Asulam sodium

Annex B (Volume 3)

B.9 Ecotoxicology

Version history

When	What
2016-04-18	Initial DAR

B.9 Ecotoxicology

Background information

'Asulox' (also referred to in this report as 'Asulam 400g/L SL') is a soluble concentrate (SL) formulation containing the active substance 'asulam sodium' – which is the sodium salt of asulam (438 g a.s./L equivalent to 400 g/L asulam). 'Representative uses' considered in this EU evaluation include use on spinach (pre- and post-emergent application) and flower bulbs (post-emergent) at a maximum application rate of 6 L product/ha, limited to one application per growing season, equating to 2628 g asulam sodium/ha (2400 g asulam/ha). Further details are summarised in the following table:

Table B.9.0.1 The representative EU crop uses for 'Asulox'

Crop and/or situation	F G or I	Pests or Group of pests controlled	Application timing	Maximum Application rate per treatment			PHI (days)
			Crop growth stage & season	number min max	Water (L/ha) min/max	kg asulam /ha min max	
Spinach	F	Pre-emergence broadleaved and grass weeds	Pre-emergence (up to 3 days after sowing) (spring/summer)	1	200-600	2.4	n.a.
Spinach	F	Post-emergence broadleaved and grass weeds	Post-emergence BBCH 12-14 (spring/summer)	1	200-600	2.4	28
Flower-bulbs	F	Composite and vetch weeds	Post-emergence (weeds 4-6 cm) (spring/summer)	1	200-600	2.4	n.a.

Asulam is a molecule that contains both basic and acidic centres, which readily forms salts. The formulated compound ('Asulox') is an aqueous solution of the sodium salt of asulam ('asulam sodium'). The majority of the ecotoxicological studies have been conducted on asulam sodium. Some studies, however, have been conducted with asulam. The Notifier has provided the following reasoning supporting the biological activity equivalence (taking into account molecule weight differences) of asulam and asulam sodium:

'Whether the compound is applied in these tests as asulam or asulam sodium (which will dissociate in solution) the protonated and deprotonated forms of asulam will be in equilibrium. This equilibrium will depend on the pH of the compound's environment and the same equilibrium will be achieved if asulam or asulam sodium has been added. The amount of compound added in the tests conducted is not sufficient to have an impact on the pHs of the various systems and so will not affect the equilibrium. Neither is the aqueous solubility of asulam exceeded in any of the tests.'

The RMS agrees that the biological activity in non-target organism toxicity studies of asulam (molecular weight 230.2) and asulam sodium (molecular weight 252.2) will be equivalent when molecule weight differences are taking into account (i.e. by multiplying by a factor of 230.2/252.2 = 0.9128 to convert from asulam sodium to asulam).

Identity of the active substance

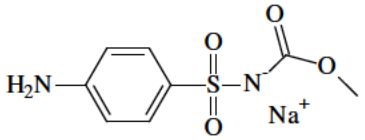
Common name: Asulam sodium

CAS number: 2302-17-2

Chemical name (IUPAC): Sodium methyl [(4-aminophenyl)sulphonyl]carbamate

Chemical name (CA): methyl N-[(4-aminophenyl)sulfonyl]carbamate sodium salt (1:1)

Molecular structure:



Biological performance:

Asulam sodium is a systemic herbicide, which may be taken up by leaves or roots from which it is translocated to other parts of the plant. . It is claimed to exert its main effect by inhibiting the process of cell division in the growing points of the plant and the greatest visual effects of the treatment therefore occur in new growth.

Degradation of active substance in soil and water

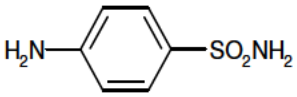
Degradation of active substance in soil

Degradation of asulam in soil occurs by the formation of a very large number of metabolites. These include sulphanilamide, methylbenzenesulphonylcarbamate (MBSC - also referred to by Notifier as 'desamino asulam'), acetyl asulam, acetyl sulphanilamide, and sulphanilic acid. However, the only metabolite that reach major levels in soil (i.e. >10% AR) is sulphanilamide – which is relatively rapidly formed as a result of hydrolysis (peak of 14% AR after 14 days in lab soil degradation study, ref. Section B.8.4 of DAR). Both asulam and its major soil metabolite sulphanilamide have fairly short half-lives in soil, with estimated geometric mean DT50s of 4.6 days and 13.4 days respectively.

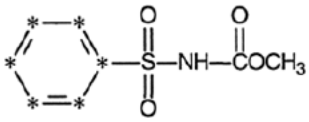
The most significant other soil metabolite is MBSC, which based on the results of a soil degradation study (Roohi and Mackenzie 2009a, Section B.8 of DAR) is concluded by the RMS to be a minor metabolite reaching 6% AR in one soil type and not exceeding 4.56% AR in the three other soils included in the study. The soil DT50 of MBSC ranges from 8.3 to 27.9 days (ref. Willems 1997a, Section B.8 of DAR) and therefore is not considered by the RMS as persistant in soil.

The structural formulae of sulphanilamide and MBSC are shown below.

Sulphanilamide (major soil metabolite):



MBSC (minor soil metabolite but potential major aquatic metabolite):



The RMS notes that the chemical structure of the metabolite MBSC is equivalent to that of asulam except for the loss of the 'NH2' group on the benzene ring.

Degradation of the active substance in water

Asulam's main route of degradation in water is via photolysis, with this varying according to pH. At pH 4 the single main photodegradate was sulphanilic acid (55% AR). At pH 9 there were a number of degradates, two of which were present in degradation studies at >10% of applied radioactivity. N-(4-aminophenyl)formamide (abbreviated to 'AP formamide') reached 24.2% AR and (4-(4-methoxycarbonyl aminophenyl)aminophenyl)carbamic acid (abbreviated to 'MCAPAP') reached 11.9% AR. In water/sediment studies, both asulam and sulphanilamide were found in significant quantities in sediment. Also, based on the results of a newly evaluated water /sediment study (Willems 1997a, Section B.8.4 of DAR), the RMS concludes that MBSC is a second potential major aquatic metabolite (reaching 10.3% AR).

Although sulphanilamide is formed slowly in water in minor amounts (maximum of 2.3% AR after 153 days in water/sediment study, ref. Section B.8.4.4 of Volume 3 DAR), the RMS concludes that it is a potential major metabolite in water - due to the potential significant drainflow and /or runoff contamination from soil (where sulphanilamide is formed as a major metabolite).

Summary of presence of active substance and major metabolites in the environment:

Details are summarised in the following table based on the conclusions of the RMS's Fate and Behaviour evaluation (see Section B.8 of Volume 3 DAR for further details):

Table B.9.02 Summary of presence in environmental compartments of the active substance and its principal (major or potential major) metabolites

Compartment:	Active substance /metabolite present:
Soil	Asulam, sulphanilamide.
Water	Asulam, sulphanilamide, sulphanilic acid, AP formamide, MCAPAP and MBSC.
sediment	Asulam, sulphanilamide.
groundwater	Asulam, sulphanilamide.

B.9.1 Effects on birds (IIA 8.1, IIIA 10.1)

B.9.1.1 Acute oral toxicity (IIA 8.1.1, IIIA 10.1.1)

B.9.1.1.1 Report: [redacted] (2000) Asulam sodium salt: Acute oral toxicity (LD_{50}) to the bobwhite quail. [redacted]

This study has previously been evaluated at EU level for the Annex I inclusion of asulam.

Guidelines:

USEPA (= EPA) series 71, § 71-1, 1982
Deviations: None

GLP:

Yes

Executive Summary:

The bobwhite quail (*Colinus virginianus*) acute oral LD_{50} for asulam sodium salt was determined to be >2000 mg asulam sodium/kg body weight, the highest dose tested, equivalent to >1826.5 mg asulam/kg body weight. The no-observed-effect-level (NOEL) was 2000 mg asulam sodium /kg, based on a lack of mortality and no treatment related effects on bodyweight changes and food consumption.

Materials and Methods

Test Material:

Asulam sodium salt, purity 896 g/kg (equivalent to 814 g asulam/kg), Batch no.: PN-24049

Test Design:

Five males and five females bobwhite quails, approximately 5 months old, weight 174-200 g (males) and 171-201 g (females) at the start of the dosing period, were given a single dose, by intubation, of either 500, 1000 or 2000 mg asulam sodium salt/kg body weight. A similar sized control was dosed in the same way receiving the vehicle only (water). The birds were acclimatised 15 days prior to dosing.

Throughout the study the temperature in the experimental area ranged from 19 to 21°C and the mean daily relative humidity was 83%. The light cycle was 10 hours light and 14 hours darkness. Access to water was available at all time. Quails were deprived of food 19 hours before test initiation.

Body weights were determined on days -15, -7, 0 (immediately prior to dosing), 7 and 14. Group mean food consumption was recorded over days -15 to -8, -7 to -1, 1 to 7 and 8 to 14. Observations included mortality, bird's health, clinical signs and *post mortem* examinations were also performed.

Results and Discussion:

The biological data are summarised in Table B.9.1.1.01

Table B.9.1.1.01 Bobwhite quail acute oral toxicity study - Effects on mortality body weight and food consumption

Dose tested (mg/kg bw)	Mortality		Mean bodyweight (g)						Group mean food consumption (g/bird/day)			
			At day 0		At day 7		At day 14		1 to 7		8 to 14	
	m	f	m	f	m	f	M	f	m	f	m	f
Control	0	0	187	188	189	192	193	195	14	16	14	16
500	0	0	182	186	184	190	188	194	13	14	14	14
1000	0	0	183	190	188	193	192	197	15	15	16	17
2000	0	0	182	184	185	188	188	191	13	14	14	14

m: male, f: female

There were no mortalities and no clinical signs of toxicity recorded. The macroscopic post mortem observations (14 days following administration) revealed no abnormalities.

There was no evidence of any treatment related effects on bodyweight changes and the food consumption was not affected by treatment.

Conclusions:

The oral LD_{50} was determined to be >2000 mg asulam sodium/kg body weight, equivalent to > 1826.5 mg asulam/kg body weight.

RMS's evaluation of bobwhite quail acute oral toxicity study:

This study has previously been evaluated at EU level for the Annex I inclusion of asulam. The conclusions presented are consistent with the previous EFSA conclusion report (2010) and no concerns were raised at EU level. CRD considers the study scientifically valid and suitable for use in a regulatory risk assessment.

Based on use of an asulam sodium (molecule weight 252.2) to asulam (molecule weight 230.2) conversion factor of 0.9128, the above concluded oral LD50 of 2000 mg asulam sodium /kg bw equates to 1825.6 mg asulam /kg bw and not 1826.5 mg asulam /kg bw - as stated above. Therefore, this minor correction to the endpoint for asulam has been included in the 'List of Endpoints' and also in the avian risk assessment.

B.9.1.2 Dietary toxicity (IIA 8.1.2)

B.9.1.2.1 Report: [redacted] (1970a) Asulox - subacute (5 days) toxicity in mallard ducklings. [redacted].

This study has previously been evaluated at EU level for the Annex I inclusion of asulam.

Guidelines:

No guidelines in place in 1970; internal company protocol broadly in line with current recommended short-term dietary toxicity study guidelines (i.e. OECD 205)
Deviations: n/a

GLP:

No (conducted prior to implementation)

Executive Summary:

The mallard duck (*Anas platyrhynchos*) dietary LC_{50} was determined to be >100000 ppm asulam in diet, equivalent to an LD_{50} of >22732 mg asulam/kg body weight. The no-observed-effect-concentration (NOEC) was determined to be 100000 ppm asulam in diet, the highest dose tested.

Materials and Methods

Test Material:

Asulox®, purity 60% w/v aqueous solution of asulam sodium salt, Batch No.: TN610

Test Design:

Experiment 1

All dietary concentrations of Asulox® are expressed in terms of asulam. 180 mallard ducks of species *Anas platyrhynchos* were used for the study. Birds were 7 days old at study initiation. Groups of 10 birds were fed for a period of 5 days with Asulox® at concentration of 10000, 15000, 22500, 33750, 50000 and 75000 ppm asulam. In the same way, groups of 10 birds were exposed to a basal diet (control group of 5 replicates of 10 birds each) and a toxic standard (DDT, at concentrations of 250, 320, 400, 500, 630, 800 and 1000 ppm). After the 5-day exposure period, the ducklings were returned to a basal diet for 3 days. Mortality was recorded daily. The weight of

the birds was assessed at study initiation and on day 5 and 8. Food consumption was recorded during the 5-day treatment period.

Experiment 2

130 mallard ducks of species *Anas platyrhynchos* were used for the study. Birds were 7 days old at study initiation. Groups of 10 birds were fed for a period of 5 days with Asulox® at concentration of 33750, 50000, 75000 and 100000 ppm asulam. In the same way, groups of 10 birds were exposed to a control (3 replicates of 10 birds each) and a toxic standard (DDT, at concentrations of 330, 500, 750, 1100, 1650 and 2500 ppm). After the 5-day exposure period, the ducklings were returned to a basal diet for 3 days. Mortality was recorded daily. The weight of the birds was assessed at study initiation and on day 5 and 8. Food consumption was recorded during the 5-day treatment period.

Results and Conclusions:

Experiment 1

No mortality was recorded in the Asulox® treatment group. Between the 5th and 8th days of the study one bird in 1000 ppm DDT group and one bird in one control replicate were found dead.

No unusual signs were observed in any bird at any time in any group.

Mean body weight and food consumption data recorded are summarised in Table B.9.1.2.01.

Table B.9.1.2.01 Mallard duck sub-acute dietary toxicity study ('Experiment 1') - Effects on body weight and food consumption

Treatment group	Mean bodyweight (g)	Mean bodyweight gain (g)			Food consumption (g/bird/day)
	Day 1	Days 1-5	Days 1-8	Days 5-8	
Control *	74.2 ± 2.3	73.8 ± 11	107.8 ± 9.5	34 ± 9.8	30.8 ± 1.8
Asulox®					
10000	72	70	104	34	29.0 ***
15000	81	71	113	42	28.4 ***
22500	73	65 **	111	46	24.4 ***
33750	75	73	115	42	25.4 ***
50000	80	64 **	114	50	23.6 ***
75000	79	49 **	91	42	22.0 ***
DDT					
250	69	67	105	38	26.5 ***
320	80	76	112	36	32.0
400	76	58 **	102	44	29.2 ***
500	69	67	103	36	26.6 ***
630	78	58 **	94	36	27.4 ***
800	74	54 **	102	48	24.8 ***
1000	76	54 **	97	43	24.0 ***

* mean value of 5 replicates.
** body weight suggest that birds grew more slowly than the control during the 5 day treatment period.
*** food consumption reduction compared with control. Palatability may have been implicated.

Conclusions

The acute LD_{50} was determined to be > 75000 ppm asulam in diet. The NOEC was determined to be 75000 ppm asulam in diet, the highest dose tested. It is not possible to convert these figures into mg asulam/kg bodyweight due to lack of data on body weight and food consumption.

Experiment 2

Mortality, body weights and food consumption during the 8-day study period are summarised in Table B.9.1.2.02.

Ducklings in all groups wasted a proportion of the food supplied. Wastage has been ignored in tabulating and evaluating the food consumption.

Table B.9.1.2.02 Mallard duck sub-acute dietary toxicity study ('Experiment 2') - Effects on mortality, body weight and food consumption

Treatment group	Total mortality (%)		Mean bodyweight (g)	Mean bodyweight gain (g)			Food consumption (g/bird/day)
	Day 5	Day 8	Day 1	Days 1-5	Days 1-8	Days 5-8	
Control *	0	0	72.7 ± 2.3	76 ± 4	134 ± 5.3	58 ± 2	29.2 ± 1.7
Asulox®							
33750	0	0	80	52	106	54	26.0 #
50000	0	0	68	56	112	56	21.6 #
75000	10	20	77	29	86	57	20.8 ** #
100000	0	0	88	36	70	34	24.0 #
DDT							
330	0	0	77	69	119	50	27.4 #
500	10	10	72	51	104	53	26.1 ** #
750	0	0	73	63	115	52	26.2 #
1100	10	30	74	41	86	45	24.0 ** #
1650	20	40	84	22	63	41	19.6 ** #
2500	80	100	78	2	-	-	15.1 ** #

- not determined because of high mortality level.
* mean value of 3 replicates.
** in groups in which deaths occurred this figure was obtained by multiplying the figure for food consumed per bird per day by 5.
# food consumption reduction compared with control. Palatability may have been implicated.

Two ducklings died at 75000 ppm asulam (on days 5 and 7) and significant mortalities were observed in the DDT treatment groups, no other mortalities were observed. No unusual signs were observed in any of the birds. All groups treated with asulam grew more slowly than the controls during the 5-day period. On withdrawal of the pesticide normal growth resumed in all groups except those which received 100000 ppm asulam, where growth rate remained significantly lower than the controls. All groups treated with asulam showed reduced food consumption when compared with the

controls (11-29% reduction throughout the groups). As in the first experiment palatability may have been implicated. The reduced growth rate of all treated groups may have been due, at least in part, to this reduced food intake

Conclusions:

The 5 day short-term dietary LC_{50} was determined to be >100000 ppm asulam in diet, equivalent to an LD_{50} of >22732 mg asulam/kg body weight (using a mean daily food consumption of 20.8 g and a mean body weight of 91.5 g). The NOEC was determined to be 100000 ppm asulam in diet, the highest dose tested.

RMS's evaluation of mallard duck sub-acute (5 day) dietary toxicity studies:

The above two studies were previously evaluated at EU level for the Annex I inclusion of asulam. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level. Although not GLP compliant, given that the studies were conducted prior to implementation of this scheme this is considered acceptable.

CRD considers the studies to be scientifically valid and suitable for consideration in the regulatory risk assessment. Based on effects from exposure at the highest test dose included in the second study, the 5 day dietary LD50 is >22732 mg asulam/kg body weight (which is as previously stated in the asulam 'List of Endpoints' dated November 2009).

B.9.1.2.2 [redacted] (1970b) Asulox - Subacute (5 days) toxicity study in pheasant chicks. [redacted].

This study has previously been evaluated at EU level for the Annex I inclusion of asulam.

Guidelines:

Guidelines not in place in 1970. Deviations: n/a

GLP:

No (conducted prior to implementation).

Executive Summary:

The pheasant (*Phasianus colchicus*) dietary LC_{50} was determined to be >75000 ppm asulam in diet. The NOEC was determined to be 75000 ppm in diet, the highest dose tested. Due to unreliable data on food consumption these figures could not be converted into mg asulam/kg.

Materials and Methods

Test Material:

Asulox®, a 60% w/v aqueous solution of asulam sodium salt, Batch no.: TN610 (experiment 1) and batch no.: UN606 (experiment 2)

Test Design:

All dietary concentrations of Asulox® are expressed in terms of asulam. In experiment 1, groups of 10 pheasant chicks, 7 days of age, were fed diets containing Asulox® at concentrations of 10000, 15000, 22500, 33750, 50000 and 75000 ppm asulam or 250, 320, 400, 500, 630, 800 and 1000 ppm DDT, for 5 days. A 3 days observation period followed.

In experiment 2, groups of 10 pheasant chicks, 7 days of age, were fed diets containing Asulox® at concentrations of 10000, 15000, 22000, 33000, 50000 and 75000 ppm asulam or 320, 400, 500, 630, 800, 1000 and 1250 ppm DDT, for 5 days. A 3 days observation period followed.

In both experiments, food consumption was recorded during the 5-day treatment period. The birds were weighed 5 and 8 days after test initiation.

Results and Discussion:

No deaths and no unusual signs occurred in the Asulox® treated groups from experiments 1 and 2.

Significant mortality occurred in the DDT treated groups in the first experiment, but fewer deaths occurred in the corresponding groups in the second experiment.

In both experiments birds in all groups wasted various varying amounts of food, and no firm conclusions can be drawn from the food consumption data. The results of experiment 2 suggested reduced food consumption in the 75000 ppm Asulox® treatment group. Palatability may have been implicated.

Conclusions:

The acute LD_{50} was determined to be >75000 ppm asulam in diet. The NOEC was determined to be 75000 ppm in diet, the highest dose tested. Due to unreliable data on food consumption these figures could not be converted into mg asulam/kg.

RMS's evaluation of pheasant sub-acute (5 day) dietary toxicity study:

This study has previously been evaluated at EU level for the Annex I inclusion of asulam. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level. Although not GLP compliant, given that the study was conducted prior to implementation of this scheme this is considered acceptable.

CRD considers the study scientifically valid and suitable for consideration in the regulatory risk assessment, although the inability to convert the derived endpoint to a dose per kg body weight limits its usefulness.

B.9.1.3 Long term/Reproductive toxicity (IIA 8.1.3)

B.9.1.3.1 Report: [redacted] (2003) Asulam sodium salt: A reproduction study with the Japanese quail (amended final report). [redacted].

This study has previously been evaluated at EU level for the Annex I inclusion of asulam.

Guidelines:

OECD draft, September 1999
Deviations: None

GLP:

Yes

Executive Summary:

For Japanese quail (*Coturnix coturnix japonica*) exposed to asulam in the diet at concentrations up to 2000 ppm asulam there were no treatment-related effects upon

measurements of adult health, egg production, embryo development, hatchability or offspring survival. However, based upon the effects upon egg shell quality noted at the 500 ppm test concentration tested, the no observed effect concentration could not be determined. This test dose is equivalent to 65 mg asulam/kg bw/day (using a mean daily food consumption of 17.7 g and a mean body weight of 136 g).

Materials and Methods

Test Material:

Asulam, purity 814 g/kg, Batch no.: OP980398

Test Design:

Test species: Fifteen weeks old Japanese quail, weighing 95 to 171 g at start of the pre-treatment period. After a two-week pre-treatment period (basal diet), birds were exposed to asulam at dietary concentrations of 0, 500, 1000 or 2000 ppm as for 6 weeks. Each treatment group had 20 pens with one male and one female per pen.

During the test, birds were maintained under a 16 or 17 hours light photoperiod. The average study room temperature was 23.8 ± 1.8 °C and the average relative humidity 76 ± 12%. All adult and offspring's were given feed and water *ad libitum* during acclimatisation and testing.

Chemical analyses of the test diet were conducted.

Adult birds were observed daily for mortality, signs of toxicity and abnormal behaviour. Body weights were measured at the start of the pre-treatment period, at the start of the treatment period, and at adult termination. Feed consumption was measured for each pen for a seven-day period every week throughout the test.

Eggs were collected daily, starting with the pre-treatment period and set weekly for incubation. Each week, eggs were selected from those eggs laid during that week for measurement of eggshell strength and thickness. Cracked or abnormal eggs were discarded and the remaining eggs were incubated. Infertile eggs or embryo mortality were detected twice during the incubation period.

On day 15 of incubation, eggs were placed in a hatcher. After hatching chicks were removed from hatcher and weighed. Chicks were fed untreated diet and were observed daily for symptoms of toxicity and abnormal behaviour. At 14 days of age, chicks were weighed and then anaesthetised.

At the end of the exposure period, necropsy analyses (weights of liver, spleen and testes or female reproductive tract) were performed.

Statistical analyses were performed to determine statistically significant differences between groups.

Results and Discussion:

The measured concentrations of samples taken from diet preparation were found to be 481 ± 38.1, 915 ± 64.0 and 1870 ± 37.1 ppm active substance. The measured diet test concentrations from samples collected from feeders during the test were 477, 934 and 2000 ppm a.s., which represented 95, 93 and 100% of nominal concentrations, respectively. The biological key information for the treatment period is summarised in the following table:

Table B.9.1.3.01 Reproductive data for Japanese quail in asulam toxicity study

Examination	Control	500 ppm	1000 ppm	2000 ppm
No. of replicates at study end (1♂ + 1♀)	20	20	20	20
No. of surviving male and female birds	20♂ + 20♀	20♂ + 20♀	20♂ + 20♀	20♂ + 20♀
Food consumption- week 3 to 8 (g/bird/day) *	18	17.7	17.7	18
Initial**/terminal body weight (g) male	124/124	121/121	122/122	122/121
female	147/147	153/150	152/150	151/149
Eggs laid (group total)	719	743	723	758
Eggs laid per hen	36	37	36	38
Mean egg shell thickness	0.221	0.210 ***	0.209 ***	0.205 ***
Mean egg strength	12.39	11.07 ***	10.58 ***	9.19 ***
Eggs cracked/eggs laid (%)	2.6	1.6	5.3	2.9
Viable embryos/eggs set (%)	87	85	82	84
Live 2-week embryos/viable (%)	98	98	99	99
Hatchlings/Live 2-week embryos (%)	90	95	92	95
14-day old survivors/hatchlings (%)	96	95	89	88***
Normal hatchlings/eggs set (%)	74	78	74	78
14-days old survivors/eggs set (%)	73	75	69	71
Normal hatchlings/hen/day	0.57	0.63	0.56	0.59
14-day old survivors/hen/day	0.55	0.60	0.53	0.54

* average of weekly mean values recorded during the treatment period.
** initial body weight = body weight at the start of the treatment period.
*** statistically different from the control group at $p < 0.01$.

There were no treatment-related mortalities, overt signs of toxicity or treatment-related effects upon body weight or food consumption at any of the concentrations tested.

Additionally, there were no treatment-related effects upon reproductive parameters measured. However, there were reductions in both egg strength and egg shell thickness at the 500, 1000 and 2000 ppm as test concentrations that were both treatment-related and concentration responsive.

Conclusions:

At test concentrations up to 2000 ppm asulam there were no treatment-related effects upon measurements of adult health, egg production, embryo development, hatchability or offspring survival.

However, based upon the effects upon egg shell quality noted at the 500 ppm test concentration tested, the no observed effect concentration for Japanese quail exposed to asulam in the diet could not be determined. This test dose is equivalent to 65 mg asulam/kg bw/day (using a mean daily food consumption of 17.7 g and a mean body weight of 136 g).

RMS's evaluation of Japanese quail reproductive toxicity study:

This study has previously been evaluated at EU level for the Annex I inclusion of asulam. CRD considers the study scientifically valid and suitable for consideration in

the regulatory risk assessment. The conclusions presented are consistent with the EFSA Conclusion report (2010) – which included the evaluation of a further additional study to enable a reproductive NOEL to be derived (for which details are presented below under B.9.1.3.2).

B.9.1.3.2 Report: [redacted] (2003) Asulam: A study to evaluate egg shell quality with the Japanese quail. [redacted]

This study has previously been evaluated at EU level for the Annex I inclusion of asulam.

Guidelines:

Draft OECD guideline (April 2000)
Deviations: None

GLP:

Yes

Executive Summary:

A second 8 week reproduction study was conducted to evaluate further effects on eggshell quality of Japanese quail (*Coturnix coturnix japonica*). Based upon the statistically significant reduction in eggshell thickness noted at 225 and 500 ppm, the NOEC is concluded to be 100 ppm, which is equivalent to 19.0 mg asulam/kg bw/day (using a mean daily food consumption of 24.5 g and a mean body weight of 129.1 g).

Materials and Methods

Test Material:

Asulam, purity 814 g/kg, Batch no.: OP980398

Test Design:

Japanese quail (100 males and 100 females) were randomly distributed into one control group and four treatment groups. The test concentrations were selected based upon the results of a reproduction study (Wildlife International, Ltd. Project Number 512-101). Each treatment and control group contained 20 pairs of birds, with one male and one female per pen. The study began with a two-week pre-treatment period, during which all Japanese quail received basal diet and measurements of adult and reproductive parameters were made. Four treatment groups were then fed diets containing 48, 100, 225, or 500 ppm as of asulam for 6 weeks. During the treatment phase, the control group was fed diet comparable to the treatment groups, but without the addition of the test substance. All adult birds were observed daily for mortality, signs of toxicity and abnormal behaviour. Adult body weights were measured at the start of the pre-treatment period, at the start of the treatment period and at adult termination. Feed consumption was measured for each pen for a seven-day period every week throughout the test. Eggs were collected daily (when available), starting with the pre-treatment period. Each week, eggs laid on the last two days of the week were selected for eggshell strength and thickness measurements. All remaining eggs were candled to detect eggshell cracks or internal abnormalities. Cracked or abnormal eggs were recorded and all eggs not selected for eggshell measurements were discarded. Endpoints measured included parental weight and feed consumption, numbers of eggs produced and eggshell quality (strength and thickness).

Results and Discussion:

There were no treatment- related mortalities, overt signs of toxicity or treatment-related effects upon body weight or feed consumption at any of the concentrations tested. Additionally, there were no treatment-related effects upon egg production, or the percentage of cracked eggs. Egg strength and eggshell thickness measurements are listed in Table B.9.1.3.02.

Table B.9.1.3.02 Effects of asulam treatment on eggshell quality parameters for Japanese quail

Experimental Group		Egg Shell Thickness (mm)				Egg Strength (N)			
Concen-tration	Daily dietary dose	Pre-treatment		Treatment		Pre-treatment		Treatment	
(ppm as)	(mg/kg bw/day)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	0	0.228	+/- 0.012	0.222	+/- 0.012	13.490	+/- 2.409	12.737	+/- 1.731
48	8.9	0.223	+/- 0.013	0.217	+/- 0.013	13.373	+/- 1.799	11.947	+/- 1.500
100	19.0	0.229	+/- 0.019	0.221	+/- 0.015	13.573	+/- 2.586	12.485	+/- 1.977
225	41.2	0.220	+/- 0.010	0.211	+/- 0.008*	12.149	+/- 1.546	11.588	+/- 1.110
500	93.8	0.229	+/- 0.012	0.216	+/- 0.013*	13.599	+/- 1.951	12.329	+/- 1.931

* statistically significant different from control group at p <0.01 (Dunnett-Hsu).

Conclusions:

Based upon the statistically significant reduction in eggshell thickness noted at 225 and 500 ppm, the NOEC is concluded by the RMS to be 100 ppm, which is equivalent to 19.0 mg asulam/kg bw/day (using a mean daily food consumption of 24.5 g and a mean body weight of 129.1 g).

RMS's evaluation of Japanese quail reproductive toxicity study:

This study has previously been evaluated at EU level for the Annex I inclusion of asulam, with the above presented conclusions being consistent with the EFSA Conclusion report (2010). CRD considers the study scientifically valid and suitable for use in the regulatory risk assessment.

A further consideration of likely population related effects from the reductions in egg shell thickness and strength reported in this study is included in the 'Risk Assessment' (Section B.9.1.5), taking into account a brief literature review on the relationship between egg shell thinning and population survival (Sections B.9.1.3.3 & B.9.1.3.4), with a NOAEL of 93.8 mg asulam /kg bw /day (500ppm asulam in diet) being proposed by the RMS (CRD). This is in line with that previously agreed for this Japanese quail reproductive toxicity study in the EFSA Conclusion report (2010).

B.9.1.3.3 Relationship between egg shell thinning and population effects – RMS's consideration of commissioned expert opinion / literature review (Thompson, HM 2004 'Impact of asulam on eggshell quality', Unpublished report No. 0039956)

The following RMS's evaluation was included in the original Volume 3 Ecotoxicology DAR for asulam (2006) and is considered still applicable at this time:

A literature review was conducted on the implications of eggshell thinning. This literature review indicates a clear correlation between egg shell thickness and reduced

egg shell strength as illustrated in Figure B.9.1.3.01 (taken from this review). Although there was variability in the reported level of effects (both between species and individual studies) 5% and 10% decreases in egg shell thickness generally resulted in approximately 10% and 20% decreases in egg shell strength.

