02?	Willems, H.	1997a	Determination Of The Degradation Rate Of Methyl-Benzene-Sulfonyl-Carbamate In Soil	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.2.3/03	Hardy, I.A.J.	2013a	Kinetic Modelling Analysis of Asulam and Sulphanilamide Rate of Degradation Studies to Derive Modelling Endpoints. Battelle UK Ltd, Ongar, Essex, England Battelle Report No. NZ/10/003C Not GLP, Unpublished ⇒ CA 7.2.1/05	N	N	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	

Data Point	Author(s)	Year	Title Company Report No. Source (where different from Company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	*Previous evaluation
CA, 7.2.3/04	Hardy, I.A.J.	2011a	Kinetic Modelling Analysis of Asulam and Sulphanilamide Rate of Degradation and Asulam Water / Sediment Studies to Derive Modelling Endpoints. Battelle UK Ltd, Ongar, Essex, England Battelle Report No. OU/10/001 Not GLP, Unpublished ⇒ CA 7.2.1/06	N	N	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.2.4/01	Yeomans, P.	2003a	[^{14}C]-Asulam sodium: Metabolism in flooded soil Covance Laboratories Ltd, Harrogate, North Yorkshire, England Covance Report No. 1849/21-D2149 GLP, Unpublished ⇒ CA 7.1.2/01	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.2.4/02	Morlock, G.	2009a	Degradation of Asulam (^{14}C- labelled) in One Soil under Anaerobic Conditions at 20 °C in the Dark. eurofins-GAB GmbH, Niefern-Oschelbronn, Germany Eurofins Report No. SOS-00327 GLP, Unpublished ⇒ CA 7.1.2/02	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.2.5/01	Yeomans, P.	2003a	[^{14}C]-Asulam sodium: Metabolism in flooded soil Covance Laboratories Ltd, Harrogate, North Yorkshire, England Covance Report No. 1849/21-D2149 GLP, Unpublished ⇒ CA 7.1.2/01	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	

Data Point	Author(s)	Year	Title Company Report No. Source (where different from Company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	*Previous evaluation
CA, 7.2.5/02	Morlock, G.	2009a	Degradation of Asulam (^{14}C- labelled) in One Soil under Anaerobic Conditions at 20 °C in the Dark. eurofins-GAB GmbH, Niefern-Oschelbronn, Germany Eurofins Report No. SOS-00327 GLP, Unpublished ⇒ CA 7.1.2/02	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.4.1/01	Lewis, C.J.	1999a	[^{14}C]-Asulam-sodium Adsorption/desorption in five soil and one sediment. Covance Laboratories Ltd, Harrogate, North Yorkshire, England Covance Report No. 68/169-D2142 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.4.1/02	Vòlkel, W.	2011a	[^{14}C]-Asulam: Adsorption/Desorption on Soil. Innovative Environmental Services (IES), Witterswill, Switzerland IES Report No.: 152 01 013 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.4.1/03	Lowden, P., Mahay, N.	1999a	[^{14}C]-Asulam: Aged desorption from four soils Rhone-Poulenc Agriculture Ltd, Ongar, Essex, England Rhone-Poulenc Report No. 15957 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.4.2/01	Simmonds, M.B.	2002a	[^{14}C]-Sulfanilamide: Adsorption to and desorption from four soils Battelle-AgriFood Ltd, Ongar Essex, England Battelle Report No.: CX/02/022 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	

Data Point	Author(s)	Year	Title Company Report No. Source (where different from Company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	*Previous evaluation
CA, 7.4.2/02	Oddy, A, Brett, R	2008a	[^{14}C]-Sulfanilamide: Adsorption to and Desorption from Four Soils. Battelle AgriFood Ltd, Ongar, Essex, England Battelle Report No. OU/07/012 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.4.2/03 ?	Willems, Ir. H.	1998	Adsorption/Desorption of Methyl-benzene-sulfonyl-carbamate on Soil	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.4.3/01	Reeves, G.L., England, D.A., Savage, E.A.	1988a	Herbicides: Asulam-[^{14}C]: Leaching study with four soils Rhone-Poulenc Agriculture Ltd, Ongar, Essex, England Rhone-Poulenc Report No. D.Ag. 1118 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.4.5/01	Reeves, G.L., England, D.A., Savage, E.A.	1988a	Herbicides: Asulam-[^{14}C]: Leaching study with four soils Rhone-Poulenc Agriculture Ltd, Ongar, Essex, England Rhone-Poulenc Report No. D.Ag. 1118 GLP, Unpublished ⇒ CA 7.4.3/01	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	