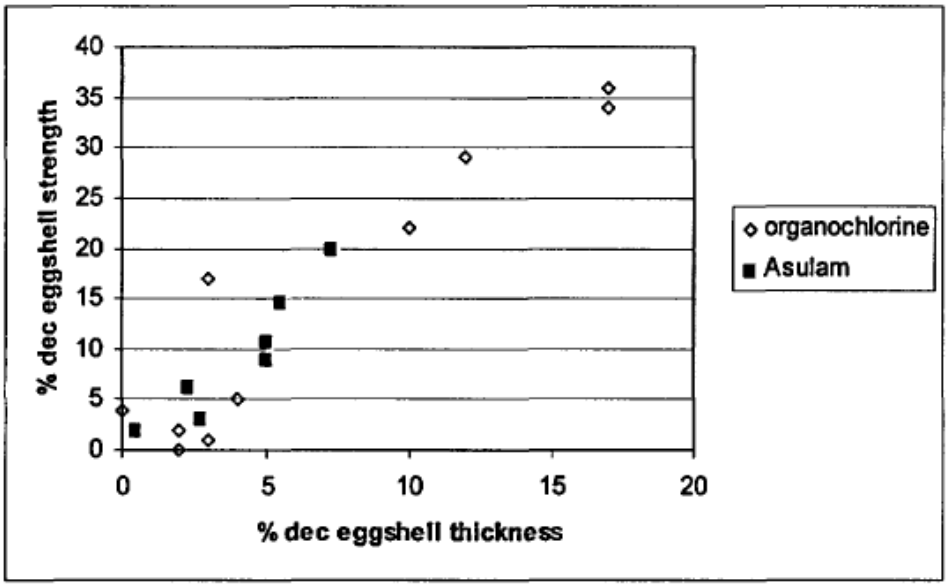


Figure B.9.1.3.01 Effects of decreasing eggshell thickness on eggshell strength, published values for organochlorines and reported values for asulam.

Effects on egg cracking and other adverse population affects were not apparent in the reviewed studies at levels of 5% egg shell thinning or less. The lowest reported values of egg shell thinning associated with the presence of cracked eggs was 5.6% and 7.2% thinning reported in two studies on ring doves. There was an increased frequency of association between egg shell thinning and adverse population effects as thinning effects increased from 10% to 20%, with reductions of 20% or higher being associated with bird population declines in numerous studies.

The conclusions of an earlier review by Cooper K (1991) which included consideration of the effects of egg shell thinning on bird population survival were also referred to within this literature review. A copy of this paper was requested by the Rapporteur Member State, with the main conclusions drawn in relation to effects of egg shell thinning being specified separately below.

The review author concluded that decreases in egg shell thickness in the two asulam reproductive toxicity studies of 5% at 225 ppm and 500 ppm asulam in diet and of 5.4% at 1000ppm asulam in diet were (based on the reviewed data) not likely to have a significant impact on breeding success. On this basis, the study NOEC was considered to be 1000 ppm asulam in diet, with 'biologically significant' decreases in eggs shell thickness of 7.2% at the next highest test dose of 2000 ppm asulam in diet. However, the Rapporteur Member State (RMS) notes that egg shell thickness decreases of 5% at 225 ppm and of 5.4% at 1000 ppm asulam in diet differ little from the 5.6% decreases associated with cracked eggs in the reviewed ring dove study. Therefore the biological significance of the results of the asulam bird reproductive toxicity studies needs further consideration (see Risk Assessment section below).

B.9.1.3.4 Relationship between egg shell thinning and population effects – RMS's consideration of additional published review (Cooper K 1991):

A paper on the effects of eggshell thinning (Cooper, K, 1991) has been assessed by the Rapporteur Member State (RMS) and was referred to in the above commissioned expert opinion /literature review. The review is considered to be well written with its conclusions in relation to the effects of egg shell thinning (from exposure to DDE and other organochloride pesticides) on shell breakage, reproductive failure and population decline being adequately supported by reference to results from published studies. The following is an extract from this review and represents the main conclusions drawn:

"The exact relation of thinning to breakage varies with circumstances. Eggs thinned to the same degree undergo breakage somewhat more when incubated naturally than when incubated artificially with special care. Thinning of 2-5% may cause some damage, but it is difficult to distinguish this damage from normal loss. Thinning of 10% leads to some cracking and to increased embryonic mortality. Thinning of 20-25% leads to the breakages of many eggs, and in nature this degree of thinning is associated with reproductive failure and population decline. The most dramatic example is that involving brown pelicans on Anacapa Island in 1969.

Some species seem essentially immune to eggshell thinning by DDE. In others, such as the Japanese quail and the mallard duck, the maximal thinning ever observed has never reached a level associated with enough breakage to produce serious reproductive failure. It seems likely that there is some variation from one species to another in the exact degree of damage, even where all the species considered are susceptible and the degree of thinning is identical."

B.9.1.4 Additional studies referred to by the Notifier in support of a higher tier risk assessment for (omnivorous) birds:

In support of a higher tier risk assessment for omnivorous birds feeding post-crop emergence in treated spinach and flower bulb crops, the Notifier has proposed the use of the skylark as a focal species. The Notifier has estimated the proportion of total diet obtained by this species in the proposed treated crops (i.e. 'PT') based on the results of a radio-tracking field study (ref. Wolf 2005) and the proportions in its diet of various food items (i.e. PD) based on the results of a skylark feeding study (ref. Muenderle and Grimm 2012). The first of these studies has previously been summarised and evaluated by the RMS in the 'Additional report' to the DAR (dated November 2009) and for completeness these details have been re-presented below (Section B.9.1.4.1), with a further (updating) comment from the RMS. The 'PD' related study is new and has therefore been evaluated by the RMS at this time (Section B.9.1.4.2) - with its comments and conclusions included following the Notifier's study summary /evaluation.

B.9.1.4.1 Ref: Wolf C 2005 Generic field monitoring of birds and mammals on maize and beet fields in Austria, Bayer Crop Science study (WFC/FS017, UK ref DP 142209)

This study has previously been evaluated at EU level for the Annex I inclusion of asulam.

a) Methodology

The study was GLP compliant and assessed the feeding behaviour of birds and mammals locating in and around 5 maize and 5 sugar beet fields located in the 'Tullner Feld' to the west of Vienna in Austria (Wolf 2005, Report WFC/FS 017). This region

is a typical area of maize and sugar beet cultivation in Europe. The study started some weeks before drilling of maize and sugar beet and was completed when the BBCH-code 14 of maize and 16 of sugar beet was reached (stated also as up to 14-18cm in crop height). Assessments of both bird and mammal foraging behaviour were provided, although the study summary included in this regulatory report focuses on the results for birds (in particular skylarks) – since it is these data that are proposed for use in the refined risk assessment.

To assess the relevance of sugar beet, maize and adjacent fields as feeding habitats for birds, census counts were carried out along 5 different transects, representing typical agrarian habitats within the region. The 5 transects areas covered 65.4 ha in total and were monitored 10 times during the whole study period. These 'transect counts' involved an observer walking slowly through fields and recording the species and number of birds within 50metres to each side of the transect track. Transect counts were taken mainly during the early morning when birds were most active in the crops. For each crop type monitored the abundance of birds was calculated. Additionally, 'scan sampling' of bird numbers was undertaken – involving a brief visual assessment with binoculars of bird species and numbers present in each of 3 maize and 3 sugar beet fields, conducted from dawn until dusk every 10 minutes on at least 3 days. Before drilling, the same fields (recorded as 'plain fields' with no planted crop) were 'scan sampled' to monitor the species composition present.

One of the main aims of the study was to provide an assessment of the use of sugar beet fields by skylarks (*Alauda arvensis*) as this species was known to occasionally use sugar beet fields as a foraging habitat. To quantify the actual relevance of sugar beet and also maize fields, a total of 16 skylarks were trapped in or close to sugar beet or maize fields, tagged with radio transmitters and were then each tracked over at least one 24 hour observation period.

During each 24 hour radio-tracking session tagged birds were tracked continuously so that their location, habitat and behaviour could be recorded, in order to obtain information on their home range (determined by the 'minimum convex polygon' of their outmost fixed positions), habitat selection and time budget while living in areas characterised by the occurrence of maize and sugar beet cultivation. 'Active' birds were determined by the occurrence of small fluctuations in the radio-tracking signal. Where possible, visual contact was maintained during the radio-tracked period, with the bird's observed behaviour being recorded. 'Active' birds were categorised as 'potentially foraging' when other activities (e.g. grooming or breeding) could not be excluded by visual observations. For the estimation of the proportion of total 'potentially foraging' time spent in beet or maize crops, data on individual birds were only included where these crops were part of their determined home range – i.e. only 'potential consumers' being included and not the wider 'non-consumer' population.

To obtain information on the food items selected by skylarks and other bird species (i.e. 'PD' data), skylark faeces were gathered in maize and sugar beet fields and analysed quantitatively for composition - based on their proportion by volume in faeces.

b) Field study results in relation to bird foraging behaviour.

The study results for the monitored birds are summarised in the study report in a single table and this is reproduced below (over).

Table B.9.1.4.01 Study author's summary of field monitoring study with respect to the presence and feeding behaviour of skylarks in newly drilled sugar beet and maize fields

PORTION OF TIME potentially foraging (PT) per habitat used by radio tracked Skylarks				
potential foraging time [1] 16 skylarks spent per habitat; [mean of sessions], (90%ile, **N**: no. of tracking sessions considered)	plain fields	19.9 %	(59.3)	**15**
	drilled maize fields	4.1 %	(14.1)	**7**
	germinated maize fields	42.1 %	(95.4)	**10**
	drilled sugar beet fields	8.1 %	(21.2)	**9**
	germinated sugar beet fields	19.7 %	(58.7)	**14**
HABITAT PREFERENCE of Skylarks according to radio tracking				
preference of crop types as a feeding habitat (Jacobs' index [D], Range: –1 to +1; MCP [100%])	plain fields	-0.47		
	drilled maize fields	-0.83		
	germinated maize fields	0.06		
	drilled sugar beet fields	-0.73		
	germinated sugar beet fields	-0.29		
DIET of Skylarks in maize and sugar beet fields				
mean portion of diet after the analysis of faeces gathered in sugar beet (63) and maize (6) fields	*food item*	*PD [%]*	*Frequency [%]* [2]	
	maize seeds	0	0	
	maize seedlings	0	0	
	sugar beet seeds[3]	< 0.7	3.2	
	sugar beet seedlings[3]	< 3.6	23.8	
	potentially sugar beet seeds[4]	< 2.1	17.5	
	potentially sugar beet seedlings[4]	< 10.2	36.5	
HABITAT of birds according to transect counts (based on population)				
abundance of Skylarks and other species after 10 transect counts covering 65.4 ha respectively [individuals/ha]	*field status*	*Skylark*	*sum of other species*	
	plain fields	0.34	0.41	
	drilled maize	0.41	0.86	
	germinated maize	0.25	1.63	
	drilled sugar beet	0.32	0.36	
	germinated sugar beet	0.31	1.1	
	all other fields	0.59	1.1	
BIRD ABUNDANCE against crop type and stage according to scan sampling				
Densities of the 3 most abundant bird species in different crop types and stages [ind./ha/scan]	plain field (28 sessions)	Skylark	0.12	
		Pied Wagtail	0.07	
		Black Redstart	0.03	
	drilled maize field (3 sessions)	Skylark	0.07	
		Common Pheasant	0.03	
		Pied Wagtail	0.03	
	germinated maize fields (6 sessions)	Grey Partridge	0.02	
		Barn Swallow	0.02	
		Common Pheasant	0.02	
	drilled sugar beet fields (4 sessions)	Skylark	0.14	
		Common Pheasant	0.05	
		Grey Partridge	0.01	
	germinated sugar beet fields (10 sessions)	Skylark	0.21	
		Whinchat	0.05	
		Common Pheasant	0.04	

[1] Sum of bird behaviours categorised as 'foraging', 'potential foraging', or 'unknown' (but active).
[2] '% frequency' based on proportion by volume of food item found in faeces
[3] Based on specifically identified food items in faeces
[4] Sum of sugar beet and unspecified seeds / seedlings – as a conservative worst case estimate

c) RMS evaluation of bird abundance data for seedling sugar beet and other fields

From the results of the 5 transect counts a total of 709 sightings of individual birds were reported, with the skylark being the most numerous species present (289 sightings). Based on transect counts, skylarks were present in both germinated maize fields (0.25 individuals /ha) and in germinated sugar beet fields (0.31 individuals /ha), although at lower densities than in 'other' surrounding fields (0.59 individuals /ha). The lower abundances recorded by 'scan sampling' than by transect counts were attributed to the scanning estimates being based on a mean for the entire day light period (taken from regular 10 minute observations), whereas the transect counts were based on observations made in the early morning - when birds were generally much more active than other parts of the day.

The common occurrence of skylarks in newly drilled sugar beet crops reported in this study provides support for the proposed use of the skylark as a focal species in the refined risk assessment – assuming that at early post-emergence crop growth stages the attractiveness to skylarks of the proposed use in spinach crops as a foraging habitat is similar to that of sugar beet crops.

d) RMS evaluation of proportion of skylark foraging time (and by extrapolation fraction of total diet) spent in seedling sugar beet fields

The individual data on which the study author / Notifier base their sugar beet and maize 'PT' related estimates (Table B.9.1.4.01) are presented in Table B.9.1.4.02 (time spent foraging in each crop /habitat) and Table B.9.1.4.03 (% of total foraging time in each crop /habitat).

Table B.9.1.4.02 Time spent by individual radio-tagged skylarks 'potentially foraging' in different habitats during each 24 hour observation session

Note: Figures only given where the home range of the tracked bird during the telemetry session included the habitat concerned i.e. they were potential consumers (e.g. 0.00 hours indicates habitat in home range but no 'potential foraging' observed).

Note: 'Sugar beet (drilled field)' = pre-crop emergence and 'sugar beet' = early post-crop emergence growth stages

	Time spent „potentially foraging" per known habitat (hh:mm) each line represents one session														
Bird No. Session No.	Plain field	Maize (drilled field)	Maize	Sugar beet (drilled field)	Sugar beet	Peas (drilled field)	Peas	Sunflower (drilled field)	Sunflower	Oilseed rape	Winter cereals	Set aside	Weed field	Path	Total time
1 – 1	8:16					3:04					0:07			0:00	11:27
1 – 2	4:10			0:00		6:53					0:10			0:00	11:13
2 – 1	0:23					0:54					2:34	7:39		0:00	11:30
2 – 2							0:13				2:47	1:17		0:00	04:17
3 – 1	2:32									10:10	0:00				12:42
4 – 1	1:43			0:00						2:31	1:06		5:22	0:00	10:42
4 – 2			9:48		0:00					0:00	2:59			0:00	12:47
5 – 1	9:16			0:00						3:26	0:00		0:00	0:33	13:15
7 – 1	0:55			0:00							13:03			0:00	13:55
8 – 1	0:12			0:00							2:56			5:23	08:31
8 – 2	0:00	1:28									6:57			2:29	10:54
8 – 3	1:41	0:00	3:03				0:00				1:57			1:21	08:02
8 – 4	0:00	0:00	11:18		0:00		0:39				1:04			0:34	13:35
9 – 1				4:36				0:25			2:24			0:00	07:25
9 – 2			0:14		8:04				0:14		1:12			0:00	09:44
9 – 3					1:29				2:23		5:28			0:18	09:38
10 – 1				0:00						0:00	12:08				12:08
10 – 2	0:00	0:00		0:00						0:08	11:53			0:00	12:01
11 – 1	4:21	1:30		1:06						0:02	0:48		2:15	0:00	10:02
12 – 1			11:34		0:00					0:00	0:00			0:00	11:34
13 – 1	0:39				5:43		2:19				1:03	0:00		0:29	10:13
13 – 2		0:00	0:19		2:52		1:31		5:38		1:09	0:00		0:00	11:29
13 – 3					1:14		7:27		2:45		0:16	0:00		0:15	11:57
14 – 1		0:00	2:07		0:00		8:23	0:00			0:05			0:00	10:35
14 – 2			0:22		0:00		10:26				0:00			0:00	10:48
15 – 1					0:30	0:00		0:00	0:00		13:35			0:00	14:05
15 – 2			0:00		2:27		0:00		1:23		7:13			0:00	11:03
16 – 1	0:00				5:06				0:14	3:11	0:00				08:31
18 – 1			11:49		0:00					0:00	0:38				12:27
N	15	7	10	9	14	4	9	3	7	10	29	5	3	25	29
50%til	0:55	0:00	1:14	0:00	0:52	1:59	1:05	0:00	1:23	0:02	1:06	0:00	2:15	0:00	11:08
90%til	6:42	1:28	10:15	1:48	5:31	5:44	7:43	0:20	3:54	3:14	7:03	5:06	4:44	1:02	13:21
mean	2:16	0:25	3:23	0:38	1:57	2:42	2:34	0:08	1:48	1:02	2:28	1:47	2:32	0:27	10:53
± sd	2:54	0:40	4:15	1:26	2:30	2:39	3:11	0:11	1:51	1:26	3:20	2:58	2:12	1:08	2:07
min	0:00	0:00	0:00	0:00	0:00	0:00	0:00	0:00	0:00	0:00	0:00	0:00	0:00	0:00	4:17
max	9:16	1:30	11:18	4:36	8:04	6:53	8:23	0:25	5:38	3:26	12:08	7:39	5:22	5:23	14:05

The shown values of time spans are rounded up (if value is >X:XX:29) or rounded down (if value is <X:XX:30)

Table B.9.1.4.03 Percentage of total 'potentially foraging' time spent in each habitat by individual radio-tagged skylarks during each 24 hour observation session

Note: Figures only included where the home range of the tracked bird during the telemetry session included the habitat concerned i.e. they were potential consumers (e.g. 0.0 indicates habitat in home range but no 'potential foraging' recorded).

Note: 'Sugar beet (drilled field)' = pre-crop emergence and 'sugar beet' = early post-crop emergence growth stages

Portion of time „potentially foraging" per known habitat (%)
each line represents 100%

Bird No. Session No.	Plain field	Maize (drilled field)	Maize	Sugar beet (drilled field)	Sugar beet	Peas (drilled field)	Peas	Sunflower (drilled field)	Sunflower	Oilseed rape	Winter cereals	Set aside	Weed field	Path
1 – 1	72.2					26.8					1.0			0.0
1 – 2	37.1			0.0		61.4					1.4			0.0
2 – 1	3.4					7.9					22.3	66.4		0.0
2 – 2							4.9				64.9	30.1		0.0
3 – 1	19.9									80.1	0.0			
4 – 1	16.0			0.0						23.5	10.3		50.2	0.0
4 – 2			76.6		0.0					0.0	23.4			0.0
5 – 1	70.0			0.0						25.9	0.0		0.0	4.1
7 – 1	6.5			0.0							93.5			0.0
8 – 1	2.3			0.0							34.5			63.2
8 – 2	0.0	13.5									63.8			22.7
8 – 3	21.0	0.0	38.0				0.0				24.2			16.8
8 – 4	0.0	0.0	83.2		0.0		4.8				7.9			4.1
9 – 1				62.0				5.6			32.4			0.0
9 – 2			2.3		82.9				2.5		12.3			0.0
9 – 3					15.5				24.7		56.7			3.1
10 – 1				0.0						0.0	100			
10 – 2	0.0	0.0		0.0						1.1	98.9			0.0
11 – 1	43.3	15.0		11.0						0.3	8.0		22.4	0.0
12 – 1			100		0.0					0.0	0.0			0.0
13 – 1	6.3				55.9		22.7				10.3	0.0		4.8
13 – 2		0.0	2.8		24.9		13.2		49.1		10.0	0.0		0.0
13 – 3					10.3		62.3		23.0		2.3	0.0		2.1
14 – 1		0.0	20.0		0.0		79.2	0.0			0.8			0.0
14 – 2			3.4		0.0		96.6				0.0			0.0
15 – 1					3.6	0.0		0.0	0.0		96.4			0.0
15 – 2			0.0		22.2		0.0		12.5		65.3			0.0
16 – 1	0.0				59.9				2.7	37.4	0.0			
18 – 1			94.9		0.0					0.0	5.1			
N	15	7	10	9	14	4	9	3	7	10	29	5	3	25
50%til	6.5	0.0	29.0	0.0	7.0	17.3	13.2	0.0	12.5	0.7	10.3	0.0	22.4	0.0
90%til	59.3	14.1	95.4	21.2	58.7	51.0	82.6	4.5	34.4	41.6	94.1	51.9	44.7	12.0
mean	19.9	4.1	42.1	8.1	19.7	24.0	31.5	1.9	16.3	16.8	29.2	19.3	24.2	4.8
± sd	24.0	6.4	39.9	19.4	26.3	23.7	35.4	2.7	16.2	24.9	33.9	26.3	20.6	13.1
min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
max	72.2	15.0	100	62.0	82.9	61.4	96.6	5.6	49.1	80.1	100	66.4	50.2	63.2

„Potentially foraging" in unknown habitat is equally distributed over all known habitats noted during the given tracking session

In relation to the percentage of foraging time spent in seedling sugar beet crops, a 50th percentile value of 7% and a 90th percentile value of 59% has been estimated by the Notifier for 'potential consumers' i.e. those radio-tracked skylarks that had the potential to forage in seedling sugar beet fields – as defined by having a home range during the observed period including such fields. However these estimated percentile values are based on 14 data points for 9 individuals – with more than one data point (24 hour tracking session) recorded for four of the nine individuals. Mean values for the nine tracked individuals from lowest to highest are: 0.0 (bird 4), 0.0 (bird 8), 0.0 (bird 12), 0.0 (bird 14 – mean of two sessions 0 & 0), 0.0 (bird 18), 12.9 (bird 15, mean of two sessions 3.6 & 22.2), 40.4 (bird 13, mean of two sessions 55.9 & 24.9),

49.2 (bird 9, mean of two sessions 82.9 & 15.5) and 59.9 (bird 16). If the data is re-analysed based on these individual mean values, the 50th percentile value for 'potential consumers' lies between 0 and 12.9% and the 90th percentile value for 'potential consumers' approximates to the highest individual value of 59.9% (i.e. closely equivalent to that based on the 14 data points). The overall mean PT value for the nine 'potential consumers' is 18%.

Therefore, based on mean values for the nine individual radio-tracked skylarks with home ranges including newly emerged sugar beet crops (i.e. 'potential consumers'), the 90th percentile value for the proportion of foraging time spent in seedling sugar beet crops approximates to 0.6.

Note: The above derived seedling sugar beet crop PT values relates to 'potential consumers'. If only the 'consumer population' is considered the data is limited to just four individuals with individual mean values of 12.9 (bird 15), 40.4 (bird 13), 49.2 (bird 9) and 59.9 (bird 16). Based on this very limited dataset and an approximate percentile analysis, the 50th percentile 'consumer' PT value lies between 40.4-49.2 and the 90th percentile PT is > 59.6. The overall mean PT value for the four 'consumers' is 40.6%.

RMS's further comments on the study (in relation to current 2015 evaluation):

The study is considered scientifically valid, with the 90th percentile PT estimates considered suitable for consideration (along with other estimates) in an avian refined risk assessment using the skylark as a relevant focal species.

However, the Notifier's concluded skylark 90th percentile 'potential consumer' PT of 0.6 (based on observations on 9 radio-tracked birds foraging in seedling sugar beet crops) is lower than an earlier UK estimated skylark 90th percentile 'consumer' PT of 0.88 -based on observations on a larger number of birds (i.e. 18 radio-tracked birds) foraging in sugar beet crops in a UK Government sponsored (CSL /FERA) avian radio-tracking field studies (ref. ACP paper SC11411, March 2006). Given this difference, the RMS previously provided in the 'Additional Report' to the DAR (2009) separate refined omnivorous bird risk assessments assuming a 90th percentile PT = 0.6 in one assessment and a 90th percentile PT = 0.88 in another. However, subsequent discussions by EFSA /Member States at the PRAPeR77 Ecotoxicology Expert meeting concluded that use of the UK's earlier estimated skylark 90th percentile PT estimate of 0.88 was most appropriate –with the risk assessment in the 'List of Endpoints' being amended to only include this PT value (ref. EFSA Conclusion Report for asulam, 2010).

B.9.1.4.2 Report: M. Muenderle, T. Grimm (2012) Diet composition of skylarks (*Alauda arvensis*) in leafy crops during spring and summer. United Phosphorus Ltd., Unpub. report No.: R11251

This study has not previously been evaluated at EU level. The Notifier states that the study 'has been conducted because of concerns raised with regards to the estimation of the proportions of food items in the diet for skylarks foraging in seedling sugar beet crops that was used in the reported field study from the original dossier'.

Guidelines:

No official test guidance available at present
Deviations: n/a

GLP: Yes

Executive Summary:

Faeces samples were collected of skylarks (*Alauda arvensis*) utilising fields of leafy crops (including spinach) as foraging habitat at two sites, one in Germany and one in the Netherlands. The proportion of different food types in their diet (PD) was estimated by analysing 35 faeces samples of the skylarks. Correction factors determined by Green (1978) were applied to take into account losses during the digestion process. Skylarks and their nests were also observed to determine the general breeding status in the study area. A total of 15 pairs of skylarks were confirmed breeding during 29 April and 01 July 2011, in most cases, the adults were observed feeding their chicks in/at the nest. Hence, these dates can be treated as the period of 'late breeding' of the local population in the study area.

Materials and Methods

Test Material:

Not applicable; generic field study

Test Design:

Faeces samples were collected of skylarks (*Alauda arvensis*) utilising fields of leafy crops (including spinach) as foraging habitat. The study was conducted at two sites in spring and summer (May to July) 2011 at one site in South-western Germany (Rhineland-Palatinate, nearby Ludwigshafen) and one in the Netherlands (Groningen, nearby Winschoten), both typical areas for the cultivation of leafy crops in Central Europe. The proportion of different food types in their diet (PD) was estimated by analysing 35 faeces samples of skylarks collected in fields of leafy crops. Correction factors determined by Green (1978) were applied to take into account losses during the digestion process.

Results and Discussion:

Breeding Status

A total of 15 pairs of skylarks were confirmed breeding during 29 April and 01 July 2011 (one pair in the study area in Germany and 14 pairs in the study area in the Netherlands). Additional activity, indicating probable breeding was also noted in both areas. In most of these cases, the adults were observed feeding their chicks in/at the nest. Hence, these dates can be treated as the period of 'late breeding' of the local population in the study area. Since the first faeces were gathered on 11 May 2011 the timing of the faeces sampling corresponds to the breeding season of skylarks in leafy crops in Central Europe (taking into account a back-calculation for the onset of breeding; i.e. egg production, egg laying and incubating period from dates where adults fed their young).

PD values

The 'fragment area' of the different food categories (e.g. invertebrates, seeds, green plant material) in the faeces was used to determine the composition of diet actually ingested. To account for different digestibility and recognisability of different food types correction factors according to Green (1978) were applied to the total fragment area of food types in the samples in order to derive the proportion each food type contributed to the actual ingested diet (PD). The combination of nest search activities with the sampling scheme of faeces (i.e. gathering faeces from skylarks observed in

study fields during defined growth stages), allowed the calculation of accurate and representative PD values for a local breeding population of skylarks settling in leafy crops. PD values were calculated for individual faeces and as overall PD values (i.e. mean, median and 90%tile) and are shown in Table B.9.1.4.4.

Table B.9.1.4.4: PD values for skylarks foraging in leafy crops in Germany and the Netherlands

Based on 35 faeces samples collected in 2011 and analysed according to Green (1978)				
Food category	Statictics[2]	PD values for skylarks utilising leafy crops in Germany (n=20) [1]	PD values for skylarks utilising leafy crops in the Netherlands (n=15) [1]	PD values for skylarks of both study sites as a whole (n=35) [1]
Arthropods	Mean	0.558	0.779	0.653
	SEM	0.090	0.073	0.062
	Median	0.690	0.962	0.758
	90%ile	0.998	1.000	1.000
Cereal grain	Mean	0.075	0.084	0.079
	SEM	0.033	0.045	0.027
	Median	0.000	0.000	0.000
	90%ile	0.352	0.432	0.401
Seeds of *Stellaria* & *Chenopodium*	Mean	0.007	0.000	0.004
	SEM	0.004	0.000	0.002
	Median	0.000	0.000	0.000
	90%ile	0.047	0.000	0.015
Weed & grass seeds	Mean	0.058	0.057	0058
	SEM	0.048	0.028	0.029
	Median	0.000	0.000	0.000
	90%ile	0.139	0.275	0.259
Dicot cotyledons & leaves	Mean	0.300	0.075	0.203
	SEM	0.085	0.045	0.055
	Median	0.077	0.000	0.024
	90%ile	0.902	0.430	0.834
Grass flowers	Mean	0.001	0.005	0.003
	SEM	0.001	0.005	0.002
	Median	0.000	0.000	0.000
	90%ile	0.001	0.030	0.002
Monocot leaves	Mean	0.000	<0.001	<0.001
	SEM	0.000	<0.001	<0.001
	Median	0.000	0.000	0.000
	90%ile	0.000	0.003	0.000

[1] Given as proportions of the dry weight of the total diet ingested; [2] SEM: Standard Error of the Mean

Conclusions:

This study provides a reliable refined parameter of PD value for skylarks for the use in higher tier risk assessments for birds foraging in fields with leafy crops in Central Europe from May to the beginning of July.

RMS's comments on the study:

The RMS (CRD) has referred to both the study summary and the more detailed submitted full study report in making its evaluation of this study. It is the RMS's view that the study summary would have benefitted from the inclusion of more detail - particularly in relation to the types of crops in the study area and also in relation to the methodology and values used to derive the 'PD' estimates. However, these details are included in the full study report, with the methodology being broadly in line with advice included in Appendix Q of EFSA (2009) guidance. With respect to the study site, the study report includes the following details:

'The selected study fields represented typical fields with leafy crops in Germany and in the Netherlands with respect to shape, surrounding and agricultural treatment. There were eleven study fields in Germany, growing broccoli, spinach, cauliflower, celery, cos lettuce and parsley and seven study fields in the Netherlands growing alfalfa. The majority of these study fields were situated in an agricultural landscape and were therefore surrounded by other leafy crops, e.g. potato, carrot and/or cereal fields. Some of the selected study fields were bordered by other elements typical of agricultural landscapes: these included tracks, fallow land, grassland, horse paddocks and hedgerows.'

Details included in the reported study are considered sufficient to confirm that skylarks were foraging in the observed commonly grown leafy dicotyledonous vegetable / forage crops – which adequately covers the EFSA (2009) 'leafy vegetable' crop scenario (including the proposed use in spinach crops). Also, the derived PD values relating to skylarks (with nestlings) foraging in 'leafy crops' between May and July are considered by the RMS as suitable for possible use (with other available dietary data) in a refined risk assessment for asulam based on the skylark as a omnivorous bird focal species. However, taking into account current risk assessment guidance (EFSA 2009) and also concerns previously raised in the EFSA (2010) peer review 'Conclusion Report' that the skylark was not the representative focal species for spinach crops, the RMS has instead conducted a refined risk assessment for 'small omnivorous birds' as a 'generic focal species' using standard dietary data estimates included in Appendix A of EFSA (2009) guidance. Therefore, <u>the dietary data including in this new 2012 study have not been relied upon in this current 'new substance' evaluation</u>.

B.9.1.5 Avian risk assessment

The risk to birds from the proposed crop uses of 'Asulox' (containing 400g asulam /L) has been evaluated based on current EFSA (2009) risk assessment guidance. This differs from that used in the previous Ecotoxicology Volume 3 DAR (2006) - which was based on earlier SANCO/4145/2000 (2002) methodology.

No additional avian toxicity studies have been reported since the previous Annex I evaluation of asulam (ref. EFSA Conclusion Report 2010). The following summary and discussion of these studies is considered still applicable and is largely unchanged from that previously included in the earlier Volume 3 DAR (dated April 2006).

Given that the formulation 'Asulox' (also referred to as 'Asulam 400g/L SL') is a simple solution of asulam in water, it does not pose any additional risk over that from the active substance. Therefore, the risk from the formulation will be covered by the asulam risk assessment.

B.9.1.5.1 Summary of avian toxicity studies

An acute oral toxicity study was conducted with bobwhite quail according to EPA Guideline 71-1 and in compliance with GLP, the results for which are suitable for use in the risk assessment. Two short-term dietary studies (one study on mallard duck and one on pheasant) were conducted in 1970, thus prior to the implementation of GLP and also prior to agreement of standard recognised guidelines. The mallard duck study is considered to have been completed using satisfactory methodologies and as such is suitable for use in the regulatory risk assessment. However, the endpoint of the pheasant study could not be converted into mg a.s./kg bw, so cannot be used in the current risk assessment.

An avian long-term reproductive study was conducted which indicated the most sensitive potential adverse effect to be effects of asulam on eggshell quality, which was further investigated in a further additional study. These studies were conducted in compliance with GLP and followed new (draft) OECD protocol guidelines on avian reproductive toxicity, which are considered acceptable by the RMS. A summary of the effects of asulam on eggshell thickness and strength from the two long-term studies is given below in B.9.1.5.01.

Table B.9.1.5.01 Percentage reduction in eggshell quality parameters for Japanese quail treated with asulam in the diet compared with control birds

Study ref.	Parameter	100 ppm asulam	225 ppm asulam	500 ppm asulam	1000 ppm asulam	2000 ppm asulam
[redacted] 2001a	Eggshell thickness (% reduction*)	-	-	5.0 #	5.4 #	7.2 #
	Egg strength (% reduction*)	-	-	10.7 #	14.6 #	20.0 #
[redacted] 2003a	Eggshell thickness (% reduction*)	0.5	5.0 #	2.7 #	-	-
	Egg strength (% reduction*)	2.0	9.0	3.2	-	-

* In comparison to the control; # Statistically significant difference from control ($p < 0.01$).

A clear NOEC (no observed effect concentration) was observed in the second reproductive toxicity study ([redacted] 2003a) – with no significant effects on eggshell thickness or strength observed at the lowest test dose (100 ppm asulam in diet). In order to conclude a NOAEC (no observed adverse effect concentration) from these long-term studies, further literature on eggshell thinning, egg strength and impact on breeding success was reviewed. This included a literature review by an expert from then UK's Central Science Laboratory - now re-named as 'FERA' ([redacted] 2004) and also an earlier review paper by [redacted] (1991).

The reviewed published literature indicates that breakages from eggshell thinning of between 2% and 5% are difficult to distinguish from that which would occur without thinning. Therefore, the statistically significant effects on eggshell thinning at 500 ppm in both avian reproduction studies (2.7 and 5.0%) are not thought likely to cause adverse effects on the reproductive success of birds. Therefore, given that 500ppm

asulam in diet is the highest test dose in the [redacted] (2001a) study, the NOAEC for this study may be concluded to be 500ppm asulam in diet (equivalent to 93.8 mg asulam /kg bw/day). However, for the [redacted] (2001a) study, it is necessary to also consider effects at the higher test doses before determining the NOAEC.

In the [redacted] (2001a) study, a 5.4% effect on eggshell thickness was observed from treatment at 1000ppm asulam in diet – which is only slightly greater than the 5% reduction obtained at 500ppm asulam – at which effects are thought not likely to be significant. However, differences in statistically significant reductions in egg shell strength between these two test doses were greater – with 14.6% reductions at 1000ppm compared with 10.7% at 500ppm asulam. Also, the 5.4% reduction in egg shell thickness obtained at 1000ppm asulam is closely similar to the reduction in egg shell thickness of 5.6% reported in the literature review ([redacted] 2004) to cause increases in levels of egg shell cracking in a ring dove study. Although the long-term toxicity studies conducted on Japanese quail recorded no increases in the number of cracked eggs in any of the treatment groups, details in published literature (ref. [redacted], 1991) suggest that the Japanese quail may be less susceptible to adverse effects on reproductive success due to egg shell thinning than other avian species. In addition, eggs incubated with special care in a laboratory environment are expected to be likely to undergo less damage than eggs incubated naturally.

It is concluded that the statistical significant reductions in eggshell thickness and eggshell strength (of 5.6% and 14.6% respectively) reported in the [redacted] (2001a) study at a dietary test dose of 1000ppm asulam may potentially have adverse effects on reproductive success. On this basis, NOAEC is considered to be the next lower test dose of 500 ppm asulam diet (equivalent to 65 mg asulam /kg bw /day) - at which reductions in egg shell thickness and strength were lower and not considered likely to have an adverse impact on reproductive success.

Details of the concluded ecotoxicological endpoints for the reported asulam avian toxicity studies are summarised in Table B.9.1.5.02. Avian toxicity tests with the formulation were not performed and are not required, since for this simple soluble concentrate spray applied product, the formulation is not considered to pose any additional risk – which can be assessed based on studies conducted with the active substance.

Table B.9.1.5.02 Overview on acute, short-term and reproductive toxicity of asulam to birds ##

Test species	Time scale/ study type	Ecotoxicological endpoint	Test guideline	References
Bobwhite quail	Acute	**LD_{50}** > 2000 mg asulam sodium/kg = **> 1825.6 mg asulam /kg bw**	EPA 71-1	[redacted] (2000)
Mallard duck	Short-term dietary	LC_{50} > 100000 mg asulam /kg diet = > 22732 mg asulam /kg bw	No guidelines in place	[redacted] (1970a)
Pheasant	Short-term dietary	LC_{50} >75000 mg asulam/kg diet *	No guidelines in place	[redacted] (1970a)
Japanese quail	Subchronic, reproduction toxicity study	NOEC = < 500 mg asulam/kg diet = < 65 mg asulam /kg bw /day **NOAEC** = 500 mg asulam /kg diet = **65 mg asulam /kg bw /day**[#]	OECD draft 1999	[redacted] (2001a)
	Subchronic, reproduction toxicity study [##]	NOEC = 100 mg a.s./kg diet = 19.0 mg a.s./kg bw/day [#] NOAEC = 500 mg a.s./kg diet = 93.8 mg asulam /kg bw/day [#]	OECD draft 2000	[redacted]. (2003)

* Not possible to convert into mg asulam /kg bw

Endpoint supported by RMS's evaluation of submitted review papers ([redacted] 1991)
Including further assessment of effects on egg shell quality
Endpoints in **bold** used in the regulatory risk assessment.

B.9.1.5.2 Background to past and current risk assessment

In the earlier EFSA conclusion report (2010), the risk to birds and mammals was assessed in accordance with the Birds and Mammals Guidance document (European Commission, 2002). The acute and short-term risk to insectivorous and herbivorous birds via dietary exposure was assessed as low at Tier 1 for the representative field use in spinach, whereas the long-term risk assessment indicated a need for further refinements. A refined long-term risk assessment was discussed at the PRAPeR 77 expert meeting. The experts agreed on the avian long-term reproductive endpoint (NOAEC = 65 mg asulam /kg bw /day) and dietary residue values to be used - including use of a foliar residue DT50 of 1.44 days derived from residue data from three residue studies in spinach – from which a 21day TWA factor of 0.0989 was calculated. Also, skylark PD and PT values for birds foraging in spinach crops were agreed - the latter using a PT= 0.88 based on a 90th percentile value supported by UK Government (CSL /FERA) data from avian radio-tracking field studies (ref. ACP paper SC11411, March 2006). However, using the concluded NOAEC of 65 mg asulam /kg bw /day, the refined focal species (skylark) long-term TER was 2.2 and therefore below the trigger value of 5. Also, 'experts raised the concern that skylark was not the representative focal species for the use in spinach' and 'Consequently, again the need for further refinements of the long-term risk assessment for insectivorous and herbivorous birds was identified at PRAPeR 77' – with this being specified both as a data gap and as a 'critical area of concern' in the EFSA Conclusion (2010).

The current (2015) risk assessment has been conducted according to the new 'EFSA Guidance Document on Risk Assessment for Birds and Mammals' (2009). After an initial screening step, this identifies specific generic focal species for different scenarios (crop and growth stage) i.e. in this case bare soil (pre-emergent applications) and leafy vegetables/bulbs and onion-like crops (post-emergent applications). This approach therefore supercedes that taken in the DAR (March 2006) and also addresses some of the concerns identified in the EFSA conclusion report (2010) e.g. the identification of relevant focal species (particularly the appropriateness of the use of the skylark as focal species in spinach crops).

The Notifier has proposed use of a skylark 90th percentile PT of 0.6 for birds foraging in spinach and flower bulb crops based on the foraging of 'potential consumers' (n=9) reported in an Austrian field study evaluated earlier in the previous Annex I evaluation of asulam (study ref. Wolf C 2005, detailed in Section B.9.1.2.2 of 'Additional report' dated November 2009). For completeness details for this study are re-presented in this document (Section B.9.1.4.1). However, given the previous decision in the 2010 EFSA 'Conclusion report' to use a PT value of 0.88 - based on UK field study data for 'consumers' conducted on a larger number of birds (n= 18), in the absence of any further evidence, CRD considers it would be more appropriate to use this value.

The Notifier has also referred to new dietary composition data for the skylark based on a field study conducted in leafy crops in Germany and the Netherlands (ref. Muenderle and Grimm 2012), for which details are included in Section B.9.1.4.2 of this report.

Need for short-term dietary risk assessment

Details for a short-term dietary toxicity study and derivation of a short-term toxicity exposure ratio are no longer a requirement under current EFSA (2009) guidance unless the dietary LD50 measured by the short-term study indicates a higher level of toxicity than that obtained in an acute oral (gavage) study – which is not the case for asulam (5 day dietary LD50 of > 22,732 mg asulam /kg bw /day c.f. acute oral LD50 of >1826.5 mg asulam /kg bw). Therefore, a short-term dietary risk assessment is not required.

B.9.1.5.3 Risk assessment for birds (via dietary route of exposure) – 'screening step'

The screening step crop groupings and critical use patterns relevant to the uses of 'Asulox' are given in Table B.9.1.5.03.

Table B.9.1.5.03: Screening step crop groupings and critical use patterns relevant to the use of Asulox.

Crop group	Critical GAP crop	Indicator species	Critical use pattern		
			Rate[a] (g a.s./ha)	No. of apps	App. Interval
'Bare soils ...'	Spinach (pre-emergence i.e. BBCH <10)	Small granivorous bird	2400	1	-
'Bulbs and onion like crops, ... leaf vegetables ...'	Spinach (post-emergence BBCH 12-14) , flower bulbs (post-emergence, all growth stages)	Small omnivorous bird	2400	1	-

[a] Maximum application rate for all applications

Estimation of acute daily dietary dose:

The acute 'daily dietary dose' (DDD) is first calculated by multiplying the 90th percentile 'shortcut value' (SV) by the application rate in kg asulam /ha.

DDD = application rate (kg asulam /ha) x SV

In the case of the use in spinach and flower bulb crops, there is only one application and so a MAF_{90} is not required to be included. The estimated acute daily dietary dose (DDD) for each of the two relevant indicator species is given in Table B.9.1.5.04.

Table B.9.1.5.04: Screening step – estimation of acute exposure to asulam

Crop group	Indicator species	Shortcut value	App. rate (kg asulam /ha)	MAF_{90}	DDD (mg a.s./kg bw/ day)
'Bare soils ...'	Small granivorous bird	24.7	2.4	1.0	59.3
'Bulbs and onion like crops, ... leaf vegetables ...'	Small omnivorous bird	158.8	2.4	1.0	381

Estimation of long-term daily dietary dose:

The long-term 'daily dietary dose' (DDD) is calculated by multiplying the mean 'shortcut value' (SV) by the application rate in kg asulam /ha and the 21 day TWA factor.

DDD = application rate (kg asulam /ha) x SV x f_{twa}

The included 'screening step' 21 day f_{twa} is 0.53 - based upon a default DT_{50} of 10 as given in the EFSA (2009) guidance document. In the case of the use in spinach and flower bulb crops, there is only one application and so a MAF_m is not required to be included. The estimated long-term daily dietary dose (DDD) for each of the two relevant indicator species is given in Table B.9.1.5.05.

Table B.9.1.5.05: Screening step – estimation of long-term exposure to asulam

Crop group	**Indicator species**	**Shortcut value**	**App. rate (kg** asulam **/ha)**	**MAF_m**	**f_{twa}**	**DDD (mg** asulam **/kg bw/ day)**
'Bare soils ...'	Small granivorous bird	11.4	2.4	1.0	0.53	14.5
'Bulbs and onion like crops, ... leaf vegetables ...'	Small omnivorous bird	64.8	2.4	1.0	0.53	82.4

Screening step acute toxicity exposure ratio (TER_A) for birds:

The acute risk to birds has been assessed by calculating toxicity exposure ratios (TER_A) using the following equation:

$$TER_A = \frac{LD_{50}\ (mg/kg bw/day)}{Acute DDD (mg/kg bw/day)}$$

The resulting TER_A values are given in Table B.9.1.5.06.

Table B.9.1.5.06 Screening step – acute risk to birds from dietary exposure

Indicator species (and related crop uses)	**App. Rate (kg** asulam **/ha)**	**LD_{50} (mg** asulam **/kg bw/day)**	**DDD (mg** asulam **/kg bw/day)**	**TER_A**
Small granivorous bird (pre-crop emergence use in spinach)	2.4	>1825.6	59.3	>31
Small omnivorous bird (post-crop emergence in spinach and flower bulbs)			381	**>4.8**

Screening step long-term toxicity exposure ratio (TER_{LT}) for birds:

The long-term risk to birds has been assessed by calculating toxicity exposure ratios (TER_{LT}) using the following equation:

$$TER_{LT} = \frac{NOAEL (mg/kg bw/day)}{Long\text{-}term\ ETE (mg/kg bw/day)}$$

The resulting TER_{LT} values are given in Table B.9.1.5.07.

Table B.9.1.5.07 Screening step – long-term risk to birds from dietary exposure

Indicator species (and related crop uses)	Indicator species	App. Rate (g asulam /ha)	NOAEL (mg asulam /kg bw/day)	DDD (mg asulam /kg bw/day)	TER_{LT}
Small granivorous bird (pre-crop emergence use in spinach)	Small granivorous bird	2.4	65	14.5	**4.5**
Small omnivorous bird (post-crop emergence in spinach and flower bulbs)	Small omnivorous bird			82.4	**0.8**

'Screening Step' acute and long-term dietary risk assessment conclusions:

The 'screening step' TER_A values are in excess of the Uniform Principles trigger value of 10 for pre-emergence use (TER >31) but in breach of this trigger for post-emergence crop uses (TER >4.8). . Therefore, based on the screening assessment, an acceptable acute risk to birds from pre-crop emergence use in spinach can be concluded. However, an acceptable risk from post-crop emergence use in spinach and flower bulb crops has not been demonstrated and therefore this is required to be assessed further in a 'Tier 1' acute risk assessment.

The 'screening step' TER_{LT} values for both relevant indicator species are less than the Annex VI trigger of 5, indicating the need for a 'Tier 1' long-term risk assessment in relation to the proposed pre and post-crop emergence uses.

B.9.1.5.4 'Tier 1' acute risk assessment for post-emergence use in spinach and flower bulbs

Acute risk from post-emergence use in spinach (Tier 1 'Leafy vegetables'):

Details of the relevant 'Tier 1' generic focal species, 90th percentile short-cut values and relevant crop stages are as follows:
Small granivorous bird ("finch") – 27.4 (BBCH 10-49)
Small omnivorous bird ("lark") – 24.0 (BBCH 10-49)
Medium herbivorous bird ("pigeon") – 90.6 (BBCH 10-19)
Small insectivorous bird ("wagtail") – 26.8 (BBCH 10-19)

Daily dietary dose (DDD) = application rate (kg a.s./ha) x shortcut value x MAF_{90} (single application so MAF not applicable)
= 2.4 x 27.4 (small granivorous bird) = 65.8 mg kg bw/day
= 2.4 x 24.0 (small omnivorous bird) = 57.6 mg kg bw/day
= 2.4 x 90.6 (medium herbivorous bird) = 217 mg kg bw/day
= 2.4 x 26.8 (small insectivorous bird) = 64.3 mg kg bw/day

Acute TER = LD50 / DDD

= >1825.6 / 65.8 = >27.7 (small granivorous bird, post-emergence in spinach)
= >1825.6 / 57.6 – >31.7 (small omnivorous bird, post-emergence in spinach)
= >1825.6 / 217 = **>8.4** (medium herbivorous bird, post-emergence in spinach)
= >1825.6 /64.3 = >28.4 (small insectivorous bird, post-emergence in spinach)

Acute risk from post-emergence use in flower bulbs (Tier 1 'Bulbs and onion like crops'):

Details of the relevant 'Tier 1' generic focal species, 90th percentile short-cut values and relevant crop stages are as follows:

Small granivorous bird ("finch") – 24.7 (BBCH 10-39)
Small omnivorous bird ("lark") – 24.0 (BBCH 10-39)
Small insectivorous bird ("wagtail") – 26.8 (BBCH 10-19)

Daily dietary dose (DDD) = application rate (kg a.s./ha) x shortcut value x MAF_{90} (single application so MAF not applicable)
= 2.4 x 24.7 (small granivorous bird) = 59.28 mg kg bw/ day
= 2.4 x 24.0 (small omnivorous bird) = 57.6 mg kg bw/ day
= 2.4 x 26.8 (small insectivorous bird) = 64.3 mg kg bw/ day

Acute TER = LD50 / DDD

= >1825.6 / 59.28 = >30.8 (small granivorous bird, post-emergence in flower bulbs)
= >1825.6 / 57.6 – >31.7 (small omnivorous bird, post-emergence in flower bulbs)
= >1825.6 /64.3 = >28.4 (small insectivorous bird, post-emergence in flower bulbs)

'Tier 1' acute risk assessment conclusions for post-emergence crop uses:

The acute TERs for relevant generic focal species are all within the Uniform Principles trigger value of 10 (indicating a low risk) with the exception of the risk to medium herbivorous birds from post-crop emergence use in spinach crops – where the acute TER of >8.4 indicates a potential risk – for which a further assessment is included below based on a refinement of the determined LD50.

In the acute toxicity study with bobwhite quail, there were neither mortalities nor any differences in weight gain or food consumption or behaviour (or other signs of toxicity - including signs of visible abnormalities at post-mortum) at the highest tested dose – which has been used to define the acute toxicity endpoint – i.e. LD50 > 1826.5 mg/kg bw. In the absence of mortality (or just a single mortality), the current guidance allows the acute LD50 to be refined by the inclusion of an 'extrapolation factor' which takes into account the numbers of animals tested at this 'limit dose' (ref. Table 1 of Section 2.1.2 of EFSA 2009). In the case of asulam, where there were 10 birds tested at each dose level, the refined LD_{50} = 1825.6 x 'extrapolation factor' (1.888) = 3446.7. The (refined) acute TER for medium herbivorous birds becomes 3446.7 / 217 = 15.9, indicating a low risk to medium herbivorous birds from post-crop emergence use in spinach crops.

A low risk of acute effects to birds from post-emergence use of asulam in spinach and flower bulb crops may therefore be concluded.

B.9.1.5.5 'Tier 1' long-term risk assessment for pre-crop emergence use of asulam in spinach

The risk from pre-crop emergence use in spinach crops (i.e. BBCH < 10) is assessed under EFSA (2009) guidance using the 'bare soil' crop scenario. Details of the relevant 'Tier 1' generic focal species and relevant mean short-cut values for this scenario are as follows:

Small granivorous bird ("finch") – 11.4 (BBCH <10)
Small omnivorous bird ("lark") – 8.2 (BBCH <10)
Small insectivorous bird ("wagtail") – 5.9 (BBCH <10)

Daily dietary dose (DDD) = application rate (kg asulam /ha) x TWA (assuming effects caused by long-term exposure) x shortcut value x MAF_m (single application so MAF not applicable)
= 2.4 x 0.53 x 11.4 (small granivorous bird) = 14.50 mg asulam /kg bw /day
= 2.4 x 0.53 x 8.2 (small omnivorous bird) = 10.43 mg asulam /kg bw /day
= 2.4 x 0.53 x 5.9 (small insectivorous bird) = 7.50 mg asulam /kg bw /day

Long-term TER = NOAEL / DDD
= 65 / 14.5 = **4.5** (small granivorous bird, pre-crop emergence in spinach)
= 65 / 10.4 = 6.3 (small omnivorous bird, pre-crop emergence in spinach)
= 65 / 7.5 = 8.7 (small insectivorous bird, pre-crop emergence in spinach)

Therefore, the 'Tier 1' long-term risk assessment for pre-crop emergence in spinach crops indicates an acceptable long-term risk to small omnivorous and small insectivorous birds but a potential risk to small granivorous birds (the long-term TER of 4.5 being marginally in breach of the trigger value of 5) – indicating the need for further consideration /refinement of the long-term risk for small granivorous birds feeding pre-crop emergence in treated spinach fields.

B.9.1.5.6 'Tier 1' long-term risk assessment for post-emergence use in spinach and flower bulbs

Long-term risk from post-emergence use in spinach (Tier 1 'Leafy vegetables'):

Details of the relevant generic focal species, mean short-cut values and relevant crop stages are as follows:
Small granivorous bird ("finch") – 12.6 (BBCH 10-49)
Small omnivorous bird ("lark") – 10.9 (BBCH 10-49)
Medium herbivorous bird ("pigeon") – 37 (BBCH 10-19)
Small insectivorous bird ("wagtail") – 11.3 (BBCH 10-19)

Daily dietary dose (DDD) = application rate (kg a.s./ha) x TWA (assuming effects caused by long-term exposure) x shortcut value x MAF_m (single application so MAF not applicable).
= 2.4 x 0.53 x 12.6 (small granivorous bird) = 16.02 mg asulam /kg bw /day
= 2.4 x 0.53 x 10.9 (small omnivorous bird) = 13.86 mg asulam /kg bw /day
= 2.4 x 0.53 x 37.0 (medium herbivorous bird) = 47.06 mg asulam /kg bw /day
= 2.4 x 0.53 x 11.3 (small insectivorous bird) = 14.37 mg asulam /kg bw /day

Long-term TER = NOAEL / DDD:
= 65 / 16.02 = **4.1** (small granivorous bird, post-emergence in spinach)
= 65 / 13.86 = **4.7** (small omnivorous bird, post-emergence in spinach)
= 65 / 47.06 = **1.4** (medium herbivorous bird, post-emergence in spinach)
= 65 / 14.37 = **4.5** (small insectivorous bird, post-emergence in spinach).

Long-term risk from post-emergence use in flower bulbs (Tier 1 'Bulbs and onion like crops' use scenario):

Details of the relevant generic focal species, mean short-cut values and relevant crop stages are as follows:
Small granivorous bird ("finch") – 11.4 (BBCH 10-39)

Small omnivorous bird ("lark") – 10.9 (BBCH 10-39)
Small insectivorous bird ("wagtail") – 11.3 (BBCH 10-19)

Daily dietary dose (DDD):
= application rate (kg a.s./ha) x TWA (assuming effects caused by LTE) x shortcut value x MAF_m (single application so MAF not applicable).
= 2.4 x 0.53 x 11.4 (small granivorous bird) = 14.50 mg kg bw/d (leafy vegetables)
= 2.4 x 0.53 x 10.9 (small omnivorous bird) = 13.86 mg kg bw/d (leafy vegetables)
= 2.4 x 0.53 x 11.3 (small insectivorous bird) = 14.37 mg kg bw/d (leafy vegetables)

Long-term TER:
= NOAEL / DDD
= 65 / 14.50 = **4.5** (small granivorous bird, post-emergence in flower bulbs)
= 65 / 13.9 = **4.7** (small omnivorous bird, post-emergence in flower bulbs)
= 65 / 14.4 = **4.5** (small insectivorous bird, post-emergence in flower bulbs).

'Tier 1' long-term risk assessment conclusions for post-emergence crop uses:

The 'Tier 1' risk assessment for post-crop emergence use of asulam indicates breaches in the 'Uniform Principles' long-term TER trigger value of 5 from the foraging of small granivorous, omnivorous and insectivorous birds in treated spinach and flower bulb crops and also from the foraging of medium herbivorous birds in treated spinach crops. Therefore, further refinements to the long-term risk assessment are required to address the potential risk from post-crop emergence use.

B.9.1.5.7 Refined risk assessment to address the risk to 'generic focal birds' failing the 'first tier' long-term /reproductive risk assessment.

Breaches in the Uniform Principles long-term TER trigger values of 5 were obtained in the 'Tier 1' risk assessment for several generic focal species and each are considered further below:

i) Refined long-term risk assessment for medium herbivorous birds: from post-emergence use in spinach ('Tier 1' long-term TER = 1.4)

As agreed in the EFSA 2010 Conclusion report, further refinement is possible by using foliar residue decline data evaluated in the previous Annex I evaluation of asulam – based on which an asulam foliar DT50 value of 1.44 days was concluded. In the asulam 'Additional Report' to the DAR (November 2009), a 21 day time weighted average factor (21d TWA factor) of 0.0989 is calculated based on this concluded DT_{50} value of 1.44 days (from three outdoor residue trials on spinach) and this value was then subsequently used to refine the long-term risk assessment for herbivorous birds and mammals. It is therefore possible to refine the 'Tier 1' medium herbivorous bird risk assessment on this same basis:

Daily dietary dose (DDD)

= application rate (kg a.s./ha) x 21d TWA factor x mean shortcut value x MAF_m (single spray – therefore MAF not applicable).

= 2.4 x 0.0989 x 37 = 8.78 mg mg asulam /kg bw /day /kg bw /day.

Therefore, the refined long-term TER calculation for the medium herbivorous bird (consuming exposed foliage) becomes:

Long-term TER = NOAEL / DDD = 65 / 8.78 = 7.4.

This is above the trigger value of 5, indicating a low and acceptable risk to medium herbivorous birds feeding in treated spinach crops.

ii) Refined long-term risk assessment for 'small omnivorous birds': from post-emergence use in spinach and flower bulb crops ('Tier 1' long-term TER = 4.7 for both crop uses)

The 'Tier 1' risk assessment results in a marginal breaching of the Uniform Principle trigger value of 5 for 'small omnivorous birds' feeding post-crop emergence in treated spinach and flower bulb crops. However, further refinement is possible by taking into account the rapid dissipation of asulam residues on foliage – asulam residues decline data indicating a foliar DT50 of 1.44 days and (by calculation) a 21 day TWA factor of 0.0989 (see refined risk assessment for herbivorous mammals for further details).

The Notifier has proposed basing the refined 'higher tier' risk assessment on the skylark as focal species and in relation to this has provided dietary data from a field study conducted with skylarks (ref. Muenderle & Grimm, 2012) and has also referred to PT data from a previously reported and evaluated study (ref. Wolf C, 2005). Details for these two higher tier studies, together with the RMS's (CRD's) evaluation, are included in Section B.9.1.4 of this report.

The RMS notes that the previous 'EFSA Conclusion' on asulam (2010) 'raised concern that the skylark was not the representative focal species for the use in spinach'. Given these concerns and also the lack of specific evidence supporting the use of the skylark as a focal species in flower bulb crops, the RMS has instead conducted a refined risk assessment for the EFSA (2009) 'Tier 1' 'small omnivorous bird' as generic focal species. This risk assessment refines the 'Tier 1' estimated levels of dietary exposure from consumption of contaminated foliage - taking into account the known relatively rapid dissipation of asulam residues on foliage, assuming a diet for the 'small omnivorous bird' consisting of 25% crop leaves, 25% weed seeds and 50% ground arthropods (as detailed in EFSA 2009 guidance).

In line with EFSA (2009) guidance, for each food item, the level of asulam exposure to birds (or 'daily dietary dose') may be estimated using the following equation:

Daily dietary dose (mg /kg bw /day) = Food intake rate per unit body weight (FIR/bw) x mean residue per unit dose (mean RUD, mg /kg fresh weight diet) x Dose (kg a.s./ha) x 21 day TWA factor.

For seed and arthropods, in the absence of specific data, as for the 'Tier 1' risk assessment, the RMS's refined risk assessment includes use of a EFSA (2009) standard 21 day TWA factor of 0.53 (based on an active substance default DT50 = 10 days). However for crop foliage, based on the available foliar residue decline data for asulam, a refined 21 day TWA factor of 0.0989 has been used – based on an asulam foliar DT50 of 1.44 days indicated by previously evaluated asulam residue decline studies.

Food intake rates per unit body weight for each dietary food item may be calculated based on the 'Tier 1' defaults 'total diet' value of 0.52 (for small omnivorous birds feeding in spinach and flower bulb crops) multiplied by the proportion that each item forms in the diet (i.e. x 0.25 for crop leaves and weed seeds, x 0.5 for arthropods), resulting in FIR/bw values of 0.13 for both crop leaves and weed seeds and 0.26 for arthropods.

Mean RUD values used in the refined long-term risk assessment are based on the maximum relevant 'Tier 1' values for the proposed post-crop emergence uses in spinach and flower bulb crops.