Data Point	Author(s)	Year	Title Company Report No. Source (where different from Company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	*Previous evaluation
CA, 7.5/01	Gohdes, M.	1989a	Hydrolysis of ^{14}C-Asulam in buffered aqueous solutions Hazleton Laboratories America Inc., Madison, Wisconsin, USA United Phosphorus Limited Report No.: R000015 Hazleton Report No. HLA 6224-122 GLP, Unpublished ⇒ CA 2.9.1/01	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.6/01	Mills, E.A, Simmonds, M.B	2003	[^{14}C]-asulam: aqueous photolysis and quantum yield at pH9 and pH4 Battelle AgriFood Ltd, Ongar, Essex, England United Phosphorus Limited Report No.: C031419 Battelle Report No. CX/02/057 GLP, Unpublished ⇒ CA 2.9.2/01	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.6/02	Mills, E.A.	2007	Asulam: [^{14}C]-Aqueous Photolysis and Quantum Yield in Buffers at pH 4 and 9. Battelle UK Ltd, Ongar, Essex, England Battelle report No. OU/07/008 GLP, Unpublished ⇒ CA 2.9.2/02	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.6/03	Mills, E.A.	2009a	Asulam: [^{14}C]-Aqueous Photolysis at pH 4 and 9: Identification of Photolytic Degradates. Battelle UK Ltd, Ongar, Essex, England Battelle report No. OU/08/001 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	

Data Point	Author(s)	Year	Title Company Report No. Source (where different from Company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	*Previous evaluation
CA, 7.6/04	Lowden, P.	2007a	Asulam: Estimation of environmental photolytic half-life in water (model calculation according to Frank and Klöepffer) Battelle UK Ltd, Ongar, Essex, England Battelle Report No. OU/07/009A Not GLP, Unpublished ⇒ CA 2.9.3/03	N	N	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.6/05	Lowden P.	2007b	Calculated theoretical lifetime in the top layer of aqueous systems and the real lifetime of asulam (GC Solar) Battelle UK Ltd, Ongar, Essex, England Battelle Report No. OU/07/009B Not GLP, Unpublished ⇒ CA 2.9.3/04	N	N	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.6/06	Mills, E.A., Caine, J.	2004a	[^{14}C]-Asulam: Aqueous photolysis in natural water Battelle UK Ltd, Ongar, Essex, England Battelle Report No. .: CX/04/047 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.7/01	Mead, C.	1999a	Assessment of ready biodegradability CO_2 evolution test Asulam (RPA 096125) Safepharm Laboratories Ltd, Derby, Derbyshire, England Safepharm Report No. 238/047 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	

Data Point	Author(s)	Year	Title Company Report No. Source (where different from Company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	*Previous evaluation
CA, 7.7/02	Feil, N.	2008	Ready Biodegradability of Asulam in a Manometric Respirometry Test. Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany IBACON Report No.: 39851163 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.8.3/01	Purser, D.	1998a	[^{14}C]-Asulam: Degradation and retention in water-sediment systems Covance Laboratories Ltd, Harrogate, North Yorkshire, England Covance Report No. 68/156-D2142 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.8.3/02	Willems, H	1997a	Degradation of asulam in aerobic aquatic environment. Notox 's-Hertogenbosch, Notox Report No. 187717 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA 7.8.3/03	Hardy, I.A.J.	2008c	Asulam: Kinetic Modelling Analysis of Data from Water/Sediment Study. Battelle UK Ltd, Ongar, Essex, England Battelle Report No. NZ/08/001A GLP, Unpublished	N	N	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA 7.8.3/04	Hardy, I.A.J	2011a	Kinetic Modelling Analysis of Asulam and Sulphanilamide Rate of Degradation and Asulam Water / Sediment Studies to Derive Modelling Endpoints Battelle UK Ltd, Ongar, Essex, England Battelle Report No. OU/10/001A GLP, Unpublished ⇒ CA 7.2.1/06	N	N	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	