Given that identical values for the FIR/bw, mean RUD, dose and 21 day TWA factor apply for both spinach and flower bulb crop uses, levels of exposure to 'small omnivorous birds' from feeding in these treated crops will be equivalent and can be estimated for both using the same input values - based on the sum of the daily dietary dose (or exposure) from asulam residues on each dietary component:

Daily dietary dose (DDD) from consumption of crop leaves
= FIR/bw (0.13) x mean RUD (28.7) x dose (2.4 kg /ha) x 21 d TWA factor (0.0989)
= 0.8856 mg asulam /kg bw /day. /kg bw /day

Daily dietary dose (DDD) from consumption of weed seeds
= FIR/bw (0.13) x mean RUD (40.2) x dose (2.4 kg mg asulam /kg bw /day /ha) x 21 d TWA factor (0.53)
= 6.6475 mg asulam /kg bw /day /kg bw /day

Daily dietary dose (DDD) from consumption of arthropods
= FIR/bw (0.26) x mean RUD (14.3) x dose (2.4 kg /ha) x 21 d TWA factor (0.53)
= 4.7293 mg asulam /kg bw /day

Therefore total DDD (or exposure)
= 0.8856 (crop leaves) + 6.6475 (weed seeds) + 4.7293 (arthropods)
= 12.2624 mg asulam /kg bw /day

On this basis the refined long-term TER calculation for the small omnivorous bird feeding in treated spinach and flower bulb crops becomes:

Refined long-term TER = NOAEL / refined total DDD = 65 / 12.2624 = 5.3

The long-term TER of 5.3 is above the trigger value of 5, indicating <u>a low and acceptable risk from long-term exposure to small omnivorous birds feeding in spinach and flower bulb crops</u>, <u>following post-crop emergence use of asulam.</u>

Additional, the RMS considers that further re-assurance of the 'low' risk can be obtained from consideration of the conservatism of the exposure estimate (DDD) in the refined risk assessment - which assumes that all of the diet of small omnivorous birds over the required time period for long-term effects (assumed to be 21 days in the absence of other evidence) is obtained by feeding only in treated spinach or flower bulb crops (i.e PT = 1.0) - which is considered unlikely. For example, values of PT of < 1 for the small omnivorous skylark foraging in vegetable row crops are supported by the results UK Government radiotracking field studies (ref. UK ACP paper SC11419 March 2009, later summarised in Prosser 2010) with a 90th percentile 'consumer' PT = 0.84 (95% confidence limits of 0.68-0.96) for vegetable row crops - based on radio-tracking data for 17 individuals foraging post-crop emergence in sugar beet crops and one individual foraging in potato crops. Also, the results of the Notifier's similar Austrian PT field study (Wolf C 2005) – summarised in Section B.9.1.4.1 of this report -indicates a 90th percentile 'potential consumer' PT = 0.6, based on radio-tracking data for a total of 9 skylarks foraging in seedling beet fields.

iii) Refined long-term risk assessment for small insectivorous birds: post-crop emergence use in spinach and flower bulbs ('Tier 1' long-term TER = 4.5 for both crop uses):

a) Small insectivorous bird refined long-term risk assessment for post-crop emergence use of asulam in spinach crops:

The Notifier has proposed to refine the risk assessment based on the use of the insectivorous yellow wagtail as focal species (which is acceptable to the RMS). The Notifier argument is presented below in italics, followed by the RMS's evaluation:

NOTIFIERS PROPOSED REFINEMENT TO THE RISK ASSESSMENT FOR INSECTIVOROUS BIRDS FROM POST-EMERGENCE USE IN SPINACH:

Yellow wagtails are exclusively insectivorous searching for invertebrates mainly on the ground with short or no vegetation (Cramp et al., 1998; Glutz von Blotzheim & Bauer, 1985b). In a comprehensive study in the state of Brandenburg, Eastern Germany, the foraging technique of yellow wagtails in agricultural landscape was studied. According to the results of this study, the most common foraging technique was picking from the soil while running on the ground. Capturing prey from a perch or collecting arthropods from vegetation were of minor importance only (Stiebel, 1997). This was corroborated by a study on the prey selection and foraging behaviour of pied and yellow wagtails in Britain (Davies, 1977). This author distinguished three types of foraging techniques:

1. Picking (84%): The bird walks and picks up prey items from the ground surface.

2. Run-picking (9%): The wagtails make quick darting runs at a prey item and pick it up, either from the ground or when it takes off.

3. Fly-catching (7%): The birds make a short sally up off the ground and catch prey mid-air.

*These main foraging techniques were also reported from a study on yellow wagtails in Sweden (Källander, 1992). All these studies suggest ground-dwelling arthropods as the main food source of yellow wagtails. As only a small amount of foliage is available on the treated field (BBCH 12-14), it is a conservative approach to assume that the portion of ground-dwelling and foliage dwelling arthropods in the diet of yellow wagtails is 90% (**PD = 0.90**) and 10% (**PD = 0.10**) respectively. On this basis the long-term risk assessment becomes:*

	Leafy vegetables (spinach) *BBCH 12-14* *Small insectivorous birds "wagtail"* *Yellow wagtail (Motacilla flava)*	
Diet	*Ground-dwelling invertebrates*	*Foliage-dwelling invertebrates*
FIR/b.w. (g/kg)	*0.79 #*	*0.79 #*
Application rate (kg asulam */ha)*	*2.4*	*2.4*
MAF	*1.0*	*1.0*
TWA	*0.53 #*	*0.53 #*
RUD (mg a.s./kg)	*7.5 #*	*21 #*
PT	*1.0*	*1.0*
PD	*0.9*	*0.1*
DDD (mg/kg b.w./d)	*6.78*	*2.11*

DDD Sum (mg/kg b.w./d)	*8.89*
NOAEL	*65*

 # Values taken from EFSA Birds and Mammals guidance document (2009)

On this basis, the long-term TER becomes 65 / 8.89 = 7.3, indicating an acceptable risk.

This is still a realistic worst-case, as it assumes that PT, the proportion of time spent in the treated area is 1.0, which for a long-term risk assessment covering an exposure period of several weeks to months is unrealistically severe. Thus, off-crop habitat plays a key role as a foraging habitat for wagtails (Dittberner & Dittberner 1984, Stiebel 1997). Wagtails displayed a preference for grassland, meadows (by following livestock), farm tracks, field paths, margins of puddles and ponds, dunghills and other areas of scant vegetation as foraging habitats (overviews in Cramp et al. (1998), Glutz & Bauer (1985b), but see also Davies (1977) or Bradbury & Bradter (2004)). Interestingly, the observed birds avoided most crop fields as foraging habitats. It therefore seems most likely that if in agricultural landscapes, wagtails prefer non-cropped areas as foraging habitat. As such, it can be expected that yellow wagtails will not obtain all their food from a single treated field i.e. PT would be less than 1 and so the margin of safety would be even greater.

RMS (CRD) Comments on Notifier's proposed refined risk assessment for insectivorous birds:

In addition to the studies referred to be the Notifier, the RMS has also considered details included in a literature review by Buxton et al (1998) and in a research study paper by Gilroy J *et al* (2009).

Gilroy *et al* (2009) observed the foraging behaviour of yellow wagtails in six areas of arable farmland in Lincolnshire and Cambridge, UK, totalling 33 km^2 on 14 different farms with 80% cropped land, including autumn sown cereals, oilseed rape, peas, potatoes, sugar beet, field beans and set aside. With respect to foraging habitat preferences, the paper includes the following conclusions which was supported by the presented observational data:

> 'Earlier nests (June) showed strong preference for foraging either close to ditches or along tracks and roads. Early nesters also used wheat (the crop in which 90% of nests were located in June) in approximate proportion to its availability, as well as potato and bean crops. Set aside, oilseed rape and sugar beet were strongly avoided by foragers. Later nesters (July) showed a switch to foraging in potato crops (the crop in which all late nests were located), while wheat crops went from being favoured for foraging to being strongly avoided. Tracks, roads and ditches remained strongly favoured, while oilseed rape was also used more than expected, in contrast to the avoidance shown in earlier nests. Beans, peas, sugar beet and set-aside were all strongly avoided in July.'

This summary, is largely in agreement with the Notifier's own literature review and supports the Notifier's assessment that off-crop habitats in arable land - such as tracks and ditches - play an important role as a foraging habitat for wagtails. However, foraging in some arable crops was also important, with the extent of this in specific crops being strongly influenced by the chosen nesting habitat – which on the arable land surveyed was predominately (in addition to tracks and ditches) autumn sown wheat in June and potato crops in July.

Based on published information reviewed by the RMS in Buxton et al (1998), Davies N B (1977), and Gilroy J et al (2009), together with the additional information provided by the Notifier, the RMS concludes that the yellow wagtail is an opportunistic feeder and as such the relative proportion of ground-dwellers to foliar dwellers in their diet is likely to be vary considerably, being influenced by their relative levels of abundance, their proximity to the nesting area and the amount of energy / time required to catch and consume them.

EFSA (2009) guidance includes the yellow wagtail as a 'representative species' for 'small insectivorous birds' foraging early post-crop emergence (BBCH 10-19) in 'leafy vegetables' – which provides some support for the selection of this species as a suitable worse case species for use in a 'small insectivore' avian risk assessment. The main foraging behaviours of the species relates to picking arthropods off surfaces (usually when walking on the ground) or by catching them from the air while flying – which could include airborne foliar dwellers. However, there is no specific evidence in relation to the relative proportions of ground-dwelling and foliar dwelling arthropods consumed to support the Notifier's dietary consumption ratio of 90% ground dwellers to 10% foliar dwellers which is used in their refined risk assessment. EFSA (2009) guidance assumes 50% ground dwelling arthropods and 50% foliar arthropods are consumed by 'small insectivorous birds' in their 'Tier 1' risk assessment and it is the RMS's view, that in the absence of specific evidence and taking into account the likely large amount of variability in this ratio due to opportunistic feeding, that the refined risk assessment should be amended to assume equivalent levels of consumption of ground and foliar dwellers – as per EFSA 'Tier 1' guidance. On this basis the long-term risk assessment becomes:

Leafy vegetables (spinach)		
BBCH 12-14		
Small insectivorous birds "wagtail"		
Yellow wagtail (Motacilla flava)		
Diet	Ground-dwelling invertebrates	Foliage-dwelling invertebrates
FIR/b.w. (g/kg)	0.79 #	0.79 #
Application rate (kg asulam /ha)	2.4	2.4
MAF	1.0	1.0
TWA	0.53 #	0.53 #
RUD (mg a.s./kg)	7.5 #	21 #
PT	1.0	1.0
PD	0.5	0.5
DDD (mg/kg b.w./d)	3.7683	12.0204
DDD Sum (mg/kg b.w./d)	15.7887	
Long-term TER	NOAEL /DDD = 65/15.7887 = 4.1	

Values taken from EFSA Birds and Mammals guidance document (2009)

The RMS's (amended) refined risk assessment for the post-emergence use of asulam in spinach crops indicates a long-term TER of 4.1 for small insectivorous birds, which remains in breach of the Uniform Principle trigger value of 5 – suggesting a potential long-term risk. However, in additional to being quite conservative in its selection of PD values for ground dwelling and foliar dwelling arthropods (details above), a PT value of 1.0 is assumed (i.e. foraging birds obtaining their entire diet from treated spinach fields) - which is not considered likely or realistic over the entire 21 day duration of exposure assumed as required (in the absence of data to the contrary) in the long-term risk assessment. This is supported by the published evidence on the foraging behaviour of the yellow wagtail as focal species, which indicates, even in predominantly arable cropped landscapes, that non-cropped areas (e.g. tracks and ditches) are important foraging areas for yellow wagtails. Also, the estimated level of exposure to asulam residues over the 21 day period deemed as required for long-term effects assumes a degradation rate on exposed insects equivalent to a DT50 of 10 days with a 21 day TWA factor = 0.53 (as per EFSA 2009 guidance), which given the known rapid degradation of asulam residues on foliage (DT50 = 1.44 days) is likely to be conservative.

Taking into account the conservative assumptions made when estimating asulam exposure levels in relation to included PT, PD and the 21 day TWA factor values, it is considered by the RMS that the overall estimated exposure level of 15.8 mg /kg bw /day is likely to be over-estimated by more than the 20% reduction required in order for the long-term TER not to exceed the Uniform Principles trigger value of 5. Therefore, taking into account the overall conservatism of exposure assessment, a long-term TER of >5 can be concluded – indicating the proposed use in spinach crops poses a low long-term (reproductive) risk from asulam exposure to small insectivorous birds.

Risk assessment conclusion: Taking into account the conservatism of the above refined focal species exposure assessment, it can be concluded that the Uniform Principles long-term TER trigger value of 5 is not likely to be breached from the proposed use of asulam in spinach crops. On this basis, the long-term reproductive risk to small insectivorous birds is low and therefore acceptable.

b) **Small insectivorous bird refined long-term risk assessment for post-crop emergence use of asulam in flower bulb crops**:

The 'Tier 1' risk assessment indicates a long-term TER of 4.5 – which is only marginally below the Uniform Principles trigger value of 5. As for post-emergence use in leafy vegetable crops (including spinach), the EFSA (2009) Tier 1 risk assessment includes the yellow wagtail as a 'representative species' for 'small insectivorous birds' foraging post-crop emergence in 'bulbs and onion like crops' (BBCH 10-19 and BBCH ≥ 20) – this supporting the appropriateness of the selection of this species as a suitable worse case species for use in a 'small insectivore' avian risk assessment. The reviewed published evidence also indicates its occurrence as a forager in crops which are sufficiently open to allow it to land and forage from the ground surface –as would be the case for the proposed use in flower bulb crops to control weeds at pre and early post-emergence growth stages.

Given the identical applied dose of asulam in flower bulb crops as that in spinach crops (i.e. one application at 2.4 kg asulam /ha) and that it is considered that the same refinements to the yellow wagtail risk assessment as that used in spinach crops also

applies to use in flower bulb crops, the results of the refined risk assessment for treated spinach crops (long-term TER > 5.0) are also applicable to treated flower bulb crops.

Risk assessment conclusion: Based on a refined long-term TER of >5, an acceptable long-term risk to small insectivorous birds foraging post-crop emergence in treated flower bulb crops can be concluded.

iv) Refined risk assessment for small granivorous birds: pre-crop emergence use in spinach ('Tier 1' long-term TER = 4.5), post-emergence use in spinach ('Tier 1' long-term TER = 4.1) and post-emergence use in flower bulbs ('Tier 1' long-term TER = 4.5):

The Notifier has conducted a refined risk assessment using the yellowhammer (*Emberiza citrinella*) as a granivorous focal species on the basis that it is a common farmland bird and is widespread in the countryside (Glutz von Blotzheim & Bauer, 1997). However, dietary data reviewed by the Notifier, and also other data previously considered by the RMS, indicate that in late spring when asulam will be used to control weeds in spinach and flower bulb crops, the yellowhammer will be breeding and consuming a large proportion of arthropods in its diet. For example Buxton *et al* (1998) describes the yellowhammer's diet as 'mostly grass seeds, invertebrates becoming important in summer' – forming up to 80% by volume of the diet. Based on published dietary data reviewed by the Notifier, PD values for the yellowhammer of 65% arthropods and 35% seeds are proposed for use in the risk assessment, with these values stated to 'reflect a conservative assumption regarding the proportion of seeds in the diet of yellowhammers during the spring/summer sowing period'. The RMS agrees with these PD estimates. However, the RMS considers that with such a low estimated level of consumption of seed in the diet (i.e. 35% fresh weight of diet) at the proposed time of pesticide use in spring and early summer, the yellowhammer does not constitute a suitable 'worst case' focal species for inclusion in a granivorous bird refined risk assessment.

The seed eating linnet (*Carduelis cannabina*) is specified in EFSA (2009) guidance as a 'representative' first tier granivorous 'generic focal species' for 'bulbs and onion like crops' with a diet consisting of 100% 'small seeds'. As such, this species is considered by the RMS as a more appropriate 'worst case' granivorous focal species for this crop than the Notifier's suggested use of the yellowhammer – which at the time of treatment in spring and early summer consumes a mixture of invertebrates and seed, with seed forming only 35% fresh weight of its diet.

Although the serin (*Serinus serinus*) is included in EFSA (2009) guidance as a 'representative' first tier granivorous 'generic focal species' for 'vegetable crops' (feeding on 'small seeds'), published data (e.g. Prosser P 2010) indicate that linnets also forage on seeds in vegetable crops and therefore this species (for which PT data are available) is also considered by the RMS to be another suitable 'representative' focal species for inclusion in a higher tier risk assessment for granivorous birds feeding in spinach crop. However, the RMS notes that due to the serin's slightly smaller body size (i.e. 11.2 g compared with 15.3 g for the linnet) it has a slightly higher estimated FIR/bw than the linnet (i.e. 0.31 compared with 0.28). This slightly greater potential FIR /bw (i.e. 10% higher), and therefore slight greater potential risk from asulam residues on weed seed, needs to be taken account of when considering the acceptability of the risk to granivorous birds foraging in treated spinach crops based on a derived 'higher tier' long-term TER for the linnet.

Refined long-term risk assessment for the seed-eating linnet (focal species) foraging pre or post-crop emergence in spinach crops and post-crop emergence in flower bulbs:

Assuming that a 100% of the diet consists of small seeds (i.e. PD = 1), a food intake rate per unit body weight (FIR/BW) of 0.28 is specified for the linnet in EFSA guidance (estimated based on the dietary energy content and the bird's energy requirements) and this value has been used in the RMS's focal species refined risk assessment.

Levels of asulam residues on small seeds consumed by linnets foraging in treated spinach and flower bulb fields have been estimated based on the EFSA (2009) 'Tier 1' generic mean 'residue per unit dose' (RUD) of 40.2, which assumes full spray interception by seed and as such represents the worse case situation. In the absence of specific residue decline data for seed, a generic 'Tier 1' DT50 of 10 days has been assumed, together with its associated 21 day time weighted average factor (F_{twa}) of 0.53.

The proportion of the daily diet obtained in treated fields (PT) for the proposed crop uses in spinach and flower bulbs has been derived based on the results of UK Government sponsored research (ref. UK's ACP paper SC11419 dated March 2009, summarised with additional related work in Prosser 2010) – using a 90th percentile PT value for the linnet of 0.59 (95% confidence intervals of 0.38-0.84) obtained from radio-tracking foraging studies conducted post-emergence in sugar beet and potato row crops with a total of 11 foraging 'consumer' birds. Although the PT data for the seed-eating linnet was obtained from foraging studies in emerged sugar beet and potato crops and the proposed uses relate to spinach and flower bulb crops, all are grown as row crops, with seed-eating birds feeding between the rows on seed produced by weeds growing within the crop or on seed present within the soil. Given that neither spinach or flower bulb crops will produce seed that would be consumed by small seed-eating birds, these row crops will not be any more attractive to linnets as a food source than sugar beet and potato crops and on this basis the PT data for linnets foraging in sugar beet and potato crops may be extrapolated to spinach and flower bulb crops. Also, given that there is likely to be an absence of seed bearing plants /weeds when asulam is applied pre-crop emergence, such crops are not considered likely to be significantly more attractive to seed-eating linnets foraging slightly later at early post-crop emergence crop growth stages (BBCH10-14) in spinach crops and therefore the same PT values may be assumed.

In line with EFSA (2009) guidance, using the above concluded exposure related inputs, the daily dietary dose for linnets foraging in spinach and flower bulb crops has been calculated as follows:

Daily dietary dose = FIR /bw x mean RUD x dose (kg /ha) x 21 day F_{twa} x PD x PT
= 0.28 x 40.2 x 2.4 x 0.53 x 1 x 0.59
= 8.4474 mg asulam /kg bw /day

Long-term TER = NOAEL / DDD = 65 / 8.4474 = 7.7.

Therefore, the refined risk assessment based on the linnet as focal species indicates a long-term TER of 7.7 which compares with a Uniform Principles trigger value of 5. Although the linnet has a slightly lower (10% less) food intake rate /unit body weight than the serin (included as a first tier representative 'generic focal species' in EFSA

guidance), the margins of safety indicated by the long-term (reproductive) TER of 7.7 for the linnet are considered by the RMS as sufficient to conclude a low and acceptable risk to the serin and other species of seed-eating birds. Also, taking into account the reported relatively rapid degradation of asulam on foliage (DT50 = 1.44 days), degradation rates of asulam on seeds are likely to be faster than the 10 days assumed in the above exposure assessment, indicating that the conducted risk assessment is likely to be conservative (with actual margins of safety being greater than that suggested by the estimated long-term TER).

v) Refined long-term dietary risk assessment conclusions:

The refined long-term risk assessments indicate the proposed use of asulam in spinach and flower bulb crops poses an acceptable long-term risk to medium herbivorous birds (required for only spinach – refined long-term TER = 7.4), small omnivorous birds (both uses – refined long-term TER = 5.3), small insectivorous birds (both uses - refined long-term TER > 5.0) and small granivorous birds (both uses - refined long-term TER = 7.7).

The refined risk assessments satisfactorily address possible potential risks to foraging birds identified in the 'first tier' risk assessment. Therefore, a low and acceptable risk to birds from the dietary route of exposure may be concluded.

B.9.1.5.8 Risk to birds from exposure to asulam via contaminated drinking water

There are two scenarios provided in the EFSA Guidance Document for assessing the risk from drinking water:

Leaf scenario

The 'Leaf scenario' is relevant for birds taking water that is collected in leaf whorls after application and applies to leafy vegetables forming heads or with a morphology that facilitates collection of rain/irrigation water sufficiently to attract birds. Since the proposed uses of 'Asulox' (pre- and early post-crop emergence use in spinach and post-crop emergence use in flower bulb crops) do not fall into these categories, the leaf scenario does not apply.

Puddle scenario

This is relevant for birds taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This is relevant for all uses of 'Asulox' and should therefore be assessed.

According to Section 5.5 of EFSA (2009) guidance, due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of the effective application rate (in g/ha) to the acute and long-term relevant endpoints (in mg/kg bw/d) does not exceed 50 for 'less sorptive substances' with a Koc of <500 L/kg (which is the case for asulam – Koc = 25.4, ref. Section B.8.6 of current Volume 3 DAR). Therefore an initial risk assessment has been conducted on this basis:

Drinking water avian acute risk assessment:

Application rate (g asulam per ha) / acute LD50 (mg/kg bw) = 2400/>1826.5 = < 1.3

Drinking water avian long-term risk assessment:

Applic. rate (g asulam per ha) / long-term NOAEL (mg/kg bw /day) = 2400/65 = 36.9

Both of these ratios are below the risk ratio trigger value of 50, indicating a low and acceptable risk to birds from potential exposure to asulam via contaminated drinking water.

B.9.1.5.9 Risk to birds from potential secondary poisoning via bio-accumulation in fish and earthworms

The log octanol water partition co-efficient of asulam (log Pow) is 0.15 which is below the trigger value of log 3.0 for which there is considered the potential for bio-accumulation. Also, there are no major soil or water metabolites with a log Pow which breach this trigger value. It is concluded that there is a lack of potential for bioaccumulation and therefore no further assessment in relation to the risk to fish-eating and earthworm-eating birds is required.

B.9.1.5.10 Risk to birds of endocrine effects

Mammalian toxicity studies conducted with asulam, including two generation and teratogenicity evaluations, produced no evidence of endocrine disrupting potential. However, no avian studies providing evidence in relation to the potential for endocrine effects in birds have been provided and therefore no conclusions can be drawn in relation to this.

Member States should note that there are currently no defined criteria for identifying avian endocrine disruptors under 1107/2009 and because of this a full regulatory assessment of asulam's endocrine disrupting properties cannot be made at this time.

B.9.1.5.11 Overall avian risk assessment conclusions

The risk assessment indicates that the proposed crop uses of asulam in spinach and flower bulb crops pose a low and acceptable risk to birds from exposure via their diet or from drinking water. There is a lack of potential for bioaccumulation in fish and earthworms (log Pow < 3.0) and therefore a low risk of secondary poisoning to fish-eating and earthworm-eating birds. Given the current absence of defined criteria for identifying avian endocrine disruptors under 1107/2009, a regulatory assessment of asulam's endocrine disrupting properties cannot be made at this time.

B.9.2 Effects on aquatic organisms (IIA 8.2, IIIA 10.2)

Aquatic life toxicity studies were conducted with the active substance asulam sodium and its metabolite sulphanilamide, for which studies summaries are presented below. Given that the representative formulation 'Asulox' contains only additional water, specific formulation studies have not been conducted – it being possible to calculate its toxicity based on that for the active substance.

B.9.2.1 Acute and chronic toxicity to fish and bioconcentration potential

B.9.2.1.1 Acute toxicity of the active substance to fish

i) Report reference: [redacted] (1988a) Acute toxicity of asulam technical to rainbow trout (*Salmo gairdneri*) under static conditions. [redacted]

This study has previously been evaluated at EU level.

Guidelines: USEPA (= EPA) 72-1
Deviations: None which affected the study results.

GLP: Yes

Executive Summary:

The acute (96-hour) LC_{50} values for rainbow trout (*Oncorhynchus mykiss* formerly *Salmo gairdneri*) with asulam sodium was determined to be >175 mg/L (equivalent to 159.8 mg asulam/L). The no-observed-effect-concentration (NOEC) was 175 mg/L, based on the lack of mortality at this concentration.

Materials and Methods

Test Material:

Asulam sodium salt, purity 88% Batch no.: XN-36111

Test Design:

Test organisms were 120-days old rainbow trout (*Salmo gairdneri*) of 0.42 ± 0.11 g wet weight and 9.9 ± 3.6 mm length. Groups of 10 fish were exposed to a control and 5 nominal concentrations of 26, 43, 72, 120 and 200 mg/L over a period of 96 hours under static conditions. The test vessels were 19 L glass jars containing 15 L of dilution water or test solution. Dilution water was well water having a pH of 8.1, a hardness of 250 mg/L as $CaCO_3$, an alkalinity of 223 mg/L as $CaCO_3$ and a specific conductivity of 360 µmhos/cm at 11°C. Treatment concentrations were prepared by direct addition of appropriate amount of asulam to 15 L of dilution water in each of the 5 jars and stirring until dissolution. The fish loading was 0.28 g/L. Fish were not fed during the exposure period.

38 mL samples were taken from control and each of the test concentrations at test initiation and test termination for determination of actual asulam concentrations.

Results and Discussion:

Throughout the study, the temperature ranged from 11 to 13°C, the dissolved oxygen concentration was ≥7.2 mg/L (≥69% of saturation) and the pH value ranged from 7.8 to 8.3.

During the test, the measured concentrations ranged from 77 to 88% of nominal test concentrations and were reported as mean measured values: 20, 37.5, 61, 100, 175 mg/L.

No mortality was observed at any of the test concentrations.

Conclusions:

Based on measured concentrations, the 96-hour LC_{50} was determined to be greater than 175 mg asulam sodium /L (equivalent to 159.8 mg asulam/L), the highest dose tested. The no-observed-effect-concentration (NOEC) was 175 mg asulam sodium /L, based on the lack of mortality at this concentration.

RMS comment:

This study has previously been evaluated at EU level. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level.

ii) Report: [redacted] (2000) Asulam sodium salt - acute toxicity to bluegill sunfish (*Lepomis macrochirus*) under static-renewal conditions. [redacted]

This study has previously been evaluated at EU level.

Guidelines:

OECD 203, 1992
Deviations: None which affected the study results

GLP:

Yes

Executive Summary:

Under the static renewal conditions used the 96-hour LC_{50} for asulam sodium to the bluegill sunfish *Lepomis macrochirus* was empirically estimated to be greater than 100 mg/L (equivalent to 91.3 mg asulam/L), the highest mean measured concentration tested. The NOEC was established to be 100 mg/L.

Materials and Methods

Test Material:

Asulam sodium salt, purity: equivalent to 814 g/kg asulam, Batch No.: OP980398

Test Design:

Bluegill sunfish (*Lepomis macrochirus*) with a mean wet weight of 0.65 g and a mean total length of 36 mm were used for the test. Based on the results of a preliminary testing, groups of 10 fish were exposed to nominal concentrations of 48, 58, 69,83 and 100 mg as/L. and a control, under static-renewal conditions (48h) over a period of 96 hours. The test was performed in 19.5 L glass aquaria, each containing 15 L of test solution or dilution water (control). A 15 mg/mL stock solution was prepared by dissolving appropriate amounts of asulam by deionised water. Appropriate addition of this 15 mg/mL stock solution to dilution water (total volume of 15 L) was made in order to obtain the above nominal test concentrations. Exposure solutions were renewed at 48 hours of exposure. The biological loading was 0.43 g/L/day.

Dilution water was well water of pH 7.1-7.2, specific conductivity of 150-160 µS/cm, total hardness of 32-36 mg/L as $CaCO_3$ and total alkalinity of 24-30 mg/L as $CaCO_3$.

The test was conducted at a temperature of 20-22 °C.

Mortality and sublethal effects (e.g. erratic swimming behaviour, lethargy) were recorded after 24, 48, 72 and 96 hours of exposure. At the same time, physical characteristics of test solutions (e.g. presence of precipitate, film on the solution's surface) were monitored. Temperature, pH and dissolved oxygen concentration were also recorded.

Water samples were taken at 0 and 48 from freshly prepared test solutions and at 96 hours from aged exposure solutions for analysis of asulam concentration via a HPLC method.

Results and Discussion:

During the study, the physical/chemical parameters were recorded as follows: pH range: 6.6-8.2; dissolved oxygen range: 4.4-8.2 mg/L (48-94% saturation); temperature range: 20-22°C.

Measured concentrations of asulam in newly prepared solutions at 0 and 48 hours and of the aged exposure solutions (96-hours) ranged from 100 to 110% of the nominal concentrations.

Measured concentrations were defined as 51, 61, 73, 90 and 100 mg asulam sodium /L.

Following 96 hours of exposure, no mortality or adverse effects were observed among organisms exposed to any treatment level tested or the control.

Conclusions:

Under the static renewal conditions used the 96-hour LC_{50} for asulam sodium to *Lepomis macrochirus* was empirically estimated to be greater than 100 mg asulam sodium /L (equivalent to 91.3 mg asulam/L), the highest mean measured concentration tested. The NOEC was established to be 100 mg asulam sodium /L.

RMS comment:

This study has previously been evaluated at EU level. The conclusions presented are consistent with the EFSA Conclusion report (2010) and no concerns were raised at EU level.

B.9.2.1.2 Acute toxicity of relevant metabolites to fish

No metabolite toxicity studies with fish have been reported.

B.9.2.1.3 Chronic toxicity of active substance to fish

i) Report: [redacted] (1997a) Asulam – fish [*Oncorhynchus mykiss*] juvenile growth test - 28 days - under flow-through conditions. [redacted]

This study has previously been evaluated at EU level.

Guidelines:

OECD draft document, proposal for fish growth test 28 days, 1994.

Deviations: None which affected the study results.

GLP: Yes

Executive Summary:

In a 28-day flow through juvenile growth test with rainbow trout (*Oncorhynchus mykiss*), the 28-day EC_{20} for asulam was estimated to be greater than 119.1 mg asulam /L. Based on the EC_{20} and the biological observations of sub-lethal toxic effects the NOEC of asulam through 28 days under the test conditions was reported to be 119.1 mg asulam /L, the highest dose tested.

Materials and Methods

Test Material:

Asulam, purity 806 g/kg, Batch no.: PN: 24004

Test Design:

A total of 96 rainbow trout (*Oncorhynchus mykiss*) (1 replicate of 16 fish per test concentration) were exposed to 5 nominal concentrations of asulam (4.7, 10.3, 22.7, 50 and 110 mg asulam /L) and a control under flow-through conditions. Six exposure aquaria (57 L clear glass containers) were used as test vessels. The flow-through test system provided approximately 7.1-7.3 (40 L) test solution renewals per 24 hours throughout the 28-day test period. The test was performed in a temperature-controlled room, where the water temperature was maintained within the range of 12.5 - 16°C. The photoperiod was 16 hours light and 8 hours darkness. The fish were fed during the test period.

Water temperature, pH values and dissolved oxygen concentrations were measured in each test aquaria on each working day except day 23. In addition, the water temperature in one test aquarium was continuously recorded throughout the test period. Water hardness was measured in the control and highest test concentration aquaria at test initiation and test termination. pH of the dilution water before its arrival to the test aquaria was also recorded at test initiation and once a week thereafter.

Weights of fish were determined at test initiation and also on test days 14 and 28. Every working day abnormal behaviour or appearance of fish was recorded. Mortalities were also recorded in the same time.

Samples of each test concentration and control were analysed at least three times during the first week of testing and once a week thereafter for determination of asulam concentrations. Chemical analyses were performed using a HPLC method (limit of quantification: 0.5 mg/L).

Results and Discussion:

Test conditions (minimum-maximum) during the exposure period were: pH values in test solutions ranged from 7.0 to 8.0, dissolved oxygen ranged from 8.1 to 9.5 mg O_2/L and temperature from 14.0 to 14.5°C.

Chemical analyses revealed that mean measured test concentrations were found to be between 100 and 113% of nominal concentrations at test initiation and between 98 and 121% during the test period. The results were expressed in term of measured concentrations, which were reported as follows: 4.9, 10.8, 23.5, 52.3 and 119.1 mg asulam /L.

The biological results of the study are summarised in the Table B.9.2.1.

Table B.9.2.1 Chronic toxicity of asulam sodium to rainbow trout

Mean measured concentrations (mg/L)	Total mortality	Wet weight (g) (mean (standard deviation))			Average percentage relative growth rate#
	Day 1 to 28	Day 0	Day 14	Day 28	0-28 days
Control	0	1.6 (0.12)	2.8 (0.32)	4.9 (0.55)	
4.9	0	1.5 (0.12)	2.8 (0.37)	4.9 (0.90)	0.40
10.8	0	1.5 (0.10)	2.8 (0.28)	5.0 (0.61)	3.15
23.5	0	1.5 (0.12)	2.8 (0.34)	4.9 (0.61)	0.06
52.3	0	1.5 (0.11)	2.9 (0.36)	5.0 (0.79)	3.49
119.1	0	1.5 (0.12)	2.9 (0.29)	5.1 (0.51)	4.30

RMS Note: This is referred to as the 'specific growth rate' in the current OECD 215 test guideline (2000) and relates to the averaged daily percentage increase in the logarithm of fish weight over the 28 day study period.

No mortalities and no sub-lethal toxicity were observed during the 28-day of exposure.

The percentage relative growth rate for each test concentration compared to the control was less than 20%, therefore the EC_{20} values were not calculated.

Conclusions:

The 28-day EC_{20} was estimated to be greater than 119.1 mg asulam /L. Based on the EC_{20} and the biological observations of sub-lethal toxic effects the NOEC of asulam through 28 days under the test conditions was reported to be 119.1 mg asulam /L, the highest dose tested.

RMS comment:

This study has previously been evaluated at EU level. Given the absence of mortality or sub-lethal effects in the study, the RMS agrees that the NOEC = 119.1 mg asulam /l (i.e. the maximum test dose). The conclusions presented are consistent with the EFSA Conclusion report (2010) and no concerns were raised at EU level.

B.9.2.1.4 Bioconcentration potential of the active substance in fish

i) Report: [redacted] (1981) Asulam - absorption, metabolism and elimination studies with fish. [redacted]

This study has previously been evaluated at EU level.

Guidelines:

Guideline not specified. Deviations: Not specified

GLP: No

Executive Summary:

A bioconcentration test was conducted on catfish (*Ameirus melas*) using radiolabelled asulam. The exposure phase lasted for 28 days and there was a 15 day depuration phase. Bioconcentration factors varied from 0.1 to 1.4 but the majority of the results were below 1.0, indicating that there was no concentration of residues within the fish. The CT_{90} (clearance time for 90% reduction of the active) was less than 7 days.

Materials and Methods

Test Material:

Asulam (non radio-labelled), purity not stated, Batch no.: AGJMA3
^{14}C-asulam, radiochemical purity 98.5%, Batch no.: DWA 2572

Test Design:

Samples of loam were treated with ^{14}C-asulam to give soil concentrations of 0.01 and 1 µg/g on air-dried basis. After aerobic incubation at ambient temperature for 36 days the soils were placed in aquaria and water and catfish (*Ameirus melas*) added. Deionised water of total hardness 20 mg/L as $CaCO_3$ was used as test water. Three 175 L glass aquaria were used for the exposure phase, one for the control group, one for the 0.1 µg/g concentration group and one for the 1 mg/g concentration group. 60 fish (5-6 cm length and weighing 2-3g) were assigned to each aquarium after introduction of water. Throughout the study the fish were fed daily.

Samples of soil, water and fish were taken at day 1 (20 hours), 3, 7, 10, 14, 21 and 28 after addition of water to the aquaria, to measure the distribution of radioactivity. Then, the fish were removed into three other soil-free aquaria in order to measure the depletion of radioactivity from the fish. During the 15 days of depletion phase, two fish from each aquarium were sampled for measurements of residues in edible and non edible tissues at day 1, 3, 7 and 15 after beginning of the depletion phase.

Results and Discussion:

The majority of the radioactivity became bound to the soil during aerobic incubation, and remained bound following transfer to the aquatic systems (approximately 20% of the applied material entered the aquarium water). The amounts of the radiolabelled material taken up by the fish were very low. In the aquarium containing soil treated at 1 µg/g a maximum of ^{14}C concentration of 16 ng/g (asulam equivalent) was found in fish tissues but variations in uptake between individual fish were quite large. In the aquarium treated at 0.01 µg/g uptake was so low that all results were below, or only slightly above, the detection limit of 0.1 ng/g (asulam equivalent). Bioconcentration factors varied from 0.1 to 1.4 but the majority of the results were below 1.0, indicating that there was no concentration of residues within the fish. After transfer of the fish to soil-free aquaria rapid depletion of radioactive residues in fish occurred and most assay results were close to, or below, the detection limit within 7 days.

Samples of soil, water and fish from the higher treatment rate system were analysed after 28 days exposure of the fish to the soil. The soil contained sulfanilamide, methyl benzene-sulfonylcarbamate and benzenesulfonamide as well as asulam. Aquarium water contained methyl benzenesulfonylcarbamate, asulam and also sulfanilic acid and benzenesulfonic acid. Because of the very low levels within the fish only tentative identification of the metabolites was possible, but the results indicated the presence of methyl benzene-sulfonylcarbamate and benzenesulfonamide in the fish.

Conclusions:

Bioconcentration factors varied from 0.1 to 1.4 but the majority of the results were below 1.0, indicating that there was no concentration of residues within the fish.

RMS comment.

This study was previously evaluated at EU level, with the 'List of Endpoints' including a bioconcentration factor of 0.1-1.4 whole fish based on the results of this study. However, this early study, conducted in 1981 prior to the requirement for GLP compliance, has not been conducted in line with the standard OECD 305 (2012) fish bioconcentration test guideline - with in particular fish not being exposed via a constant level of exposure of active substance in the test water (as in the standard study) but via the presence of asulam and its metabolites in treated sediment /soil. Therefore, the route of exposure is totally different from that in the standard OECD 305 study (i.e. via sediment and not water) and the derived endpoints (bioconcentration factor of 0.1-1.4) need to make clear that they relate to a comparison of the concentration in whole fish to that in sediment and not water - which is usually the case).

B.9.2.2 Acute and chronic toxicity to aquatic invertebrates

B.9.2.2.1 Acute toxicity of active substance to aquatic invertebrates

i) **Report: C.S. Manning (1988b) Asulam technical acute toxicity to the water flea (*Daphnia magna*) under flow-through conditions. United Phosphorus Limited, Unpublished report No.: R001268, CA 8.3.1.1/01.**

This study has previously been evaluated at EU level.

Guidelines:

USEPA (= EPA) 72-2. Deviations: None which affected the study results

GLP: Yes

Executive Summary:

The acute (48-hour) EC_{50} value for *Daphnia magna* with asulam sodium was determined to be 63.4 mg/L (95% confidence limits: 51.0-89.8 mg/L), equivalent to 57.87 mg asulam/L. The no-observed-effect-concentration (NOEC) was 25.5 mg/L, based on immobilisation at 48.5 and 83 mg/L.

Materials and Methods

Test Material:

Asulam sodium salt, purity 88%, Batch no.: XN-36111

Test Design:

Daphnia magna, less than 24-hours old were used as test organisms. 20 Daphnids (2 replicate of 10 per concentration) were exposed to nominal concentrations of 0 (water control), 14, 24, 40, 66 and 110 mg asulam/L for a period of 48 hours under flow-through conditions (approximately 6.4 daily volume turnover). The test was performed under a photoperiod of 16-hour light and 8-hour dark.

The dilution water used for the study was well water with pH of 7.7, conductivity of 450 µmhos/cm at 21°C, and a hardness and alkalinity of 235 mg/L and 247 mg/L as $CaCO_3$, respectively.

Observations of dead animals were made at 24 and 48 hour of exposure.

One water sample was collected from each control and test solution at test initiation and termination to monitor actual exposure concentrations of asulam.

Results and Discussion:

During the test, the temperature was 20 to 21°C; dissolved oxygen concentrations were ≥8 mg/L (88% saturation) and pH values ranged from 7.6 to 7.7.

Average measured concentrations of asulam during 48-hour exposure ranged from 64 to 75% of nominal. Mean measured concentrations were 9.3, 17.5, 25.5, 48.5 and 83 mg/L.

The biological key information can be summarised in Table B.9.2.2.

Table B.9.2.2 Acute toxicity of asulam sodium to *Daphnia magna*

Concentration (mg/L) mean measured	Cumulative percent mortality after	
	24 Hour	48 Hour
-	0	0
9.3	0	0
17.5	0	0
25.5	0	0
48.5	0	45
83.0	10	55
EC_{50} (mg/L): (95% confidence limits):	≤83	63.4 (51.0-89.8)

Conclusions:

Based on mean measured concentrations, the 48-hour EC_{50} of asulam sodium in *Daphnia magna* was determined to be 63.4 mg/L (95% confidence limits: 51.0-89.8 mg/L), equivalent to 57.87 mg asulam/L. The no-observed-effect-concentration (NOEC) was 25.5 mg/L (equivalent to 23.28 mg asulam/L), based on immobilisation at 48.5 and 83 mg/L.

RMS comment:

This study has previously been evaluated at EU level. The conclusions presented are consistent with the EFSA Conclusion report (2010) and no concerns were raised at EU level.

B.9.2.2.2 Acute toxicity of relevant metabolites to aquatic invertebrates

No metabolite toxicity studies with aquatic invertebrates have been reported.

B.9.2.2.3 Chronic toxicity of active substance to aquatic invertebrates

i) Report: A. McElligott (1997b) Asulam - chronic toxicity (21-day) to Daphnids under static renewal conditions. United Phosphorus Limited, Unpublished report No.: R005639, CA 8.3.2.1/01.

This study has previously been evaluated at EU level.

Guidelines:

OECD 211, 1997 USEPA (= EPA) 72-4, 1987. Deviations: None which affected the study results.

GLP: Yes

Executive Summary:

The chronic (21-day) no-observed-effect-concentration (NOEC) for *Daphnia magna* with asulam was 6.4 mg asulam /L, based on significant reproductive effects observed at concentrations of 16.1 mg asulam /L and above.

Materials and Methods

Test Material:

Asulam, purity: 806 g/kg , Batch no.: PN: 24004

Test Design:

10 daphnids (less than 24 hours old) were exposed to each of the following nominal concentrations: 2.6, 6.4, 16.0, 40.0 and 100 mg asulam /L. A dilution water (containing 10 *Daphnia magna*) was also included in the test. There were 10 replicate of 1 daphnia per concentration level. Test vessels were 250 mL glass beakers containing 200 mL of test solution. Reconstituted water (80% DSW + 20% LC-oligo) was used as dilution test water (characteristics: mean total hardness 170 mg/L as CaCO3, mean conductivity 540 µS/cm, mean pH value 7.8 and total organic carbon <2 mg/L). The test organisms were fed three times a week according to the test renewal schedule.

The test was performed in a temperature-controlled room at 20 ± 2°C under a photoperiod of 16 hours light and 8 hours darkness. The test solutions were not aerated during the test.

Biological observations of daphnids were made at test initiation and test termination and at days 2, 5, 7, 9, 12, 14, 16 and 19 before transfer of the parent animals to fresh solutions. Biological observation included: survival of first generation daphnids in all test vessels, time at which the first offspring are produced, number of offspring (alive and dead), presence of eggs in the brood pouch, number of non hatched eggs, presence of any winter eggs (ephippia), any observations of abnormal appearance or behaviour of first and second generation daphnids. Additionally at test termination total length and dry weight of all surviving parental daphnids were measured.

Measurements of pH, temperature, total hardness and conductivity were performed in the dilution water at test initiation and at each stock solution preparation. Dissolved oxygen,

temperature and pH were measured at each media renewal, in each test level in the bulk preparations of the fresh stock solutions and from a pool of all replicate of the old test solutions. The photoperiod and light intensity were continuously recorded.

At the beginning and end of a renewal period, the concentrations of asulam in dilution water were verified (twice a week). Samples from each fresh bulk preparations of test solutions and pooled samples from each concentration level and the control were taken before media renewal of the 1st, 2nd, 6th and 9th test solutions renewals and at the end of these test solution renewals, respectively. Quantification was performed using a HPLC method (QL: 0.5 mg asulam/L).

Results and Discussion:

Test conditions (range of recorded values) recorded in the test solutions (fresh and old) during the exposure period were: temperature 20.0-21.2°C, pH 7.3-8.4, dissolved oxygen 7.6 mg/L, total hardness 163-170 mg/L as $CaCO_3$ in the control group and 165-178 mg/L as $CaCO_3$ in the highest test concentration.

Test conditions (range of recorded values) recorded in the dilution water at each media renewal were within the following ranges: pH 7.7-7.9, total hardness 160-175 mg/L as $CaCO_3$ and specific conductivity 537-562 µS/cm.

The measured concentrations of asulam in test solutions (beginning and end of renewal period) were close to the nominal (88-112% recovery and 86-117% recovery, respectively).

The biological parameters recorded are summarised in Table B.9.2.3.

Table B.9.2.3 Chronic toxicity of asulam to *Daphnia magna*

Test concentrations (mg asulam /L)		% survival at day 21	Mean number of live offspring/parent alive on day 21	Mean total length (mm)	Mean dry weight (g)
Nominal	Mean measured		Mean (standard deviation)	Mean (standard deviation)	
Control		80	138.4 (26.8)	4.25 (0.18)	1.00 (0.26)
2.6	2.6	80	146.3 (11.1)	4.26 (0.11)	1.19 (0.34)
6.4	6.4	100	147.7 (9.0)	4.35 (0.11)	1.26 (0.30)
16.0	16.1	100	102.1 (33.1) *	3.96 (0.32)	0.57 (0.21)
40.0	39.9	90	14.7 (9.2) **	3.71 (0.16)	0.52 (0.13)
100.0	99.0 (a)	0	-	-	-

(a) this concentration was excluded from the statistical analysis of the reproductive data as there is no daphnids alive
* mean value was significantly different (α =0.05) from the control (Kruskall-Wallis test followed by Mann Witney test)
** mean value was significantly different (α =0.01) from the control (Kruskall-Wallis test followed by Mann Witney test)
° mean value was significantly different (α =0.05) from the control (ANOVA and Dunnett's test)
°° mean value was significantly different (α =0.01) from the control (ANOVA and Dunnett's test)

The number of live young produced per parent daphnia alive on Day 21 of the test and the total lengths and dry weights of these daphnids were significantly reduced compared to the control group at the concentrations of 16.1 and 39.9 mg asulam /L. No significant effects of the test substance (for reproductive output, total length or dry weight) were observed at the lower measured concentrations of 2.6 and 6.4 mg asulam /L.

Conclusions:

Based on mean measured concentrations, the EC_{50} (adult survival) in *Daphnia magna* exposed to asulam was estimated to be 57.1 mg asulam /L. Based on the statistical analysis of the test data, the NOEC was found to be 6.4 mg/L.

RMS comment:

This study has previously been evaluated at EU level. The conclusions presented are consistent with the EFSA Conclusion report (2010) and no concerns were raised at EU level.

ii) Report: Herrmann, Voigt, Benz (1992) Toxicity of Asulox 80 SG to water-fleas (*Daphnia magna*) (20 days). United Phosphorus Limited, Unpublished report No.: R001808; CA 8.3.2.1/02.

This study has previously been evaluated at EU level.

Guidelines:

OECD 202 II, 1984. Deviations: None which affected the study results

GLP: Yes

Executive Summary:

The chronic (20-day) no-observed-effect-concentration (NOEC) for *Daphnia magna* with asulam was 8.96 mg/L, based on reproductive effects observed at concentrations of 22.4 mg/L and above.

Materials and Methods

Test Material:

'Asulox 80 SG', purity 80% asulam sodium, Batch no.: not stated

Test Design:

10 daphnids (less than 24 hours old) were exposed to each of the following nominal concentrations: 3.58, 8.96, 22.4, 56, 140 and 350 mg/L. A dilution water was also included in the test as control. The test was carried out under semi-static conditions.

The test was performed in a temperature-controlled room at 21 ± 1°C under a photoperiod of 16 hours light and 8 hours darkness. The test solutions were not aerated during the test. No analysis was conducted.

Results and Discussion:

The biological parameters recorded are summarised in Table B.9.2.4.

Table B.9.2.4 Chronic toxicity of asulam sodium to *Daphnia magna*

Nominal test concentrations (mg/L)	% survival at day 20 (compared to control)	% live offspring on day 20 (compared to control)
Control	100	100
3.58	102.7	66.9
8.96	100	219.7
22.4	93.7	70
56.0	46	0
140.0	0	-

Nominal test concentrations (mg/L)	% survival at day 20 (compared to control)	% live offspring on day 20 (compared to control)
350.0	0	-

The immobility of the animals in the control was about 7.5% until the end of the test. The first young animals could be found after 9 days.

Conclusions:

Based on nominal concentrations (converted to pure asulam), the 20 day EC_{50} (reproduction rate) was 21.48 mg asulam/L and the 20 day EC_{50} (immobilisation) was 45.6 mg asulam/L. The NOEC can be stated to be 8.96 mg asulam /L.

RMS comment:

This study has previously been evaluated at EU level. The conclusions presented are consistent with the EFSA Conclusion report (2010). However, the RMS notes that the test concentrations were not confirmed by chemical analysis – which is considered an important omission affecting the reliability of the derived study endpoints. This therefore needs to be made clear when reporting the study endpoints, with endpoints derived based on measured concentrations in the other more reliably McElligott (1997b) *Daphnia* chronic toxicity study being used in preference in the regulatory risk assessment.

B.9.2.2.4 Chronic toxicity of active substance to sediment dwelling invertebrates

Report: A. Heintze (2002) Assessment of side effects of asulam sodium salt on the larvae of the midge, *Chironomus riparius* with the laboratory test method. United Phosphorus Limited, Unpublished report No.: C030755; CA 8.5.2/01.

This study has previously been evaluated at EU level.

Guidelines:

OECD Draft guideline no 219 (using spiked water), 2000, BBA Guideline proposal 1995
Deviations: None which affect the study results.

GLP:

Yes.

Executive Summary:

Under the conditions of the test, and based on the nominal concentrations of the test substance, the effects of asulam sodium salt to the sediment-dwelling life stage of the midge, *Chironomus riparius* in a sediment water system are reported as follows: 28-day LOEC >100 mg/L (equivalent to >91.3 mg asulam/L) and 28-day NOEC = 100 mg/L.

Materials and Methods

Test Material:

Asulam sodium salt, purity 822 g/kg, Batch no.: OP210109.

Test Design:

The nominal test solutions were prepared by dilution of a stock solution in test medium. Test organism: *Chironomus riparius* (Diptera, Chironomidae). The test organisms were introduced into the test system as 1st instar larvae. The test substance was applied 24 hours after introduction of the test organisms. The test organisms were exposed during their subsequent larval

development for 28 days or until emergence as adults. Test system: 2 L glass beakers (12 cm in diameter); 2-3 cm sediment layer (310 g wet sediment, artificial sediment prepared according to OECD 207); 13.5 cm (1.6 L) medium column. Test concentrations and replications: during the range-finding test each test concentration level was tested with 2 replicates and 25 larvae per replicate. Control was tested with 6 replicates. The range finder was performed at 5 concentrations between 0.01 and 100 mg/L differing from each other by a factor of 10. During the main test each treatment rate and control was tested with 6 replicates and 25 larvae each. Nominal concentrations: 25, 50 and 100 mg/L. Test parameter: imaginal emergence rate (ER), larval development rate (DR) of the test organisms. Statistical analysis: The mean ER and DR values observed in the test groups were compared to the control values using statistical methods (Dunnett or pairwise U-test).

Results and Discussion:

During the range-finding test emergence and development rate were not significantly reduced at or below treatment concentrations of 100 mg/L. Therefore, the main test was conducted as a limit test at concentrations of 25, 50 and 100 mg/L. Following 28 days of exposure to the test substance there was no significant difference between the emergence rate of adult midges at any of the test concentrations and the control group. No significant differences in development rate of midges during the test period were observed between the control group and the test concentrations.

Therefore, the NOEC (28 d) was estimated to be 100 mg/L and the LOEC (28 d) to be >100 mg/L. The EC_{50} could not be determined, because the highest concentration tested was below the dose response value of 50%.

Conclusions:

Under the conditions of the test, and based on the nominal concentrations of the test substance, the effects of asulam sodium salt to the sediment-dwelling life stage of the midge, *Chironomus riparius* in a sediment water system are reported as follows: 28-day LOEC >100 mg/L (equivalent to >91.3 mg asulam/L) and 28-day NOEC = 100 mg/L.

RMS comment:

This study has previously been evaluated at EU level. The conclusions presented are consistent with the EFSA Conclusion report (2010). However, the RMS notes that the test concentrations were not confirmed by chemical analysis – which is considered an important omission affecting the reliability of the derived study endpoints. This therefore needs to be made clear when reporting the study endpoints.

B.9.2.3 Effects on algal growth and growth rate inhibition

B.9.2.3.1 Effects of active substance on algal growth and growth rate inhibition

i).a Report: J.R. Hoberg (1992a) Asulam sodium: Toxicity to the freshwater green alga, S*elenastrum capricornutum*. United Phosphorus Limited, Unpublished report No.: R003656; CA 8.4/01a.

Guidelines:

FIFRA guideline § 122-2 and 123-2, 1982. Deviations: pH ranged from 7.3-7.5 (test initiation) to 9.7-10.8 (test termination). This pH change is common in static algae cultures due to photosynthesis and respiration by the algae. This is considered to have no impact on the outcome of the study.

GLP: Yes

Executive Summary:

Based on mean measured concentrations, the 120-hour EC_{50} for S*elenastrum capricornutum* (*Pseudokirchneriella subcapitata*) with asulam sodium was calculated to be 0.19 mg/L, equivalent to 0.17 mg asulam/L, with 95% confidence limits of 0.071-0.49 mg/L. The 120-hour No Observed Effect Concentration (NOEC) was determined to be 0.020 mg/L (0.018 mg asulam/L), based on the inhibition of cell growth seen at concentrations of 0.049 mg/L and above.

Materials and Methods

Test Material:

Asulam sodium, purity 89.5%, Batch no.: EN50005

Test Design:

Test organisms were the freshwater green algae *Selenastrum capricornutum* (now named *Pseudokirchneriella subcapitata*). Alga were exposed to nominal concentrations of 0.020, 0.051, 0.13, 0.32, 0.80 and 2.0 mg asulam sodium/L over a period of 120 hours under temperature controlled conditions (room temperature of 24-25°C) and continuous shaking (100 rpm) and illumination. The initial cell density in each test level was 0.3 x 10^4 cells/mL. MBL medium (pH 7.5) was used as dilution water and as control. All treatment levels consisted of three 125 mL Erlenmeyer flasks.

The cell density was assessed in each treatment level every 24 hours. Observations of the health of the cells were also made and recorded each 24-hour interval.

Temperature was measured continuously. Conductivity and pH were measured prior to test initiation and at test termination. At test initiation and termination, a sample of each treatment level was removed for analysis of asulam concentration via a HPLC method (LOQ: 3.6 µg/L).

Results and Discussion:

The physical/chemical parameters were recorded as follows: conductivity ranged from 130-150 µS/cm, pH ranged from 7.3-7.5 at test initiation and increasing to 9.7-10.8 at test termination, temperature ranged from 24-25°C.

Analysis of asulam concentrations showed that measured concentrations averaged 94% of nominal concentrations throughout the study period. Mean measured concentrations were found to be 0.020, 0.049, 0.12, 0.29, 0.71 and 1.9 mg/L.

The key biological information is summarised in Table B.9.2.5.

Table B.9.2.5 Effects of asulam sodium on the growth of *Selenastrum capricornutum*

Concentration (mg/L)		Mean (standard deviation) cell density (x10^4 cells/mL) after				
Nominal	Mean measured	24 hours	48 hours	72 hours	96 hours	120 hours
Control	-	2 (1)	6 (2)	40 (4)	70 (17)	115 (18)
0.020	0.020	2 (1)	4 (2)	33 (4)	51 (13)	105 (10)
0.051	0.049	2 (<1)	4 (1)	10 (3)	58 (16)	88 (6) *
0.13	0.12	2 (1)	3 (1)	18 (3)	56 (26)	56 (3) *
0.32	0.29	1 (1)	2 (2)	10 (3)	47 (19)	43 (6) *
0.80	0.71	1 (<1)	3 (1)	8 (2)	37 (14)	36 (10) *

Concentration (mg/L)		Mean (standard deviation) cell density (x10^4 cells/mL) after				
Nominal	Mean measured	24 hours	48 hours	72 hours	96 hours	120 hours
2.0	1.9	1 (<1)	3 (1)	3(1)	14 (4)	15 (1) *
EC_{50} (mg/L)		0.85	0.74	0.060	0.91	0.19
95% confidence limits		0.0008-17x10^4	-	0.032-0.77	- -2.8	0.71-0.49

* significantly different ($p \leq 0.05$) when compared to control data, based on Williams' test.

Conclusions:

Based on mean measured concentrations, the 120-hour [biomass] EC_{50} was calculated to be 0.19 mg asulam sodium /L, equivalent to 0.17 mg asulam/L, with 95% confidence limits of 0.071-0.49 mg/L. The 120-hour No Observed Effect Concentration (NOEC) was determined to be 0.020 mg/L (0.018 mg asulam/L).

RMS comment:

This study has previously been evaluated at EU level (details included in Volume 3 B.9. DAR dated March 2006). The conclusions presented are consistent with the EFSA Conclusion report (2010) and no concerns were raised at EU level. It is considered scientifically valid and suitable for consideration in the regulatory risk assessment.

In line with the current OECD 201 test guideline and also current regulatory requirements, in addition to the calculated biomass EbC50, a growth rate ErC50 is required to be calculated. The RMS notes that the most sensitive derived endpoint from the study was a 72 hour EbC50 = 0.06 mg asulam sodium /L (equivalent to 0.9128 x 0.06 = 0.055 mg asulam /L). It is therefore appropriate to calculate the regulatory ErC50 based on this time interval – as detailed below in the related subsequent report.

i).b Report: M. Dorgerloh (2004a) Non-GLP Recalculation Report: Influence of asulam sodium on the growth of the green alga, *Pseudokirchneriella subcapitata* (formerly named *Selenastrum capricornutum*), originally reported from Springborn Laboratories, Inc., 1992, Report No.: 92-8-4391. United Phosphorus Limited, Unpublished report No.: C040380; CA 8.4/01b

This report has previously been evaluated at EU level.

Guidelines:

OECD 201 (June 1984) under consideration of the new draft revised proposal for updating OECD 201 (Feb. 18, 2004). Deviations: None.

GLP: N/A

Executive Summary:

This non-GLP recalculation fulfils the new OECD guideline 201 requirements, which ask for the EC_{50} for growth rate for the study with *Pseudokirchneriella subcapitata* (CA 8.4/01a): E_rC_{50} (72-hour) = 1.90 mg/L (equivalent to 1.73 mg asulam/L).

Materials and Methods

Test Material:

Test and results of the study with the freshwater alga, *Pseudokirchneriella subcapitata* (J.R. report no. R003656) have been summarised in CA 8.4/01a. The recalculation was done using the commercial ToxRat Professional.

Test Design:

Refer to report number R003656 (CA 8.4/01a).

Results and Discussion:

This non-GLP recalculation fulfils the new OECD guideline 201 requirements, which ask for the EC_{50} for growth rate. The factor of cell number, measured in the control between 0 and 72 hrs, was 133.0. The test fulfils the validation criterion.

Conclusions:

Result based on OECD 201: 72h E_rC_{50}: 1.90 mg/L (equivalent to 1.73 mg asulam/L). The 72h NOErC was 0.02 mg asulam sodium /L (equivalent to 0.018 mg asulam /L).

RMS comment:

This 're-calculation report' has previously been evaluated at EU level (details in Volume 3 B.9. DAR dated March 2006). It is considered scientifically valid and suitable for consideration in the regulatory risk assessment. The RMS agrees with the assessed 72 hour time period – which resulted in the most sensitive derived EbC50 value in the original report.

ii).a Report: J.R. Hoberg (1992b) Asulam sodium - Toxicity to the freshwater alga, *Anabaena flos-aquae*. United Phosphorus Limited, Unpublished report No.: R003654; CA 8.4/02a.

This study has previously been evaluated at EU level.

Guidelines:

USEPA (= EPA) 122-2 and 123-2, 1982
Deviations: pH ranged from 7.4-7.5 (test initiation) to 9.9-10.2 (test termination). This pH change is common in static algae cultures due to photosynthesis by the algae. This is considered to have no impact on the outcome of the study.

GLP: Yes

Executive Summary:

Based on mean measured concentrations, the 120-hour EC_{50} for *Anabaena flos-aquae* with asulam sodium was calculated to be 0.74 mg/L, equivalent to 0.68 mg asulam/L, with 95% confidence limits of 0.43-1.2 mg/L. The 120-hour No Observed Effect Concentration (NOEC) was determined to be 0.19 mg/L (0.17 mg asulam/L), based on the inhibition of cell growth seen at concentrations of 0.37 and 0.72 mg/L.

Materials and Methods

Test Material:

Asulam sodium, purity 89.5%, Batch no.: EN50005

Test Design:

Test organisms were the freshwater algae *Anabaena flos-aquae*. Algae were exposed to nominal concentrations of 0.025, 0.050, 0.10, 0.20, 0.40 and 0.80 mg asulam sodium/L over a period of 120 hours under temperature controlled conditions (room temperature of 24-25°C) and continuous shaking (100 rpm) and illumination. The initial cell density in each test level was 1.0 x 10^4 cells/mL. AAP medium (pH 7.6) was used as dilution water and as control. All treatment levels consisted of three 125-mL Erlenmeyer flasks.

The cell density was assessed in each treatment level every 24 hours. Observations of the health of the cells were also made and recorded each 24-hour interval. Temperature was measured continuously. Conductivity and pH were measured prior to test initiation and at test termination.

At test initiation and termination, a sample of each treatment level was removed for analysis of asulam concentration via a HPLC method (LOQ: 3.6 µg/L).

Results and Discussion:

The physical/chemical parameters were recorded as follows: conductivity ranged from 90-100 µS/cm, pH ranged from 7.4-7.5 at test initiation and increasing to 9.9-10.2 at test termination, temperature ranged from 24-25°C.