Data Point	Author(s)	Year	Title Company Report No. Source (where different from Company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	*Previous evaluation
CA, 7.10/01	Van der Gaauw, A.	2001a	Estimation of the degradation of asulam by photo-oxidation in air RCC Ltd, Itingen, Switzerland RCC Report No. 802168 Not GLP, Unpublished ⇒ CA 2.10/01	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.12/01	Thorvardarson A., Kristiansen, H.	1992a	Sampling of groundwater from 'Feddet' Plantation, Denmark, for the determination of Asulam Geological Survey of Denmark (GSD) Geochemical Division GSD Report No. No.: Jour.no. 072-051 Not GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.12/02	Norris, F.A.	1992a	A small scale prospective groundwater monitoring study with asulam-sodium, the active ingredient of asulox® brand herbicide, applied to sugar cane and to turf Rhone-Poulenc Ag Company, Research Triangle Park, North Carolina, USA Rhone-Poulenc Report 41121, GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 7.12/03	Gustafson, D.I.	1993a	Drinking water monitoring for residues of asulam in high-use areas of the United States Rhone-Poulenc Agro. Co., El Dorado Hills, California, USA Rhone-Poulenc Report No.: 41293 GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	

Data Point	Author(s)	Year	Title Company Report No. Source (where different from Company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	*Previous evaluation
CA, 7.12/04	Boussemart, M.J.	1994a	Asulam monitoring programme in North Yorkshire Rhone-Poulenc Agriculture, Ongar, Essex, England Rhone-Poulenc Report No.: 200636 Not GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	

Plant Protection Product – 'Asulox'

*details to be added during Peer review

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CP 9.4/01	M. Patel, I.A.J. Hardy and P. Lowden	2013a	Predicted Environmental Concentrations of Asulam and its Major Metabolite Sulphanilamide in Soil, Following Application to Spinach or Flower-bulbs of the Formulation ASULOX® Generated by Battelle UK Ltd, Chelmsford, Essex UK Report No.: NZ/13/017A Not GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CP 9.5/01	M. Patel, I.A.J. Hardy and P. Lowden	2013a	Predicted Environmental Concentrations of Asulam and its Major Metabolite Sulphanilamide in Soil, Following Application to Spinach or Flower-bulbs of the Formulation ASULOX® Generated by Battelle UK Ltd, Chelmsford, Essex UK Report No.: NZ/13/017A Not GLP, Unpublished ⇒ CP 9.4/01	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CP 9.6/01	M. Patel, I.A.J. Hardy and P. Lowden	2013c	Predicted Environmental Concentrations of Asulam and the metabolite Sulphanilamide in Groundwater (PEC_{GW}) Following Application to Spinach or Flower-bulbs. Generated by Battelle UK Ltd, Chelmsford, Essex UK Report No.: NZ/13/017C Not GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CP 9.6.2/01	M. Patel, I.A.J. Hardy and P. Lowden	2013c	Predicted Environmental Concentrations of Asulam and the metabolite Sulphanilamide in Groundwater (PEC_{GW}) Following Application to Spinach or Flower-bulbs. Generated by Battelle UK Ltd, Chelmsford, Essex UK Report No.: NZ/13/017C	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	

Data Point	Author(s)	Year	Title Company Report No. Source (where different from Company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	*Previous evaluation
			Not GLP, Unpublished ⇒ CP 9.6/01					
CP 9.7/01	M. Patel, I.A.J. Hardy and P. Lowden	2013b	Predicted Environmental Concentrations in Surface Water (PEC_{SW}) and Sediment (PEC_{Sed}) for Asulam and Its Metabolites Following Application to Spinach or Flower-bulbs. Generated by Battelle UK Ltd, Chelmsford, Essex UK Report No.: NZ/13/017B Not GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CP 9.8/01	M. Patel, I.A.J. Hardy and P. Lowden	2013b	Predicted Environmental Concentrations in Surface Water (PEC_{SW}) and Sediment (PEC_{Sed}) for Asulam and Its Metabolite Following Application to Spinach or Flower-bulbs. Generated by Battelle UK Ltd, Chelmsford, Essex UK Report No.: NZ/13/017B Not GLP, Unpublished ⇒ CP 9.7/01	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	