Analysis of asulam concentrations showed that measured concentrations averaged 91% of nominal concentrations throughout the study period. Mean measured concentrations were found to be 0.023, 0.045, 0.089, 0.19, 0.37 and 0.72 mg asulam sodium /L.

The biological key information is summarised in Table B.9.2.6.

Table B.9.2.6 Effects of asulam sodium on the growth of *Anabaena flos-aquae*

Concentration (mg/L)		Mean (standard deviation) cell density (x 10^4 cells/mL) after				
Nominal	Mean Measured	24 hours	48 hours	72 hours	96 hours	120 hours
Control	-	8 (2)	18 (9)	103 (15)	94 (5)	123 (23)
0.025	0.023	2 (1)	11 (10)	85 (20)	98 (20)	119 (5)
0.050	0.045	7 (5)	10 (3)	57 (14)	105 (25)	113 (10)
0.10	0.089	3 (4)	19 (9)	62 (17)	68 (29)	154 (44)
0.20	0.19	2 (4)	7 (5)	49 (4)	73 (8)	112 (8)
0.40	0.37	4 (2)	11 (1)	25 (4)	57 (12)	115 (6)
0.80	0.72	0 (0)	4 (2)	46 (42)	46 (9)	64 (6) *
EC_{50} (mg/L)		-	-	0.16	0.67	0.74
95% confidence limits		-	-	0.0009-39	0.62-22	0.43-1.2

* significantly reduced ($p \leq 0.05$) when compared to control data, based on Williams' test.

Conclusions:

Based on mean measured concentrations, the 120-hour [biomass] EC_{50} was calculated to be 0.74 mg/L, equivalent to 0.68 mg asulam/L, with 95% confidence limits of 0.43-1.2 mg/L. The 120-hour No Observed Effect Concentration (NOEC) was determined in the DAR to be 0.19 mg/L (0.17 mg asulam/L).

RMS comment:

This study has previously been evaluated at EU level (details in Volume 3 B.9. DAR dated March 2006). It is considered scientifically valid and suitable for consideration in the regulatory risk assessment using the 72h EC50 = 0.16 mg asulam sodium /L (which is the usual time period and also the most sensitive value in this study) – equivalent to 0.15 mg asulam /L In line with the current OECD 201 test guideline and also current regulatory requirements, in addition to the calculated biomass EbC50, a growth rate ErC50 is required to be calculated and this is estimated below in a separate later report (Dorgerloh 2004b).

ii).b Report: M. Dorgerloh (2004b) Non-GLP re-calculation report: influence of asulam sodium on the growth of the green alga, *Anabaena flos-aquae*, originally reported from Springborn Laboratories, Inc., USA, 1992, Report No.: 92-10-4457. United Phosphorus Limited, Unpublished report No.: C040383; CA 8.4/02b.

This report has previously been evaluated at EU level.

Guidelines:

OECD 201 (June 1984) under consideration of the new draft revised proposal for updating OECD 201 (Feb. 18, 2004). Deviations: None.

GLP: N/A

Executive Summary:

This non-GLP recalculation fulfils the new OECD guideline 201 requirements, which ask for the EC_{50} for growth rate for the study with *Anabaena flos-aquae* (CA 8.4/02a): E_rC_{50} (72-hour) = >0.72 mg/L (equivalent to >0.66 mg asulam/L).

Materials and Methods

Test Material:

Test and results of the study with the freshwater alga, *Anabaena flos-aquae* (report no. R003654) have been summarised in CA 8.4/02a. The recalculation was done using the commercial ToxRat Professional.

Test Design:

Refer to report number R003654 (CA 8.4/02a).

Results and Discussion:

This non-GLP recalculation fulfils the new OECD guideline 201 requirements, which ask for the EC_{50} for growth rate. The factor of cell number, measured in the control between 0 and 72 hrs, was 103.0. The test fulfils the validation criterion.

Conclusions:

Result based on OECD 201: 72 hour E_rC_{50}: >0.72 mg as/L (equivalent to >0.66 mg asulam/L). The 72 hour NOErC = 0.19 mg asulam sodium /L (equivalent to 0.17 mg asulam /L)

RMS comment:

This 're-calculation report' has previously been evaluated at EU level (details in Volume 3 B.9. DAR dated March 2006). It is considered scientifically valid and suitable for consideration in the regulatory risk assessment.

iii) Report: J.R. Hoberg (1992c) Asulam sodium - toxicity to the freshwater diatom, *Navicula pelliculosa*. United Phosphorus Limited, Unpublished report No.: R003655; CA 8.4/03.

This study has previously been evaluated at EU level.

Guidelines:

USEPA (= EPA) 122-2 and 123-2, 1982
Deviations: None which affected the study result

GLP:

Yes

Executive Summary:

Based on mean measured concentrations, the 120-hour EC_{50} for *Navicula pelliculosa* with asulam sodium was calculated to be 2.3 mg/L, equivalent to 2.10 mg asulam/L, with 95% confidence limits of 1.2-4.7 mg/L (in the DAR, the 72-hour EC_{50} was calculated to be 3.4 mg/L, equivalent to 3.1 mg asulam/L). The 120 hour No Observed Effect Concentration (NOEC) was determined to be 0.15 mg/L (0.14 mg asulam/L), based on the inhibition of cell growth and effects on algal cells seen at concentrations of 0.30 mg/L and above.

Materials and Methods

Test Material:

Asulam sodium, purity: 89.5%, Batch no.: EN50005

Test Design:

Test organisms were freshwater diatom N*avicula pelliculosa*. Algae were exposed to nominal concentrations of 0.16 0.31, 0.63, 1.3, 2.5 and 5.0 mg asulam sodium/L over a period of 120 hours under temperature controlled conditions (room temperature of 24-25°C) and continuous shaking (100 rpm) and illumination. The initial cell density in each test level was 1.0×10^4 cells/mL. AAP medium (pH 7.6) was used as dilution water and as control. All treatment levels consisted of three 125 mL Erlenmeyer flasks.

The cell density was assessed in each treatment level every 24 hours. Observations of the health of the cells were also made and recorded each 24-hour interval. Temperature was measured continuously. Conductivity and pH were measured prior to test initiation and at test termination.

At test initiation and termination, a sample of each treatment level was removed for analysis of asulam concentration via a HPLC method (LOQ: 3.6 µg/L).

Results and Discussion:

The physical/chemical parameters were recorded as follows: conductivity ranged from 100-110 µS/cm, pH ranged from 7.4-7.6 at test initiation and increasing to 8.0-9.2 at test termination, temperature ranged from 24-25°C. Analysis of asulam concentrations showed that measured concentrations averaged 91% of nominal concentrations throughout the study period. Mean measured concentrations were found to be 0.15, 0.30, 0.54, 1.3, 2.1 and 4.4 mg/L. The biological key information is summarised in Table B.9.2.7.

B.9.2.7 Effects of asulam sodium on the growth of N*avicula pelliculosa*

Concentration (mg/L)		Mean (standard deviation) cell density (x 10^4 cells/mL) after				
Nominal	Mean measured	24 hours	48 hours	72 hours	96 hours	120 hours
Control	-	1 (1)	3 (1)	12 (3)	57 (8)	81 (11)
0.16	0.15	1 (<1)	4 (1)	12 (1)	57 (5)	85 (3)
0.31	0.30	1 (<1)	5 (2)	11 (2)	37 (10)	71 (7) [A;B]
0.63	0.54	1 (<1)	5 (4)	10 (1)	44 (2)	66 (7) [A,B]
1.3	1.3	1 (<1)	4 (2)	9 (2)	43 (2)	48 (5) [A,B] *
2.5	2.1	1 (<1)	2 (1)	7 (1)	37 (4)	41 (5) [A,B] *
5.0	4.4	0 (0)	2 (1)	5 (1)	31 (2)	32 (3) [A,B] *
EC_{50} (mg/L)		-	4.1	3.4	9.0	2.3
95% confidence limits		-	0.16-640000	1.6-5.6	0.54-1300	1.2-4.7

A: cultures were observed to contain cell fragments.
B: cultures were observed to contain bloated cells.
*: significantly reduced ($p \leq 0.05$) when compared to control data, based on Williams' test.

Conclusions:

Based on mean measured concentrations, the 120-hour cell number (biomass) EC_{50} was calculated to be 2.3 mg/L, equivalent to 2.10 mg asulam/L, with 95% confidence limits of 1.2-4.7 mg/L. The 120 hour No Observed Effect Concentration (NOEC) was determined to be 0.15 mg/L (0.14 mg asulam/L).

RMS comment:

This study has previously been evaluated at EU level (details included in Volume 3 B.9. DAR dated March 2006), with no concerns /issues identified. However, the RMS evaluator for the current submission notes that although there was active cell growth in the control after 24 hours (as required by the OECD test validity criteria), there appears to have been no increase in cell numbers in the control during the first 24 hours of the study. The Notifier has stated that due to the age of the study it has not been possible to consult the raw data of the study to investigate this further. However, they have referred the matter to another study laboratory (Ibacon) – who have provided the following comments /explanation:

"The data from 1992 are not very detailed due to the fact that the values are rounded. Therefore it is not so easy to say if there was an increase in cell growth or not after 24 hours. Therefore it might be helpful to see more decimal places for the cell number. We observed several times that Navicula needs a little bit longer to start growing (> 24h). Moreover, we start with 10.000 to 20.000 cells in order to meet the validity criteria. The OECD provides validity criteria for the green algae. Sometimes these specification can not be met by Navicula or Anabaena as their growth is slow compared to the green algae. There must be a 16 fold increase for cell growth. However, this is hardly achievable with Navicula. But as long as the second criteria The coefficient of variation on the sectional (daily) growth rates in the control cultures during the course of the test must not exceed 35 %. is met one can argue that the test is valid."

Given that the endpoints from this study do not 'drive' the algal risk assessment, the RMS has not considered the issue of the initially slow growth rates in the untreated control in further detail and accepts the explanation given by the Notifier that this is not uncommon for the tested species *Navicula pelliculosa*. Details in the study summary (Table B.9.2.5) indicate a 20 fold increase in cell number in the untreated control between 24 and 72 hours – which is above the minimum of a 16 fold increase in the first 72 hours period required under current OECD 201 validity criteria. Calculations for the coefficient of variation of control daily growth rate were not included in the study report and therefore it is not possible to comment on this (which is a requirement for more recently conducted studies performed using the OECD 201 (2006) test guideline).

The usual time period considered in EU regulatory work is 72 hours, for which the 72 hour EbC50 = 3.4 mg asulam sodium /L, which equates to 0.9128 x 3.4 = 3.10 mg asulam /L. However, in this study, the 120 hour EbC50 is lower at 2.3 mg asulam sodium /L (equivalent to 2.1 mg asulam /L) – indicate a possible increase in sensitivity with an increased duration of exposure. Therefore this value has also been considered in the regulatory risk assessment (Section B.9.2.5.4).

iv).a Report: J.R. Hoberg (1992d) Asulam sodium - toxicity to the marine diatom, *Skeletonema costatum*. United Phosphorus Limited, Unpublished report No.: R003657; CA 8.4/04a.

This study has previously been evaluated at EU level.

Guidelines:

USEPA (= EPA) 122-2 and 123-2, 1982. Deviations: None which affected the study result

GLP: Yes

Executive Summary:

Based on mean measured concentrations, the 120-hour EC_{50} for *Skeletonema costatum* with asulam sodium was calculated to be 0.43 mg/L, equivalent to 0.39 mg asulam/L, with 95% confidence limits of 0.15-1.2 mg/L. The 120-hour No Observed Effect Concentration (NOEC) was determined to be 0.022 mg/L (0.020 mg asulam/L), based on the inhibition of cell growth seen at concentrations of 0.058 mg/L and above.

Materials and Methods

Test Material:

Asulam sodium, purity batch 89.5%, Batch no.: EN50005

Test Design:

Test organisms were the marine diatom *Skeletonema costatum*. Algae were exposed to nominal concentrations of 0.020 0.051, 0.13, 0.32, 0.80 and 2.0 mg asulam sodium/L over a period of 120 hours under temperature controlled conditions (room temperature of 20-22°C) and continuous shaking (60 rpm) and a photoperiod of 16 hours light and 8 hours darkness. The initial cell density in each test level was 1.0 x 10^4 cells/mL. AES medium (pH 8.1) was used as dilution water and as control. All treatment levels consisted of three 125 mL Erlenmeyer flasks.

The cell density was assessed in each treatment level every 24 hours. Observations of the health of the cells were also made and recorded each 24-hour interval. Temperature was measured continuously. Conductivity and pH were measured prior to test initiation and at test termination.

At test initiation and termination, a sample of each treatment level was removed for analysis of asulam concentration via a HPLC method (LOQ: 3.6 µg/L).

Results and Discussion:

The physical/chemical parameters were recorded as follows: conductivity ranged from 42000-43000 µS/cm, pH ranged from 8.1 at test initiation and increasing to 8.8-9.0 at test termination, temperature ranged from 20-21°C.

Analysis of asulam concentrations showed that measured concentrations averaged 101% of nominal concentrations throughout the study period. Mean measured concentrations were found to be 0.022, 0.058, 0.13, 0.33, 0.74 and 1.8 mg/L.

The key biological key information is summarised in Table B.9.2.8.

Table B.9.2.8 Effects of asulam sodium on the growth of *Skeletonema costatum*

Concentration (mg/L)		Mean (standard deviation) cell density (x10^4 cells/mL) after				
Nominal	Mean measured	24 hours	48 hours	72 hours *	96 hours	120 hours
Control	-	7 (1)	20 (3)	65 (3)	79 (3)	113 (9)
0.020	0.022	6 (3)	24 (3)	70 (6)	80 (9)	106 (10)
0.051	0.058	5 (4)	15 (2)	68 (9)	80 (10)	98 (3) **
0.13	0.13	4 (3)	9 (1)	63 (6)	51 (11)	79 (8) **
0.32	0.33	3 (1)	7 (1)	54 (2)	32 (5)	70 (5) **

Concentration (mg/L)		Mean (standard deviation) cell density ($x10^4$ cells/mL) after				
Nominal	Mean measured	24 hours	48 hours	72 hours *	96 hours	120 hours
0.80	0.74	2 (1)	8 (1)	50 (4)	34 (6)	42 (12) **
2.0	1.8	2 (1)	7 (1)	47 (2)	18 (6)	26 (4) **
EC_{50} (mg/L)		0.33	0.33	Not determined	0.16	0.43
95% confidence limits		0.00-290000	0.034-3.5		0.044-0.59	0.16-1.2

* twin-walled cells (abnormal) were inadvertently counted in addition to normal cells at the 72-hour observation interval.
** significantly different ($p \leq 0.05$) when compared to control data, based on Williams' test.

Conclusions:

Based on mean measured concentrations, the 120-hour EC_{50} (95% confidence limits) was calculated to be 0.43 (0.15-1.2) mg asulam sodium /L. The 120-hour No Observed Effect (NOEC) was determined to be 0.022 mg asulam sodium /L.

RMS comment:

This study has previously been evaluated at EU level (details in Volume 3 B.9. DAR dated March 2006). It is considered scientifically valid and suitable for consideration in the regulatory risk assessment. The usual time period assessed for EU regulatory work is 72hours, although in this case a 72h EbC50 has not been determined. The most sensitive derived EbC50 for the study is the 96 EbC50 of 0.16 mg asulam sodium /L, which is equivalent to 0.9128 x 0.16 = 0.146 mg asulam /L. This value is therefore considered to be the relevant regulatory EbC50 . The concluded 120 hour NOEC of 0.022 mg asulam sodium /L is equivalent to 0.9128x 0.022 = 0.02 mg asulam /L.

In line with the current OECD 201 test guideline and also current regulatory requirements, in addition to the calculated biomass EbC50, a growth rate ErC50 is required to be calculated and this is estimated below in a separate later report (Dorgerloh 2004c).

iv).b Report: M. Dorgerloh (2004c) Non-GLP re-calculation report: influence of asulam sodium on the growth of the marine diatom, *Skeletonema costatum*, originally reported from Springborn Laboratories, Inc., 1992, Report No.: 92-8-4395. United Phosphorus Limited, Unpublished report No.: C040376; CA 8.4/04b.

This report has previously been evaluated at EU level.

Guidelines:

OECD 201 (June 1984) under consideration of the new draft revised proposal for updating OECD 201 (Feb. 18, 2004)
Deviations: None

GLP:

N/A

Executive Summary:

This non-GLP recalculation fulfils the new OECD guideline 201 requirements, which ask for the EC_{50} for growth rate for the study with marine diatom, *Skeletonema costatum* (CA 8.4/04a): E_rC_{50} (72-hour) = >1.8 mg/L (equivalent to >1.64 mg asulam/L); the 72-hour NOEC was considered in the DAR to be 0.33 mg/L (equivalent to 0.30 mg asulam/L).

Materials and Methods

Test Material:

Test and results of the study with the marine diatom, *Skeletonema costatum* (report no. R003657) have been summarised in CA 8.4/04a. The recalculation was done using the commercial ToxRat Professional.

Test Design:

Refer to report number R003657 (CA 8.4/04a).

Results and Discussion:

This non-GLP recalculation fulfils the new OECD guideline 201 requirements, which ask for the EC_{50} for growth rate. The factor of cell number, measured in the control between 0 and 72 hrs, was 64.7. The test fulfils the validation criterion.

Conclusions:

Result based on OECD 201: 72 hour E_rC_{50}: >1.8 mg asulam sodium /L (equivalent to >1.64 mg asulam /L). The 72 hour NOErC = 0.33 mg /L (equivalent to 0.3 mg asulam /L).

RMS comment:

This 're-calculation report' has previously been evaluated at EU level (details in Volume 3 B.9. DAR dated March 2006). It is considered scientifically valid and suitable for consideration in the regulatory risk assessment. The RMS notes that effects in the study (Table B.9.2.8) suggest a greater sensitivity at 96 hours than at 72 hours and therefore ideally a 96 hour ErC50 should also have been calculated. However, in view of other reported more sensitive algae growth inhibition studies, these study results do not 'drive' the regulatory risk assessment and therefore an additional calculation of the 96h ErC50 value for this study is not considered essential.

B.9.2.3.2 Effects of relevant metabolites on algal growth and growth rate inhibition

An algal growth inhibition study has been conducted with asulam's major soil metabolite sulphanilamide.

Report: H. Gosch, P. Sowig (2003) Algal growth inhibition - *Pseudokirchneriella subcapitata* under static testing conditions AE C473799; substance, pure Sulfanilamide, metabolite of Asulam Code: AE C473799 00 1B99 0001. United Phosphorus Limited, Unpublished report No.: C027726; CA 8.4.1/01.

This study has previously been evaluated at EU level.

Guidelines:

OECD 201, 1984, EU (= EEC) 92/69/EWG C.3, 1992 and US EPA (= EPA) J § 123-2, 1982
Deviations: None

GLP: Yes.

Executive Summary:

The 72, 96 and 120-hour E_bC_{50} of AE C433799 to the green algae *Pseudokirchneriella subcapitata* were 7.6, 5.5 and 6.9 mg test item/L (based on the mean measured concentration), respectively. The 72, 96 and 120-hour E_rC_{50} were greater than 21.15 mg test item/L (based on the mean measured concentration). The No Observed Effect Concentration was considered to be 2.78 mg/L according to the area under the growth curve.

Materials and Methods

Test Material:

Sulfanilamide, purity 99.9% (w/w), Batch no.: AZ 09839

Test Design:

The test organisms were the unicellular planktonic green algae *Pseudokirchneriella subcapitata*. Triplicate algal cultures with an initial cell density of 10^4 algal cells/mL were incubated in a synthetic medium at 25 ± 1°C for 120 hours.

Nominal test item concentrations were 1, 2, 4, 8, 16 and 32 mg/L with three replicates each, together with an untreated control and a solvent control with six replicates.

Samples of the algal populations were removed daily from each test vessel and cell concentrations were determined after 24, 48, 72, 96 and 120 hours test duration. Algal morphology was also observed daily using a light microscope and counting chamber.

Chemical analysis of the freshly prepared and aged (120 hours) test solutions was performed for all tested concentrations of AE C473799 using HPLC with UV detection. The concentrations were diluted prior analyses.

Results and Discussion:

Analyses of freshly prepared water for AE C473799 resulted in test item concentrations ranging from 81.2% to 121.6% of nominal values. Analyses of aged water (120 h) for AE C473799 at experimental termination ranged from 38.8% to 57.8% of nominal values. The mean measured values over the time of exposure ranged from 65.7% to 80.2%. Therefore all concentrations were corrected by their mean measured factor. The mean measured values were used for reporting the results. The key biological information is summarised in Table B.9.2.9.

Table B.9.2.9 Effects of sulphanilamide on the growth of *Pseudokirchneriella subcapitata*

Concentration (mg/L)		Mean cell numbers x10^4 cell/mL					Percent inhibition according to					
							Mean area under the growth curve after			Mean growth rate after		
Nominal	Mean measured	24h	48h	72h	96h	120h	72h	96h	120h	72h	96h	120h
Control		1.72	13.01	50.03	207.14	336.86	-10.6	-13.0	-8.4	-2.2	-2.8	-0.1
Untreated control		1.50	11.52	46.28	180.16	334.35	0.0	0.0	0.0	0.0	0.0	0.0
1	0.69	2.14	16.59	64.75	217.71	351.93	-44.4	-29.5	-17.5	-8.2	-3.7	-0.9
2	1.60	2.07	13.40	52.19	212.48	363.38	-16.0	-16.8	-13.7	-3.5	-3.4	-1.4
4	2.78	2.20	11.08	42.33	176.96	355.69	5.1	3.6	-0.9	2.2	0.2	-1.1
8	5.42	1.57	8.89	23.04	87.31	265.42	42.1	49.5	38.1	17.9	13.8	4.0
16	10.51	1.91	7.27	15.18	39.81	114.39	57.6	72.1	70.9	30.1	29.8	18.8
32	21.15	1.23	3.96	8.44	17.19	35.98	79.5	87.2	89.0	44.2	45.1	38.4

The mean measured concentration of test item inhibiting the growth and the resulting E_bC_{50} in comparison with the untreated control after 72, 96 and 120 hours was 7.6, 5.5 and 6.9 mg test item/L, respectively.

The mean measured concentration of test item inhibiting the growth and the resulting E_rC_{50} in comparison with the untreated control after 72, 96 and 120 hours was >21.15 mg test item/L.

Significant inhibition of growth based on a comparison of slopes of the growth curves (α= 0.05) was observed in treatment levels of and above 5.42 mg/L (measured concentration) after 120 hours test duration.

This 120 hour NOEC according to the area under the growth curve is a consequence of a slight retardation of growth at the higher treatment levels during the first 24 hours. Since an obvious recovery was observed during the consecutive test period, the No Observed Effect Concentration can be considered as mean measured 2.78 mg/L.

Conclusions:

The 72, 96 and 120 hours E_bC_{50} of AE C433799 to green algae *Pseudokirchneriella subcapitata* was 7.6, 5.5 and 6.9 mg test item/L (based on the mean measured concentration), respectively.

The 72, 96 and 120 hours E_rC_{50} of AE C433799 to green algae *Pseudokirchneriella subcapitata* were all greater than 21.15 mg test item/L (based on the mean measured concentration).

The No Observed Effect Concentration can be considered as mean measured 2.78 mg/L.

RMS comment:

This study has previously been evaluated at EU level (details in Volume 3 B.9. DAR dated March 2006). It is considered scientifically valid and suitable for consideration in the regulatory risk assessment.

B.9.2.4 Effects on aquatic plants

B.9.2.4.1 Effects of the active substance on the growth of aquatic plants

i) Report: J.R. Hoberg (1992e) Asulam sodium - Toxicity to the duckweed (*Lemna gibba*). United Phosphorus Limited, Unpublished report No.: R003653; CA 8.6/01.

This study has previously been evaluated at EU level.

Guidelines:

USEPA (= EPA) 122-2 &123-2, 1982
Deviations: pH ranged from 5.1-5.2 at test initiation, increasing to 6.0-6.5 at test termination. This pH change is due to respiration and photosynthesis of the plants and is common in static *Lemna* cultures. This is not considered to have any impact on the outcome of the study

GLP:

Yes

Executive Summary:

Based on initial measured concentrations, the 14-day EC_{50} (95% confidence limit) for *Lemna gibba* with asulam sodium based on frond density was calculated to be 0.30 (0.020-0.61) mg/L, equivalent to 0.27 mg asulam/L. The 14-day No Observed Effect Concentration (NOEC) was determined to be 0.12 mg/L. The 14-day EC_{50} (95% confidence limit) based on biomass was calculated to be 0.32 (0.12-0.54) mg/L, equivalent to 0.29 mg asulam/L. The corresponding NOEC was determined to be 0.12 mg/L (initial measured concentration).

Materials and Methods

Test Material:

Asulam sodium, purity 89.5%, Batch no.: EN50005

Test Design:

The test organism was the duckweed *Lemna gibba*. Groups of 15 plants (3 replicates of 5 plants per test group) of 3 fronds each were exposed to a control and nominal concentrations of 0.031, 0.063, 0.13, 0.25 and 0.50 mg/L under 'static exposure' laboratory conditions (24 ± 2°C, continuous illumination) over a period of 14 days. M-type Hoagland's medium was used as dilution water and as control. The number of fronds present in each replicate was counted and observations were recorded at each 3-day interval (day 3, 6, 9 and 12) and at test termination (day14). At test termination, Lemna plants were dried for determination of dry weight. Temperature was measured continuously. pH values were determined in each treatment at test initiation and test termination.

Results and Discussion:

During the test, the temperature was recorded to be 24-25°C. The pH ranged from 5.1-5.2 at test initiation, increasing to 6.0-6.5 at test termination.

Concentrations of asulam averaged 99% of nominal at test initiation and decreasing to 15% of nominal at test termination. Therefore concentrations used for EC_{50} calculations were based on initial measured concentrations. Initial measured concentrations were found to be 0.035, 0.065, 0.12, 0.26 and 0.44 mg/L.

The key biological information is summarised in Table B.9.2.10.

Table B.9.2.10 Effects of asulam sodium on the growth of *Lemna gibba*

Initial measured concentrations (mg/L)	Frond production after mean of 3 replicates (standard deviation)					Biomass (dry weight) at 14-days mean (SD)
	day 3	day 6	day 9	day 12	day 14	
Control	43 (2)	100 (22)	206 (46)	328 (70)	406 (81)	0.0711 (0.0194)
0.035	40 (1)	96 (4)	198 (14)	312 (33)	403 (54)	0.0801 (0.0055)
0.065	42 (6)	93 (16)	191 (33)	310 (42)	393 (67)	0.0722 (0.0127)
0.12	45 (1)	103 (16)	207 (46)	301 (93)	410 (189) [A]	0.0702 (0.0254)
0.26	42 (5)	67 (8)	124 (20)	200 (43)	236 (52) *, [B]	0.0413 (0.0167) *
0.44	35 (2)	43 (3)	59 (2)	64 (3)	59 (5) *, [B]	0.0178 (0.0009) *
14-day EC_{50} (mg/L)	0.30					0.32
95% confidence limits	0.020-0.61					0.12-0.54

A: all fronds in one replicate observed to be slightly chlorotic, with less root formation in comparison to control fronds.
B: all fronds observed to be chlorotic, with very little root formation in comparison to control fronds.
* significantly reduced ($p \leq 0.05$) when compared to control, according to Williams' Test.

Conclusions:

The 14-day EC_{50} (95% confidence limit) based on frond density was calculated to be 0.30 (0.020-0.61) mg asulam sodium /L, equivalent to 0.27 mg asulam/L. The 14-day No Observed Effect Concentration (NOEC) was determined to be 0.12 mg asulam sodium /L, equivalent to 0.11 mg asulam /L. The 14-day EC_{50} (95% confidence limit) based on dry weight (biomass) was calculated to be 0.32 (0.12-0.54) mg asulam sodium /L, equivalent to 0.29 mg asulam /L.

RMS comment:

This laboratory static exposure study has previously been evaluated at EU level with no significant concerns regarding the scientific validity of the study (details in Volume 3 B.9 DAR dated March 2006 and EFSA Conclusion report dated 2010). However, in this current (2015) re-evaluation the RMS notes some issues with the study which are discussed below, although none of these are considered sufficient to invalidate the derived endpoints.

The design of the study is in line with the current standard OECD 221 (2006) guideline except for the 14 day duration of the study – which compares with a 7 day duration recommended in the current OECD guideline.

A reduction in the rate of (frond number) growth in the study over the last 6 days of the study (equivalent to a doubling time of 5 days) is noted by the RMS – with rates below the minimum doubling time of 2.5 days specified as a 'validity requirement' in the current OECD 221 (2006) test guideline. However, taking into account that growth rates in the first 9 days of the study were in excess of this minimum rate (0 and 9 day frond numbers per replicate of 15 and 206 respectively) this is not considered sufficient reason to invalidate the study.

The RMS also notes that the test concentrations were not maintained over the duration of the study (measured concentrations 99% of nominal at study start and 15% of nominal on day 14 at study end). Because of this lack of maintenance of exposure concentrations, the Notifier has expressed the derived endpoints in terms of 'initial measured concentrations'. This is in line with the previous Annex I evaluation of asulam, with the EFSA 'Conclusion on Pesticide Peer Review' (2010) report specifying the regulatory endpoint from this study to be a frond number (biomass) EC50 = 0.27mg asulam /Litre (based on initial measured concentrations).

For risk assessment purposes, the RMS agrees with the Notifier's use of 'initial measured concentrations' in deriving the regulatory frond number 14 day EbC50 of 0.27 mg asulam /Litre (as opposed to use of geometric mean test concentrations specified in the current OECD 221 2006 test guideline) provided it can be demonstrated that rates of dissipation of asulam in this lab study (approximating to a DT50 of 5 days) would be representative of (or slower than) dissipation rates in natural surface waters. This is considered further in Section B.9.2.5 of this assessment report ('Aquatic life toxicity, hazard classification and risk assessment').

For asulam sodium hazard classification purposes, CRD requested that growth rate ErC50 and NOErC values be provided based on mean measured concentrations – in relation to which the following calculated 'mean measured' endpoints were subsequently provided (ref. Notifier's email of 21st September 2015):

- 6 days: ErC50: 0.205 mg asulam sodium /L
- 9 days: ErC50: 0.186 mg asulam sodium /L
- 14 days: ErC50: 0.160 mg asulam sodium/L
- 6, 9 and 14 day NOErC: 0.051 mg asulam sodium/L

Therefore <u>for hazard classification purposes</u>, the RMS considers the relevant endpoints from the study are the most sensitive derived values obtained after 14 days i.e. an 14 day ErC50 = 0.16 mg asulam sodium /L and a 14 day NOErC = 0.051 mg asulam sodium /L.

ii) Report: A. Michel, R.D. Johnson, S.O. Duke, Scheffler B.E. (2004) Dose-response relationships between herbicides with different modes of action and growth of *Lemna paucicostata*: an improved ecotoxicological method. Environmental Toxicology and Chemistry, 23(4), 1074-1079. CA 8.6/02.

This published study has not previously been evaluated at EU level and was identified as part of a literature search.

Guidelines:

International Organisation for Standardization (2001). Water quality – duckweed growth inhibition: determination of the toxic effect of water constituents and waste water to duckweed (*Lemna minor*). ISO/WD 20079 (draft). Geneva, Switzerland.
Deviations: none reported.

GLP: No.

Executive Summary:

In this published study, 26 herbicides with up to 19 different modes of action were tested on the leaf area growth of the duckweed *Lemna paucicostata*, in order to establish complete dose-response relationships. The 7-day EC_{50} value for asulam was 407 µM, equivalent to 93.8 mg/L. Asulam showed the lowest toxicity of all 26 herbicides tested.

Materials and Methods

Test Material:

Asulam; purity not specified

Test Design:

The test organism was the duckweed *Lemna paucicostata*, which was grown in cultures on modified Hoagland medium (pH 5.5). For the tests, plants were taken while still in exponential growth from a 4- to 5-day old.

26 herbicides with up to 19 different modes of action were tested on the leaf area growth of the duckweed. The herbicides used were analytical grade and the solvent used for asulam was acetone (final concentration, 1% by volume). An initial screening test, with a 10-fold dilution concentration series (starting with 1mM as the highest concentration) was used to obtain an estimate for the concentration range. Based on these results, a 2-fold dilution concentration was used to narrow the range from no effect at the lowest concentration to no growth at the highest concentration. Each concentration within this range was tested in three replicates and the whole experiment was replicated at least twice.

Tests were conducted in an incubator with white light (94.2 $\mu E/m^2/s$ photosynthetically active radiation at the plant level). Initial inoculums comprised two three-frond colonies of approximately the same size. Total frond area was recorded by the image analysis system Scan analyser once per day from day 0 to day 7.

The total frond area for each day, at days 0 through 7, were used to calculate the growth rate, as determined by:

$r = \ln x_{t2} - \ln x_{t1} / t_2 - t_1$

where, r is the growth rate per day; x_{t1} is the value of the observation parameter at t_1 in days and x_{t2} is the value of the parameter at t_2 days; and $t_2 - t_1$ is the time period between x_{t1} and x_{t2} in days. Based on the average growth rate from day 0 to day 7, dose-response curves were

calculated using a logistic regression in order to produce EC_{50} values (% reduction in growth rate).

Results and Discussion:

In all tests, untreated *L. paucicostata* grew exponentially, and the growth rate of the control and solvent control remained the same throughout the experiment.

The EC_{50} values obtained ranged over five orders of magnitude, from 0.003 µM for sulcotrione and 0.005 µM for chlorsulfuron to 388 µM for glyphosate isopropylamine salt and 407 µM for asulam i.e. asulam showed the lowest toxicity to L. *paucicostata* of all the herbicides tested. In the case of asulam, an EC_{50} value of 407 µM is equivalent to 93.8 mg/L.

The key regression information is summarised in Table B.9.2.11.

Table B.9.2.11 Median effective concentration (EC_{50}) of the herbicide used (asulam), along with regression parameters

Herbicide	Upper level of curve	Lower level of curve	Slope	EC_{50} (µM)
Asulam	0.278 ± 0.007	0.001 ± 0.020	2.07 ± 0.444	407.3 ± 50.73

Conclusions:

In this published study, 26 herbicides with up to 19 different modes of action were tested on the leaf area growth of the duckweed *Lemna paucicostata*, in order to establish complete dose-response relationships. *L. paucicostata* was used because it is smaller than *L. minor* and *L. gibba*, thus facilitating miniaturisation. However, it was considered that the principles of this study with *L. paucicostata* should be transferable to other *Lemna* species. The 7-day EC_{50} value for asulam was 407 µM, equivalent to 93.8 mg/L. Asulam showed the lowest toxicity of all 26 herbicides tested.

RMS comment:

This study has not previously been evaluated at EU level. The paper includes a summary of the test results, which in addition to asulam included the testing of 25 other herbicides - with a total of 17 different modes of action.

Based on the information provided, although not GLP compliant and not conducted to the standard *Lemna* growth inhibition OECD 221 test guideline, the study appears to have been conducted to an acceptable standard although the lack of chemical analysis to confirm test substance concentrations is considered a significant deficiency affecting the reliability of the study. Also, although growth in the untreated control is stated as 'exponential', details for frond doubling time in the control were not included in the study summary or full publication and hence it is not possible to verify that the study passes the OECD221 test guideline 'validity' requirement of a doubling time of 2.5 days. However, given asulam's high solubility in water, solubility issues affecting the test concentrations are not likely. Taking account of the available evidence, the study has been classified by the RMS as 'Reliable with restrictions' .

The reported *Lemna paucicostata* 7-day growth rate EC50 of 93.8 mg asulam /L suggests a much lower toxicity of asulam to this species than to *Lemna gibba* – for which a 14 day frond density (≡ biomass) EC50 of 0.27 mg a.s. /L has been estimated (Hoberg 1992e) – indicating that this published study does not constitute 'adverse data'.

iii) Report:G.H.P. Arts & J.D.M. Belgers (2013) Aquatic macrophyte toxicity tests with asulam. United Phosphorus Limited, Unpublished report, CP 10.8.2.1/01.

This study has not previously been evaluated at EU level.

Guidelines:

In-house methodology. Deviations: Not applicable.

GLP: No

Executive Summary:

Toxicity tests were performed with five rooted aquatic macrophytes and the test item asulam in the laboratory. Asulam was applied as the 400 g/L SC. Tests followed a worst-case approach as aquatic macrophytes were exposed in a water-medium and sediment was not included. EC_{50} values were based on nominal and time-weighted-average concentrations of the test item. Samples from the experimental systems taken 1 h post-treatment showed that on average 86 to 101% of the nominal concentrations of asulam were present in the test systems and that the asulam concentrations remained relatively stable during the exposure period of 21 days. Of all macrophyte species tested, *Myriophyllum spicatum* was the most sensitive species. The most sensitive endpoint was total dry weight, of which the EC_{50} values were 9.6 and 10.7 µg/L based on TWA and nominal concentrations, respectively. The EC_{50} values for length endpoints were 16.9 and 18.1 µg/L based on TWA and nominal concentrations, respectively. In the 2012 experiments *Elodea canadensis* was the next most sensitive macrophyte species. Dry weight of new shoots and length of new shoots were the most sensitive macrophyte endpoints.

Materials and Methods

Test Material:

Asulox, purity 400 g/L, Batch no.: not stated

Test Design:

Non-GLP tests with aquatic macrophytes were performed in an aqueous growth medium in the laboratory. All tested macrophytes were submerged, rooted aquatic macrophytes. Tests were performed without a sediment compartment. Species tested included *Myriophyllum spicatum*, *Elodea nuttallii*, *Elodea canadensis*, *Ranunculus circinatus* and *Potamogeton crispus*. Tests were performed in 2005, 2009 and in 2012 in the spring, during the growing season of the macrophytes. All tests were performed in a controlled climate room with a constant temperature of the water medium of 20 ± 2°C with 14 hours of light per day and 10 hours darkness and a light intensity of 190 ± 20 ($\mu E.m^{-2}.S^{-1}$). Three macrophyte apical top shoots were introduced into each test vessel (1.5 L glass vessel containing 1.2 L test solution). The shoots were non-flowering shoots with a length of 10 cm. Side shoots and roots were removed before the start of the experiments.

In 2005, macrophytes were exposed to 7 different concentrations of asulam: 0, 1.4, 5.6, 14, 42, 140, 420 and 1260 µg/L asulam. Tests were performed in duplicate. In 2009 and 2012, macrophytes were exposed to asulam at 6 different concentrations: 0, 0.1, 1, 10, 100 and 1000 µg/L. In 2009, tests were performed in duplicate. In 2012, tests were performed in triplicate. Samples for chemical analysis were taken from each test solution at t=0.04 (1 hour), 7 and 21 days in the 2005 experiment. In the 2009 experiment samples for chemical analysis of the test substance were taken at t = 0.04 (1 hour), 1, 3, 7, 14 and 21 days. During the 2012 experiment samples were taken at t = 0.04 (1 hour) and 21 days.

The regulatory endpoints shoot length and biomass of the macrophytes were measured at the end of the experiment (t = 21 days): total wet weight (2005 experiment), total dry weight, length of the main shoot and total length of new shoots. Ten extra macrophyte shoots were weighed at t=0 to determine the dry weight at the start of the experiment. To monitor macrophyte performance in the laboratory tests, values for relative growth rates (RGR) were calculated on the basis of the dry biomass and total length in the control vessels

Results and Discussion:

Samples from the experimental systems taken 1 h post-treatment showed that on average 86 % (2005), 101 % (2009) and 86 % (2012) of the nominal concentrations of asulam were present in the test systems. Asulam concentrations remained relatively stable during the exposure period of 21 days. On average 79 % (2005), 79 % (2009) and 81 % (2012) of the compound was still present in the test systems at the end of the experiment (day 21).

The EC_{50} values were based on nominal and time-weighted-average concentrations of the test item. However, test concentrations at the start were on average 86 to 101% of the nominal concentrations and remained stable throughout the test so the nominal values can be considered valid. If EC_{50} values were calculated to be higher than the highest test concentration they were reported as 'greater than' because extrapolation values are not reliable. The EC_{50} values for various macrophytes and endpoints are presented in Table B.9.2.12. Of all macrophyte species tested, *Myriohyllum spicatum* was the most sensitive species. This was true for biomass as well as for length endpoints. The most sensitive endpoint was total dry weight, for which the EC_{50} values are 9.6 and 10.7 µg/L based on TWA and nominal concentrations, respectively. The EC_{50} values for length endpoints are 16.9 and 18.1 µg/L based on TWA and nominal concentrations, respectively. *Elodea canadensis* in the 2012 experiments was the next most sensitive macrophyte species. Dry weight of new shoots and length of new shoots were the most sensitive endpoints.

Table B.9.2.12 Effects of asulam sodium on the growth of aquatic macrophytes (as indicated by EC50 values for the specified parameters)

species	concentration	wet weight		dry weight							
		total	CI	total	CI	growth	CI	main shoot	CI	new shoots	CI
		ng/mL	min-max	ng/mL	min-max	ng/mL	min-max	ng/mL	min-max	ng/mL	min-max
R. circinatus (2005)	nominal	-	-	-	-	-	-	-	-	-	-
P. crispus (2005)	nominal	>1260		-	-	-	-	-	-	723.5	477-1097
E. nuttallii (2005)	nominal	>1260		-	-	-	-	-	-	-	-
E. canadensis (2005)	nominal	>1260		>1260		1093	457-2611	-	-	-	-
R. circinatus (2005)	*TWA*	-	-	-	-	-	-	-	-	1063	34-33031
P. crispus (2005)	*TWA*	>1260		-	-	-	-	-	-	617	401-950
E. nuttallii (2005)	*TWA*	>1260		-	-	-	-	-	-	-	-
E. canadensis (2005)	*TWA*	>1260		>1260		970	391-2407	-	-	>1260	
E.canadensis (2012)	*TWA*	nm	nm	>1000		128	*-*	nm	-	nm	-
	nominal	nm	nm	>1000		160	*-*	nm	-	nm	-
M. spicatum (2009)	*TWA*	nm	nm	9.6	1-67	0.95	0.09-10.1	nm	-	nm	-
	nominal	nm	nm	10.7	2-74	1.07	0.1-11.5	nm	-	nm	-

species	concentration	length							
		total	CI	growth	CI	main shoot	CI	new shoot	CI
		ng/mL	min-max	ng/mL	min-max	ng/mL	min-max	ng/mL	min-max
R. circinatus (2005)	nominal	-	-	-	-	-	-	863	23-32560
P. crispus (2005)	nominal	>1260		962	539-1716	>1260		1108	609-2016
E. nuttallii (2005)	nominal	-	-	-	-	-	-	1033	304-3515
E. canadensis (2005)	nominal	>1260		85	18-396	>1260		>1260	
R. circinatus (2005)	*TWA*	-	-	-	-	>1260		965	*-*
P. crispus (2005)	*TWA*	>1260		834	453-1534	>1260		967	516-1810
E. nuttallii (2005)	*TWA*	-	-	-	-	-	-	-	-
E. canadensis (2005)	*TWA*	>1260		75	13-407	>1260		>1260	
E.canadensis (2012)	*TWA*	>1000		>1000		-	-	207	56-765
	nominal	>1000		>1000		-	-	251	72-877
M. spicatum (2009)	*TWA*	>1000		>1000		-	-	16.9	4-68
	nominal	>1000		>1000		-	-	18.1	5-71

For *Elodea canadensis* the EC_{50} values for new shoots were above the highest test concentration in 2005. In the tests conducted in 2012 the EC_{50} values for new shoots were much lower (251 (nominal) and 207 (TWA) µg/L). It was considered that this variation might be due to:

- Differences in plant material. An optimal growth is necessary to detect changes compared to the controls; this is very much dependent on the quality of the material;
- Differences in nitrogen, phosphorous and carbon concentrations in the growth media;
- In 2005 surface water from the Sinderhoeve experimental station was used to solve the nutrients and prepare the test media. In 2009 and 2011 demineralized tap water from the Alterra ERA laboratories was used to prepare nutrients and test media.

Conclusions:

Of all macrophyte species tested, *Myriophyllum spicatum* was the most sensitive species. The most sensitive relevant endpoint was total dry weight, of which the EC_{50} values were 9.6 and 10.7 µg/L based on TWA and nominal concentrations, respectively. The EC_{50} values for length endpoints were 16.9 and 18.1 µg/L based on TWA and nominal concentrations, respectively. In the 2012 experiments *Elodea canadensis* was the next most sensitive macrophyte species. Dry weight of new shoots and length of new shoots were the most sensitive macrophyte endpoints.

RMS comment:

Details included in this study summary and in the submitted study report have been evaluated by the RMS.

The conducted chemical analysis indicates adequate levels of recovery (greater than 80% of nominal values) during the study and therefore supports the reporting of the derived endpoints

based on nominal test concentrations, with a concluded total dry weight (biomass) EbC_{50} of 10.7 µg/L (nominal) for the most sensitive test species *Myriophyllum spicatum* based on total dry weight differences from the untreated control. However, it is noted that although recently conducted (2013) the study is not GLP compliant – which is a usual standard requirement for regulatory studies. Also, 95% confidence intervals for the most sensitive total dry weight EbC50 of 10.7 µg/L are large i.e. 2-74 µg/L - indicating uncertainty in the accuracy of this determined endpoint. Given these deficiencies, the results of the Seeland-Fremer, V. Wydra (2014) *Myriophyllum* study are considered more reliable for regulatory use.

iv) Report: A. Seeland-Fremer, V. Wydra (August 2014, with amendment of September 2015) 'Toxicity of Asulam 400 g/L SL to the Aquatic Plant *Myriophyllum spicatum* in a Static Growth Inhibition Test with a Prior Rooting Phase'

Study guideline:

The study protocol was based on the following guidelines /recommendations:

Draft OECD Guideline for a Proposed Test Method for the Rooted Aquatic Macrophyte, *Myriophyllum* sp., in a water-sediment System, July 07, 2013.

Ring Test Protocol for a Proposed Test Method for the Rooted Aquatic Macrophyte, *Myriophyllum spec*., 2009.

Ring Test Protocol: Standardized method for investigating test substance impact on rooted aquatic macrophytes, 2011

GLP compliance: Yes

Purpose: The purpose of this test was to determine the inhibitory effect of the test item Asulam 400 g/L SL on the vegetative growth of the freshwater aquatic plant *Myriophyllum spicatum*. Plants of *Myriophyllum spicatum* were exposed in a static test to various concentrations of the test item under defined conditions. The inhibition of growth in relation to control cultures was determined over a test period of 14 days.

The purpose of the analytical part of this study was to verify the concentration of the test item in the test medium.

Material and Methods

Test Item: Asulam 400 g/L SL; Batch No.: 686A; content of Asulam: 400 g /L (nominal); 391 g/L (analytical) according to certificate of analysis

Test Species: *Myriophyllum spicatum*

Test Design: This study encompassed 6 treatment groups (5 dose rates of the test item and a control) with three replicates per test concentration and six replicates for the control.

A water sediment test system was used with plants being grown within small 500ml pots containing sediment which were placed within larger (2 litre) test vessels. The sediment surface was >70% of the test beaker's surface, with a minimum overlaying water depth of 12cm. In line with that recommended in the draft test guideline, the sediment consisted of 5% (sphagnum) peat,

75% quartz sand and 20% kaolinite clay, with calcium carbonate added to adjust the pH (which at pH 6.9 was within the recommended range of 6.5-7.5).

After a pre-rooting phase of 7 days, 3 plants per replicate were incubated for 14 days under static conditions. The shoot length was determined at test start and at test end (day 14). Sublethal effects were recorded at test start, at day 7 and at the end of the test. On day 14, the fresh and dry weight of each replicate was determined.

The samples collected at start and after 14 days were analysed via LC-MS/MS method.

Endpoints: Yield and growth rate based on total shoot length, wet and dry weight

Test Concentrations: 2.56, 0.64, 0.16, 0.04 and 0.01 mg a.i./L and a control.

Test Conditions: Water temperature: 18 - 21 °C; light regime: 16 h light : 8 h dark; mean light intensity: 9207 lux (8320 - 9560 lux); pH values at test start 7.8 – 8.0, on day 7 8.8 – 9.3, at the end of the test 8.6 – 9.8; oxygen concentrations at test start 8.6 – 8.8 mg/L, on day 7 10.2 - 12.6 mg/L, at the end of the test 5.8 – 12.1 mg/L.

Table B.9.2.13 Summary of biological results.

Parameter	Yield (shoot length) [mg a.i./L]	Specific growth rate (shoot length) [mg asulam /L]	Yield (wet weight) [mg asulam /L]	Specific growth rate (wet weight) [mg asulam /L]	Yield (dry weight) [mg asulam /L]	Specifc growth rate (dry weight) [mg asulam /L]
EC_{50} (14-day)	0.987	> 2.56	0.390	> 2.56	> 2.56.	> 2.56.
95 % conf. limits	0.368- > 2.56	n.d.	0.135 – 2.01	n.d.	-	-
EC_{20} (14-day)	0.013	0.128	< 0.01	0.038	1.23	> 2.56
95 % conf. limits	< 0.01 - 0.047	0.011 – 0.393	n.d.	< 0.01 -0.138	n.d.	n.d.
EC_{10} (14-day)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.052
95 % conf. limits	n.d.	n.d.	n.d.	< 0.01 -0.022	n.d.	n.d.
14-day NOEC	0.01	0.01	0.01	0.01	0.01	0.04
14-day LOEC	0.04	0.04	0.04	0.04	0.04	0.16
n.d.: could not be determined Values refer to nominal test concentrations						

Analytical Results: At the start of the test 104% of the nominal test concentration was found in the analysed water phase (average of all test concentrations). After 14 days test duration, 80% of the nominal value was determined (average of all test concentrations). During the test the plants were exposed to a mean of 92% of nominal. Therefore, all reported results refer to nominal concentrations.

Conclusion: The influence of Asulam 400 g/L SL on the growth of the

dicotyl freshwater plant *Myriophyllum spicatum* was assessed in a static dose-response test.

The 14-day biomass E_yC_{50} was calculated to be 0.987, 0.390 and > 2.56 mg asulam /L for shoot length, wet weight and dry weight, respectively.

The 14-day specific growth rate E_rC_{50} was calculated to be > 2.56 mg a.i./L for shoot length and wet weight. For dry weight no E_rC_{50} value could be calculated [due to low level effects only, although an ErC50 of > the highest test dose of 2.56 mg asulam /L was concluded].

The 14-day NOE_yC and the LOE_yC were determined to be 0.01 and 0.04 mg asulam /L for shoot length, wet and dry weight.

The 14-day NOE_rC and the LOE_rC were determined to be 0.01 and 0.04 mg asulam /L for shoot length and wet weight and 0.04 and 0.16 mg asulam /L for dry weight, respectively.

RMS comment:

Details included in this study summary and in the submitted study report have been evaluated by the RMS. The study is GLP compliant and has been conducted to the most recent (draft) OECD test method guideline (July 2013) for the rooted aquatic macrophyte *Myriophyllum* sp. in a water-sediment test system.

The draft guideline states that in order for the test to be valid, the total shoot length and shoot fresh weight in control plants must at least double during the exposure phase of the test, control plants must not show visual symptoms of chlorosis, and the mean coefficient of variation for yield based on shoot fresh weight must not exceed 35% over the study duration. Details included in the full study report indicate that these criteria were met. Compared with initial values for the control (100%), after 14 days shoot length values were 524% and wet weight values 371%. The coefficient of variation for yield wet weight was 3.8% and therefore well within the maximum acceptable value of 35%.

The included chemical analysis of test concentrations indicates adequate levels of recovery (i.e. within 80% of nominal values) during the study and supports the use of nominal test concentrations to derive the effects endpoints – which are considered suitable for regulatory use. The most sensitive relevant regulatory endpoints are a 14-day wet weight (biomass) E_yC_{50} of 0.39 mg asulam /L (with 95% confidence intervals of 0.135 – 2.01 mg asulam /L), a 14-day specific growth rate E_rC_{50} of > 2.56 mg a.i./L for shoot length and wet weight and a 14 day NOErC = 0.01 mg asulam /L for shoot length and wet weight (equivalent to 0.01/0.9128 = 0.011 mg asulam sodium /L).

v) Report: R. Vinken, V. Wydra (2007) Final Report IBACON Project 33533240 Toxicity of Asulam 400g/L SL to the Aquatic Plant Lemna gibba in a Static Growth Inhibition Test

This study was not included in the Notifier's original submission but became available to the RMS (as a PDF document) at a late stage in the evaluation process. The study summary included below has been taken directly from the full study report (which has been evaluated), followed by comments from the RMS regarding the study's acceptability for regulatory use:

1. Summary

Title: Toxicity of Asulam 400 g/L SL to the Aquatic Plant *Lemna gibba* in a Static Growth Inhibition Test

Guidelines/Recommendations:
- OECD Guideline 221: "*Lemna* sp. Growth Inhibition Test", adopted March 23, 2006.

Purpose: The purpose of this test was to determine the inhibitory effect of the test item Asulam 400 g/L SL on the growth of the freshwater aquatic plant *Lemna gibba*. Cultures of *Lemna gibba* were exposed to various concentrations of the test item under defined conditions. The inhibition of growth in relation to control cultures was determined over a test period of 7 days.

The test method of application and the test system are recommended by the test guidelines and *Lemna gibba* is one of the recommended test species.

The purpose of the analytical part of this study was to verify the concentration of the active ingredient of the test item in the test medium.

Test Concentrations: 10, 3.2, 1.0, 0.32, 0.10 and 0.032 mg test item/L and a control.

Biological Results:

Parameter	Growth rate (frond number) [mg/L]	Yield (frond number) [mg/L]	Growth rate (dry weight) [mg/L]	Yield (dry weight) [mg/L]
EC_{50} (7-day)	2.56	0.32	>10	1.83
95% conf. limits	0.39 – >10	0.02 – 1.25	2.85 - >10	0.34 - >10
7-day NOEC	0.10	0.10	0.32	0.32
7-day LOEC	0.32	0.32	1.0	1.0

n.d.: could not be determined

Analytical Results:

Table 1. Summary of analytical results

sample description [mg/L]	% of nominal[1]	RSD [%]	n
control	n.a.	n.a.	2
0.032	n.d.	-	-
0.1	88	13	4
0.32	95	8	4
1	90	5	4
3.2	98	4	4
10	98	4	4

[1] mean value of all measured samples per treatment group
RSD relative standard deviation per treatment group
n number of analysed samples
n.d. could not be determined

At the start of the test just before introduction of the *lemna* 97% of the nominal test concentrations were found. After 7 days test duration, 90% of the nominal values were determined. Thus, during the test period the *lemna* were exposed to a mean of 94% of nominal. Therefore, all reported results are related to nominal concentrations of the test item.

RMS comment:

This study has not previously been evaluated at EU level. The study is GLP compliant and has been conducted in line with the current OECD 221 (2006) test guideline (with no significant deviations). Additional, the validity criteria for this guideline is satisfied, with the frond number doubling time in the control of 1.7 days (corresponding to a 16 fold increase over the 7 day study duration) being faster than the minimum specified as required of 2.5 days. Therefore, the study is considered scientifically valid and suitable for consideration in the regulatory risk assessment. Given that the analysed concentrations over the study duration were within 90% of the nominal test test concentrations, the RMS agrees that (as calculated) the determination of endpoints may be based on use of nominal test concentrations. The specific growth rate ErC50 of 2.56 mg 'Asulam 400g/L SC' /L (based on changed in frond number) is considered to be the most relevant regulatory endpoint – which is equivalent (based on the reported analytical concentration of 389.9g asulam /L and density of 1.181 kg /L) to 0.845 mg asulam /L. The reported frond number EbC50 of 0.32 mg product /L and NOEC of 0.1 mg product /L are equivalent (assuming toxicity relates to the asulam content) to 0.106 mg asulam /L and 0.033 mg asulam /L

B.9.2.4.2 Effects of relevant metabolites on the growth of aquatic plants

An aquatic macrophyte (*Lemna gibba*) growth inhibition study has been conducted with asulam's major soil metabolite sulphanilamide.

Report: D. Juckeland (2011) Effects of Sulfanilamide on *Lemna minor* in growth inhibition test under semi-static test conditions. United Phosphorus Limited, Unpublished report No.: 11 10 48 021 W; CA 8.6.1/01.

This study has not previously been evaluated at EU level.

Guidelines:

OECD 221, 2006
Deviations: None

GLP: Yes

Executive Summary:

Based on nominal concentrations, the 7-day EC_{50} (95% confidence limit) for *Lemna gibba* with asulam sodium based on frond number was calculated to be 5.82 mg test item/L for growth rate and 2.30 mg test item/L for yield. The 7-day No Observed Effect Concentration (NOEC) was determined to be 0.67 mg test item/L and the LOEC was determined to be 2.14 mg test item/L, based on nominal concentrations. The 7-day EC_{20} based on biomass was calculated to be 25.1 mg test item/L for growth rate and the EC_{50} value was 33.8 mg test item/L for yield. The corresponding NOEC was determined to be 2.14 mg test item/L and the LOEC was determined to be 6.84 mg test item/L, based on nominal concentrations.

Materials and Methods

Test Material:

Sulphanilamide, analysed purity 101.4%, Lot no.: L010098499

Test Design:

Test organisms were the duckweed *Lemna minor*. Groups of 27 plants (3 replicates of 9 plants per test group) of 3 fronds each were exposed to a control and nominal concentrations of 0.67, 2.14, 6.84, 21.9, 70.0 mg/L under laboratory conditions (22 to 23.6°C, continuous illumination) over a period of 7 days, with test substance renewal on days 2 and 4 in this semi-static study. Steinberg medium was used as dilution water and as control. The number of fronds present in each replicate was counted and observations were recorded (day 0, 2 and 4) and at test termination (day 7). At test termination, Lemna plants were dried for determination of dry weight. Temperature was measured continuously. pH values were determined in each treatment at test initiation and test termination.

Results and Discussion:

During the test, the temperature was recorded to be 22.0-23.6°C. The pH ranged from 5.41-5.49 at test initiation, increasing to 5.82-6.59 at test termination.

Concentrations of sulphanilamide were measured at the test start (day 0), at the test end (day 7) and after each test renewal in the 'fresh' solutions and the 'spent' solutions. The measured solutions remained within a range of 100-103% of the nominal values in freshly prepared test solutions at the start of the test (day 0) and at each renewal, and within a range of 85-97% of nominal values in the spent solutions at each renewal and at the end of the test (day 7). Accordingly the toxicity results are based on the nominal concentrations.

The key biological information is summarised in Table B.9.2.14.

Table B.9.2.14 Effects of sulphanilamide on the growth of *Lemna minor*

Nominal concentrations (mg/L)	Final frond number (mean of 3 replicates) Day 7	Biomass (dry weight) Day 7 (mg)	% inhibition			
			Average specific growth rate (% I_r)		Yield (% I_y)	
			Frond number	Biomass	Frond number	Biomass
Control (untreated test medium)	71.3	12.3	-	-	-	-
0.67	71.0	12.6	0.2	0.0 (-1.1)[1]	0.5	0.0 (-2.8)[1]
2.14	38.3	12.3	30.1+	0.1	52.9+	0.4
6.84	19.3	7.9	63.1+	16.3+	83.4+	38.2+
21.9	15.0	6.8	75.4+	22.2+	90.4+	48.3+
70.0	13.3	6.1	81.0+	26.0+	93.0+	53.8+
7-day EC_{50} (mg/L)			E_rC_{50} 5.82	E_rC_{20}* 25.1	E_yC_{50} 2.30	E_yC_{50} 33.8
95% confidence limits			(4.24 - 7.97)	15.4 – 41.0)	1.88 – 2.80)	21.0 – 67.5)

+ statistically significantly different to the untreated control (Williams t-test; $p \leq 0.05$, one-sided)
[1] negative values indicate a higher growth relative to the untreated control
*EC_{50} not determined due to mathematical reasons or inappropriate data

Conclusions:

The 7-day EC_{50} frond number was calculated to be 5.82 mg test item/L for growth rate and 2.30 mg test item/L for yield. The 7-day No Observed Effect Concentration (NOEC) was determined to be 0.67 mg test item/L and the LOEC was determined to be 2.14 mg test item/L, based on initial measured concentrations. The 7-day EC_{20} based on biomass was calculated to be 25.1 mg test item/L for growth rate and the EC_{50} value was 33.8 mg test item/L for yield. The 7-day NOEC was determined to be 2.14 mg test item/L and the LOEC was determined to be 6.84 mg test item/L, based on initial measured concentrations.

RMS comment:

This study has not previously been evaluated at EU level. The study is GLP compliant and has been conducted in line with the current OECD 221 test guideline. Additional, the validity criteria for this guideline is satisfied, with the frond number doubling time in the control of 2.3 days being faster than the minimum specified as required of 2.5 days. Therefore, the study is considered scientifically valid and suitable for consideration in the regulatory risk assessment. The specific growth rate ErC50 of 5.82 mg sulphanilamide /L (based on changed in frond number) is considered to be the relevant regulatory endpoint.

B.9.2.5 Aquatic life toxicity, hazard classification and risk assessment

B.9.2.5.1 Summary and review of aquatic life toxicity data.

Aquatic life toxicity studies have been conducted with asulam or its salt 'asulam sodium' and for algae and aquatic plants also with its major metabolite sulphanilamide. As discussed under Section B.9.0 (Background information), asulam and asulam sodium can be regarded as biologically equivalent when molecule weight differences are taken into account. To enable a comparison in the risk assessment with maximum surface water exposure values (PECsw) given in terms of concentrations of asulam, study endpoints derived from use of asulam sodium have been converted into

asulam equivalents using a conversion factor of 0.9128 - based on the molecule weight differences of asulam (230.2) and asulam sodium (252.2).

The purity of the asulam or 'asulam sodium' test material specified in the aquatic life toxicity study summaries is in line with the previously published FAO specification (1998) – which is detailed in the 'Identity' section of the 'List of Endpoints' as 'not less than 800g /kg asulam, equivalent to 876g /kg asulam sodium (+ or – 25g/kg)'. Therefore, this aspect of the conduct of the studies is acceptable.

Given that the representative formulation 'Asulox' (also referred to as 'Asulam 400 g/L SL') contains only additional water, acute toxicity formulation studies have not been conducted for all aquatic groups – it being possible instead to calculate its toxicity based on toxicity values derived from studies conducted with the technical active substance.

It is a usual requirement for ecotoxicology studies submitted in support of approval of the active substance or product authorisations to be GLP compliant - with the possible exception of relevant and reliable peer reviewed published studies and also early studies conducted prior to the implementation of this requirement. The reported studies were all GLP compliant , with the exception of the published *Lemna paucicostata* growth inhibition study (Michel et al 2004) which is considered to be relevant and 'reliable with restrictions' and also the recently conducted multi-species macrophyte study (Arts and Belgers 2013). These two studies are considered further below.

The Michel et al 2004 study, indicates a much lower toxicity to *Lemna paucicostata* than to the two standard test species *Lemna minor* or *Lemna gibba* which were evaluated in two other lab growth inhibition studies. Therefore, the results of this *Lemna paucicostata* study do not 'drive' the aquatic macrophyte risk assessment and do not need to be considered further.

The Arts and Belger 2013 study, although not GLP compliant, appears to have been conducted to a satisfactorily standard, with the inclusion of chemical analysis supporting the adequate maintenance of test concentrations over the study period, although it is noted that the methodology used is non-standard and not in line with the current (2014) draft OECD test guideline for *Myriophyllum* growth inhibition studies. The very high toxicity of asulam to *Myriophyllum spicatum* reported in this study (21 day EbC50 = 0.0107 mg a.s. /L) indicates that this test species may be more sensitive than the standard *Lemna* test species used in the other reported macrophyte studies (most sensitive reported total dry weight EbC50 = 0.27 mg asulam /L). To address this point, the Notifier has recently conducted a single species laboratory GLP compliant growth inhibition study with *Myriophyllum spicatum,* with a concluded 14 day biomass EyC50 = 0.39 mg asulam /L (Seeland-Fremer & Wydra 2014) – indicating a lower level of sensitivity than the Arts and Belger (2013) study.

The newly reported (Seeland-Fremer & Wydra 2014) *Myriophyllum* study was conducted according to the most recent (2014) draft OECD test guideline for this test species and as such is considered by the RMS as more reliable than the Arts and Belgers (2013) study – which was conducted to a different 'in-house methodology' and additional unlike the 2014 study was not GLP compliant. Therefore, the results of the Seeland-Fremer & Wydra (2014) study - in relation to the sensitivity of *Myriophyllum* to asulam - have been used in the aquatic life risk assessment in preference to that from the Arts and Belgers (2013) study.

The 2014 study derived *Myriophyllum spicatum* EyC50 of 0.39 mg asulam /L is less sensitive than the *Lemna gibba* EbC50 of 0.27 mg asulam /L (based on initial measured concentrations) - which was used in the earlier Annex I evaluation risk assessment for asulam. Therefore, in relation to effects on aquatic macrophytes, the relevant most sensitive endpoint for use in the regulatory risk assessment remains the earlier used *Lemna gibba* 14 day EbC50 of 0.27 mg asulam /L derived from the Hoberg (1992) 'first tier' laboratory growth inhibition study. However, when reviewing this regulatory endpoint (which was previously agreed in the EFSA Conclusion Report of 2010), the RMS has identified some issues with the use of this endpoint - which are discussed further below.

Chemical analysis to confirm test concentrations was included in the reported studies with the exception of the *Lemna paucicostata* growth inhibition study (Michel et al 2004) which has been discussed above and also the spiked water sediment dweller chronic toxicity study with *Chironomus riparius* from which a NOEC of 91.3 mg asulam /L has been derived based on nominal test concentrations. This is not considered ideal by the RMS, however given that the derived chironomid endpoint was agreed in the earlier EFSA (2010) Peer review Conclusion Report for asulam, its use has been retained in this re-evlauation.

The other GLP compliance aquatic life toxicity studies and derived endpoints are considered by the RMS as scientifically valid, with the majority of these studies having been previously evaluated in the earlier Annex I evaluation for asulam - with no specific concerns raised in the original Volume 3 B.9 DAR /Addendum or in the EFSA Conclusion report (2010).

Further consideration of previously used most sensitive aquatic macrophyte toxicity endpoint:

The RMS notes that the *Lemna gibba* 14 day EbC50 endpoint of 0.27 mg asulam /L is based on measured initial concentrations, with test concentrations not being maintained over the duration of the study –measured concentrations being 99% of nominal values at the study start but only 15% of nominal at the study end on day 14 (ref. Hoberg 1992, summarised in Section B.9.2.4.1i of this DAR). The RMS agrees with the Notifier's use of initial measured concentrations in deriving the endpoint provided it is made clear in the 'List of Endpoints' that this endpoint relates to a declining level of exposure and that it can be demonstrated that rates of dissipation of asulam in this lab study (approximating to a DT50 of 5 days) would be representative of (or slower than) dissipation rates in natural surface waters. Such an exposure profile comparison approach is common practise in regulatory risk assessment when considering the relevance of results of higher tier 'refined exposure' studies and is considered equally applicable here in relation to this standard 'first tier' laboratory study.

Information in this DAR on the Fate and Behaviour of asulam in water (summarised in Section B.8.4.6) indicates that asulam is hydrolytically stable and that in laboratory aerobic natural water /sediment test systems it declines relatively slowly in surface water from initial levels of between 87-94% of nominal levels to 13-18% after 153 days. However, the results of aqueous photolysis studies indicate relatively rapid photodegradation. Results of an aqueous photolysis study using sterile buffered solutions indicate DT50s of 0.44 days (pH 4) and 0.87 days (pH9) following artificial illumination – which is stated as equivalent to 0.78 and 1.56 days from summer sunlight at central European latitudes (52°N). In another study conducted to a

Japanese guideline using natural water at pH 7.8 the DT_{50} under artificial light was estimated to be 0.84 days – which was stated to be equivalent to 4.21 days spring sunlight at 35°N in Japan, which is comparable to Athens, Southern Europe. Additionally, estimated photolytic half life of asulam in natural surface waters calculated from the quantum yield, ranged from 7 to 119 hours (4.96 days) at pH 4 and 8 to 135 hours (5.6 days) at pH 9 in central European latitudes (52°N).

In summary, the evidence indicates that due to photodegradation asulam is likely to degrade quite rapidly in natural surface waters - with natural sunlight photolysis DT50s estimates ranging between 0.78-5.6 days. Additional, dissipation from surface waters is likely to be further enhanced due to dilution from drainflow and possibly runoff . Therefore, dissipation rates of asulam in natural surface waters are likely to be at least as rapid as that present in the Hoberg (1992) *Lemna gibba* 'first tier' growth inhibition study (i.e. a DT50 of approximnately 5 days) and therefore it is acceptable to use the derived 14 day EbC50 endpoint of 0.27 mg asulam /L (based on initial measured concentrations) in a regulatory acute risk assessment – based on a comparison of this endpoint with the maximum estimated PECsw.

<u>Selection of endpoints for use in the risk assessment:</u>

The concluded asulam and sulphanilamide regulatory study endpoints for fish, aquatic invertebrates (including sediment dwellers), algae and higher aquatic plants are summarised in Table B.9.2.14. For fish and aquatic invertebrates, the most sensitive acute (LC50) and chronic (NOEC) toxicity endpoints have been used in the regulatory risk assessment.

For algae and higher aquatic plants, current aquatic life risk assessment guidance (EFSA 2013) recommends use of a specific growth rate ErC50 in the aquatic risk assessment where this value is available, or the use of a biomass EbC50 as a conservative alternative where the specific growth rate value has not been calculated. With respect to the exposure duration to be considered, for standard first tier laboratory studies the EU assessment usually considers results after 72 hour exposure for algae and after 7 or 14 days exposure for *Lemna* – although this may vary depending on which duration of exposure results in the most sensitive endpoint.

Based on the above criteria, the algal (*Anabaena flosaquae*) 72h ErC50 of >0.66 mg asulam /L has been used in the algal risk assessment. For aquatic plants, not all of the GLP compliant asulam (or asulam sodium) studies calculated an ErC50 value and in these cases the reported EbC50 value has been considered instead, resulting in the most sensitive relevant endpoint for use in the risk assessment relating to the 14 day EbC50 = 0.27 mg asulam /L derived from the Hoberg (1992) first tier *Lemna gibba* growth inhibition study.

Table B.9.2.15 Summary of aquatic life toxicity data for asulam and its major metabolite sulphanilamide

Type of study	Species (test duration)	Test material	EC/LC$_{50}$ #	NOEC #	Reference
Fish					
Acute toxicity	*Oncorhynchus mykiss* (Rainbow trout) (96-hour acute)	Asulam sodium #	>159.8 mg asulam/L (m.m.)	159.8 mg asulam/L (m.m.)	Manning (1988a)
	Lepomis macrochirus (Bluegill sunfish) (96-hour acute)	Asulam sodium #	**>91.3 mg asulam/L** (m.m.)	91.3 mg asulam/L (m.m.)	Machado (2000)
Chronic toxicity	Oncorhynchus mykiss (Rainbow trout) (28-day chronic)	Asulam	>119.1 mg asulam/L (m.m.)	**119.1 mg asulam/L** (m.m.)	McElligott (1997a)
Aquatic invertebrates (including sediment dwellers)					
Acute toxicity	*Daphnia magna* (48-hour acute)	Asulam sodium	**57.87 mg asulam/L** (m.m.)	23.28 mg asulam/L (m.m.)	Manning (1988b)
Chronic toxicity	*Daphnia magna* (21-day chronic)	Asulam	57.1 mg asulam/L (m.m.)	**6.4 mg asulam/L** (m.m.)	McElligott (1997b)
	Daphnia magna (20-day chronic)	'Asulam 80 SG'	21.48 mg asulam/L (nominal) ##	8.96 mg asulam/L (nominal) ##	Herrmann *et al.* (1992a)
	Chironomus riparius (28-day spiked water study in a sediment/water test system)	Asulam sodium	>91.3 mg asulam/L (nominal- assuming all applied active in water phase) ##	**91.3 mg asulam/L** (nominal- assuming all applied active in water phase) ##	Heintze (2002)
Algae					
Standard laboratory (OECD/ EPA) algal growth inhibition studies	*Pseudokirchneriella subcapitata* (120-hour growth)	Asulam sodium	E_bC50 (72h) = 0.06 mg asulam /L (m.m.) E_rC_{50} (72 h) 1.73 mg asulam/L (m.m.)	0.018 mg asulam/L (120h, m.m.)	Hoberg (1992a)/Dorgerloh (2004a)
	Anabaena flos-aquae (120-hour growth)	Asulam sodium	EbC50 (72h) = 0.15 mg asulam /L (m.m.) **E_rC_{50} (72 h) >0.66 mg asulam/L** (m.m.)	0.17 mg asulam/L (120h, m.m.)	Hoberg (1992b)/Dorgerloh (2004b)
	Navicula pelliculosa (120-hour growth)	Asulam sodium	EbC50 (72h) = 3.1 mg asulam /L (m.m.) EbC50 (120h) = 2.1 mg asulam /L (m.m.) E_rC_{50} not calculated.	0.14 mg asulam/L (120h, m.m.)	Hoberg (1992c)
	Skeletonema costatum (120-hour growth)	Asulam sodium	EbC50 (96h) = 0.146 mg asulam /L (m.m.) E_rC_{50} (72 h) >1.64 mg asulam/L (m.m.)	0.02 mg asulam/L (120h, m.m.)	Hoberg (1992d)/Dorgerloh (2004c)

Type of study	Species (test duration)	Test material	EC/LC$_{50}$ #	NOEC #	Reference
	Pseudokirchneriella subcapitata (120-hour growth)	Sulphanil-amide	E_bC_{50} (72 h) 7.6 **E_rC_{50} (72 h) >21.15 (m.m.)**	2.78 mg metabolite /L (120h, m.m.)	Gosch & Sowig (2003)
Higher aquatic plants					
Standard laboratory (OECD/ EPA) *Lemna* growth inhibition studies	*Lemna gibba* (14-day growth)	Asulam sodium	**EbC50 (14 day)** = 0.3 mg asulam sodium /L = **0.27 mg asulam/L** [1] **(initial measured)** ErC50 = 0.16 mg asulam sodium /L = 0.146 mg asulam /L [2] (mean measured) [Mean analysed concns: 99% of nominal at test start & 15% at test end]	0.11 mg asulam/L (14 days, initial measured) 0.051 mg asulam sodium /L (14 days, mean measured)	Hoberg (1992e)
	Lemna gibba (7-day growth)	'Asulam 400g/L SL' (≡ Asulox)	EbC50 (7 day) = 0.32 mg product /L = 0.106 mg asulam /L (nominal) ErC50 (7 day) = 2.56 mg product /L = 0.845 mg asulam /L (nominal) [Mean analysed concns: 97% of nominal at test start & 90% at test end]	0.1 mg product /L = 0.033 mg asulam /L (based on nominal test concns. supported by chemical analysis)	Vinken & Wydra (2007)
	Lemna paucicostata (7-day growth)	Asulam	E_rC50 (14 day) 93.8 mg asulam /L (unsupported nominal conc.[##]) Note: Study not GLP compliant.	-	Michel *et al.* (2004)
	Lemna minor (7-day growth)	Sulphanil-amide	**E_rC_{50} 5.82** E_yC_{50} 2.30 (Based on nominal test concentrations - supported by results of chemical analysis)	0.67 mg/L	Juckeland (2011)

Type of study	Species (test duration)	Test material	EC/LC$_{50}$ #	NOEC #	Reference
Non-standard replicated (x 2-3) study in which two or three excised 10cm long apical shoots of each test species were grown in 1.5 litre vessels (containing test solution without sediment)	Five aquatic macrophytes including: *Myriophyllum spicatum*, *Elodea nuttallii*, *Elodea canadensis*, *Ranunculus circinatus* and *Potamogeton crispus* (21 day study duration, effects assessed on shoot length and biomass).	'Asulox' (400g asulam /litre)	Most sensitive test species *Myriophyllum spicatum* EbC50 (21 day) = 0.0107 mg /L (Based on nominal test concentrations - supported by results of chemical analysis) ###	Not reported	Arts & Belgers (2013)
Lab growth inhibition study conducted to *Myriophyllum* draft OECD test guideline (dated July 2014), using rooted plants in a water sediment test system.	*Myriophyllum spicatum* (14 day growth)	'Asulam 400 g/L SL' (≡ Asulox)	14-day (wet weight) biomass EyC50 = 0.39 mg asulam /L 14-day (wet weight) specific growth rate ErC50 > 2.56 mg asulam /L (Based on nominal test concentrations - supported by results of chemical analysis)	0.01 mg asulam /L (or 0.011 mg asulam sodium /L)	Seeland-Fremer & Wydra (2014)

[#] Effects endpoints assessed at end of study unless indicated otherwise and expressed for active substance studies in term of amounts of asulam, using where required a conversion factor from amounts of asulam sodium to asulam of 0.9128.
[##] These studies are considered to be of limited reliability due to no chemical analysis included to confirm test concentrations at the test start and /or over the duration of the study.
Study non-GLP compliant and 95% confidence intervals for the most sensitive total dry weight EbC50 of 10.7 µg/L are large i.e. 2-74 µg/L - indicating uncertainty in the accuracy of this determined endpoint. Given these deficiencies, the results of this study are not considered sufficiently reliable for regulatory use.
[1] Asulam frond number (biomass) toxicity endpoint (EbC50) derived based on initial measured test concentrations used in aquatic risk assessment.
[2] Asulam sodium specific growth rate toxicity endpoints (ErC50 and NOErC) derived based on mean measured test concentrations used in the CLP active substance hazard classification.

B.9.2.5.2 Aquatic life environmental hazard classification

Asulam sodium has previously been classified under the 'Dangerous Substances Directive 1999/45/EC' as R50 'Very toxic to aquatic organisms' and R53 'May cause long term adverse effects in the aquatic environment'. Under the 'Dangerous Preparations Directive 1999/45/EC' the same classification has also been agreed for its 400g a.s./L soluble concentrate formulated product (which is a simple solution of asulam in water). However, new requirements under the 'Classification, Labelling and packaging of Sustances and Mixtures (CLP) Regulations (reg. EC No 1272/2008, with amendments under Reg. EU No 286/2011) will shortly replace these classification requirements – for which details are included below.

i) Active substance environmental hazard classification under the CLP Regulations

The aquatic life toxicity data indicates that the most sensitive aquatic groups are algae and higher aquatic plants and therefore it is toxicity data relating to these groups which will determine the aquatic hazard classification. With respect to the acute toxicity classification, the most sensitive relevant endpoint relates to a *Lemna gibba* ErC50 of 0.16 mg asulam sodium /L (derived using mean measured test concentrations – as agreed for aquatic hazard classification where the active substance is not maintained within 80-120% of nominal values over the duration of the study). This acute endpoint is less than the CLP acute classification trigger of ≤ 1.0 and on this basis the active substance is classified as 'Acute category 1'.

With respect to chronic toxicity classification, the most sensitive relevant endpoint relates to that reported in *Myriophylum spicatum* growth inhibition study conducted by Seeland-Fremer and Wydra (2014) with a reported 14 day NOErC = 0.011 mg asulam sodium / L. For substances such as asulam sodium that are not 'readily degradable', this chronic endpoint is within the trigger value of ≤ 0.1 mg /L, indicating the need for classification under CLP as 'Chronic category 1' (with an M-factor of 1).

It is concluded that asulam sodium's toxicity to aquatic life indicates the need for classification under the CLP Regulation as 'Acute Category 1' and 'Chronic category 1', with the following labelling requirements:

'GHS09 Pictogram' and associated signal word 'Warning'

Hazard statement: H410 'Very toxicity to aquatic life with long lasting effects' (which also covers need for 'acute category 1' related 'H400' hazard statement)

Precautionary statements: P391 'Collect spillage' and P501 'Dispose of contents container to …' [wording to be completed in accordance with local/ regional/ national/ international regulation].

ii) Formulation environmental hazard classification under the CLP Regulations

The formulation has been classified using the CLP's 'Summation method'. As a simple solution in water, the only 'relevant component' in 'Asulam 400g /L SL' is asulam sodium. In order to classify the formulation, it is first necessary to determine the acute and chronic multiplying factor ('M-factor') - based on the level of toxicity indicated by the most sensitive relevant acute and chronic toxicity endpoints. Using the *Lemna gibba* acute ErC50 of 0.16 mg asulam sodium /L, the acute M-factor is 1. Using the *Myriophyllum spicatum* 14 day NOEC = 0.011 mg asulam sodium /L, for 'non-rapidly degradable' substances (such as asulam sodium) the chronic M-factor is 1.

<u>Determination whether the formulation's aquatic hazard classification is 'Acute category 1':</u>

% w/w of acute category 1' x M-factor = 40 x 1 = 40. This is greater than the CLP trigger value of ≥ 25%, indicating the need for classification of the formulation as 'Acute category 1'.

<u>Determination whether formulation's aquatic hazard classification is 'Chronic category 1:</u>

% w/w of chronic category 1' x M-factor = 40 x 1 = 40. This is greater than the CLP trigger value of ≥ 25%, indicating the need for classification of the formulation as 'Chronic category 1'.

It is concluded that the formulation 'Asulam 400g /L SL' should be classified similarly to its component active substance asulam i.e. as 'Acute Category 1' and 'Chronic Category 1', with the following identical labelling requirements:

'GHS09 Pictogram' and associated signal word 'Warning'

Hazard statement: H410 'Very toxicity to aquatic life with long lasting effects' (which also covers 'Acute Category 1' H400 hazard statement)

Precautionary statements: P391 'Collect spillage' and P501 'Dispose of contents container to …' [wording to be completed in accordance with local/ regional/ national/ international regulation].

B.9.2.5.3 Potential for bioaccumulation /bioconcentration in fish (IIA 8.2.3)

The log Pow values of asulam and its metabolites are below the bioaccumulation trigger value of log 3.0 (asulam log Pow = 0.15), indicating a lack of potential for bioaccumulation in fish and other aquatic organisms. Therefore, the use of asulam does not pose a potential risk to aquatic life from bioaccumulation /bioconcentration .

The lack of potential for bioaccumulation is also supported by the results of an early non-GLP compliant bioaccumulation study, conducted in a static sediment water test system with bottom dwelling catfish (*Ameirus melas*), with levels of bioaccumulation in fish being variable and low – with bioconcentration factors (based on a comparison of concentrations in fish with that in sediment) ranging from 0.1-1.4 and post-exposure 90% clearance times in fish (CT90) of < 7 days (study ref. Jones 1982, summarised in Section B.9.2.1.4 of this DAR). However, the RMS notes that this study differs in important ways from the standard OECD 305 bioconcentration study, particularly in including treated sediment in a static water-sediment test system – as opposed to treated water in a 'constant exposure' flow-through test system. Also, the derived endpoint relates to a comparison of the concentration in whole fish to that in sediment and not that in water (which is usually the case). Theses significant differences both in study methodology and method of determination therefore need to be made clear when quoting the determined 'bioconcentration factors' in the 'List of Endpoints'.

B.9.2.5.4 Aquatic life risk assessment for asulam

i) FOCUS 'Step 1' risk assessment – for aquatic organisms including sediment dwellers

The most sensitive relevant endpoints from the acute and chronic aquatic toxicity studies have been compared against the 'Step 1' scenario PEC_{sw} values (estimated in Table B.8.160 of Section B.8.6 of this report) to derived Step 1 TER values presented in Table B.9.2.16. With the exception of comparison with the chironomid endpoint (see below), the initial (maximum) Step 1 PECsw value has been used.

Given the lack of chemical analysis in the 'spiked water' sediment dweller (*Chironomus riparius*) toxicity study to indicate levels of partitioning of asulam between the water and sediment phases, the study NOEC has been based on a nominal initial water phase concentration calculated assuming all of the applied dose is present in the water phase . Therefore, in order to compare against equivalent exposure values in toxicity exposure ratio calculations , this toxicity endpoint has been compared

against the FOCUS Step 1 'total load' PEC_{sw} (which is similarly estimated - assuming asulam entering surface water is present entirely in the water phase) .

Table B.9.2.16 Asulam TER estimates for aquatic and sediment dwelling organisms – using FOCUS Step 1 maximum PECs for proposed use in spinach and flower bulb crops

Test organism	Test type and duration	Toxicity endpoint (mg asulam/L)	Initial (maximum) PEC_{sw} (mg asulam/L)	TER	TER trigger
Fish (*Lepomis macrochirus*)	Acute, 96 h static test	LC_{50} > 91.3	0.796 #	>114.7	100
Fish (*Oncorhynchus mykiss*)	Chronic, 28 h flow through juvenile growth test	NOEC = 119.1		149.6	10
Daphnia magna	Acute, 48 h flow through test	EC_{50} = 57.87		**72.7**	100
	Chronic 21 day semi-static test	NOEC = 6.4		**8.0**	10
Chironomus riparius	Chronic 28 day spiked water test	NOEC = 91.3		111.1	10
Algae (*Anabaena flosaquae)*	Acute, 120 hour static test	72h E_rC_{50} > 0.66		**> 0.8**	10
Lemna gibba	Acute, 14 day static test	E_bC_{50} = 0.27 *		**0.3**	10

Note: Figures in bold indicate a breaching of the Uniform Principles trigger value.
* Based on effects on frond number
\# Note: Focus Step 1 initial (maximum) PECsw values for proposed crop uses (summarised in Table B.8.160 of Section 8.6) have been used in TER calculations except for sediment dwellers (Chironomid) where a FOCUS Step 1 'total load' PECsw of 822 µg asulam/L has been used (for which details included in paragraph immediately following Table B.8.160 of Section B.8.6) – see above text for explanation.

The TER values for fish and sediment dwellers (*Chironomus riparius*) are greater than the Uniform Principles trigger values, indicating an acceptable risk from asulam exposure to these groups of organisms.

The TER values for *Daphnia*, algae and higher aquatic plants are in breach of Uniform Principles trigger values, indicating a potential risk for these groups of organisms. A 'Step 2' risk assessment is therefore required.

ii) FOCUS 'Step 2' risk assessment

Since the TER values for *Daphnia*, algae and aquatic plants breach the Uniform Principles trigger values at the FOCUS Step 1 exposure level, the risk assessment is taken to FOCUS Step 2 for these groups of organisms using exposure values reported in Section B.8.6. Maximum PEC_{sw} values have been used in the TER calculations. Details are presented in Table B.9.2.17.

Table B.9.2.17 Asulam TER estimates for aquatic organisms – FOCUS Step 2

Scenario	Test organism	Test type & duration	Toxicity endpoint (mg asulam/L)	Maximum PEC_{sw} (mg asulam/L)#	TER	TER trigger
Spinach - Southern Europe (March – May)	*Daphnia magna*	Acute, 48 h flow through test	EC50 57.87	0.19015	304.3	100
		Chronic 21 day semi-static test	NOEC 6.4		33.7	10
	Algae (*Anabaena flosaquae)*	Acute, 72h static test	ErC50 > 0.66		**> 3.5**	10
	Lemna gibba	Acute, 14 day static test	EC50 0.27		**1.4**	10
Spinach - Northern Europe (March to May)	*Daphnia magna*	Acute, 48 h flow through test	EC50 57.87	0.10554	548.3	100
		Chronic 21 day semi-static test	NOEC 6.4		60.6	10
	Algae (*Anabaena flosaquae*)	Acute, 72h static test	ErC50 > 0.66		**> 6.3**	10
	Lemna gibba	Acute, 14 day static test	EC50 0.27		**2.6**	10
Flower bulbs - Southern Europe (March – May)	*Daphnia magna*	Acute, 48 h flow through test	EC50 57.87	0.17321	334.1	100
		Chronic 21 day semi-static test	NOEC 6.4		36.9	10
	Algae (*Anabaena flosaquae)*	Acute, 72h static test	ErC50 > 0.66		**> 3.8**	10
	Lemna gibba	Acute, 14 day static test	EC50 0.27		**1.6**	10
Flower bulbs - Northern Europe (March to May)	*Daphnia magna*	Acute, 48 h flow through test	EC50 57.87	0.09698	596.7	100
		Chronic 21 day semi-static test	NOEC 6.4		66.0	10
	Algae (*Anabaena flosaquae*)	Acute, 72h static test	ErC50 > 0.66		**> 6.8**	10
	Lemna gibba	Acute, 14 day static test	EC50 0.27		**2.8**	10

Note: Figures in bold indicate a breaching of the Uniform Principles trigger value.
PECsw values taken from Table B.8.160 of Section B.8.6 of Volume 3.

The acute and chronic TER values for *Daphnia* are above the Uniform Principles trigger values indicating an acceptable risk to aquatic invertebrates.

The TER values for algae and aquatic plants are in breach of the Uniform Principles trigger values - indicating a potential risk for these groups of organisms. A Step-3 risk assessment is therefore required.

iii) FOCUS 'Step 3' risk assessment

Step 3 PEC_{sw} values 0-100 days after 'peak' exposure levels have been calculated in Section 8.6 for a total of nine water body plus drain flow or run-off scenarios (with two application timings being considered for 6 of these exposure scenarios). These exposure estimates are considered to be representative of EU 'leafy vegetable' growing areas in which surface water is potentially vulnerable to pesticide

contamination via drain-flow or run-off and will effectively cover potential exposure from the proposed use in spinach and flower bulb crops.

The estimated peak (maximum) exposure levels for each of the nine FOCUS Step 3 scenarios have been used in the Step 3 risk assessment – conducted for each of the two aquatic groups which failed at 'Step 2' i.e. algae and higher aquatic plants – see Tables B.9.2.18 and B.9.2.19 respectively.

Table B.9.2.18 Asulam TER estimates for algae using maximum FOCUS Step 3 surface water PECs

Scenario type	**Exposure from spray drift plus drain flow #**				**Exposure from spray drift plus run-off #**				
	D3	**D4**	**D4**	**D6**	**R1**	**R1**	**R2**	**R3**	**R4**
Water body	**ditch**	**pond**	**stream**	**ditch**	**pond**	**stream**	**stream**	**stream**	**stream**
Use in spinach crops:									
PEC_{sw} * [µg asulam /L]	15.253	0.527	12.102	15.350	0.525	10.032	21.850	36.279	62.969
72 h ErC_{50} [µg asulam /L]	> 660								
TER	> 43.3	> 1252.4	> 54.5	> 43.0	>1257.1	> 65.8	> 30.2	> 18.2	> 10.5
Use in flower bulb crops:									
PEC_{sw} * [µg asulam /L]	15.199	0.527	11.827	15.350	0.570	24.052	21.145	36.379	61.081
72 h ErC_{50} [µg asulam /L]	> 660								
TER	> 43.4	> 1252.4	> 55.8	> 43.0	>1157.9	> 27.4	> 31.2	> 18.1	> 10.8

* In scenarios where it is possible to grow a second crop, the maximum PECsw value for the crop which gives the higher exposure value is included.
# Maximum (peak) PECsw values taken from Table B.8.162 and B.8.163 of Section B.8.6 of Volume 3.

Table B.9.2.19 Asulam TER estimates for higher aquatic plants using maximum FOCUS Step 3 surface water PECs

Scenario type	**Exposure from spray drift plus drain flow #**				**Exposure from spray drift plus run-off #**				
	D3	**D4**	**D4**	**D6**	**R1**	**R1**	**R2**	**R3**	**R4**
Water body	**ditch**	**pond**	**stream**	**ditch**	**pond**	**stream**	**stream**	**stream**	**stream**
Use in spinach crops:									
PEC_{sw} * [µg asulam /L]	15.253	0.527	12.102	15.350	0.525	10.032	21.850	36.279	62.969
72 h ErC_{50} [µg asulam /L]	270								
TER	17.7	512.3	22.3	17.6	514.3	26.9	12.4	**7.4**	**4.3**
Use in flower bulb crops:									
PEC_{sw} * [µg a.s asulam /L]	15.199	0.527	11.827	15.350	0.570	24.052	21.145	36.379	61.081
72 h ErC_{50} [µg asulam /L]	270								
TER	17.8	512.3	22.8	17.6	473.7	11.2	12.8	**7.4**	**4.4**

* In scenarios where it is possible to grow a second crop, the maximum PECsw value for the crop which gives the higher exposure value is included.
# Maximum (peak) PECsw values taken from Table B.8.162 and B.8.163 of Section B.8.6

For algae, for both crop uses, all TER values for the Step 3 scenarios pass the Uniform Principles trigger value of 10, indicating an acceptable risk to this group of organisms.

For higher aquatic plants, for both crop uses, TER values at Step 3 pass the Uniform Principles trigger, except for the 'R3 stream' and 'R4 stream' scenarios – for which further refinement / evaluation is required.

iv) FOCUS 'Step 4' risk assessment

For the proposed crop uses in spinach and flower bulbs, the 'Step 3' risk assessment indicates a potential risk to higher aquatic plants for the 'R3 stream' and 'R4 stream' scenarios and therefore the need for a 'FOCUS Step 4' risk assessment.

FOCUS Step 4 PECs for asulam have been estimated by the RMS in Section B.8.6 of the Volume 3 DAR, with inclusion of '80% run-off' mitigation (Section 8.6 Table B.8.166 for spinach and Table B.8.167 for flower bulb crops) or of '80% run-off mitigation plus a 5 metre spray drift buffer' (Section 8.6 Table B.8.170 for spinach and Table B.8.171 for flower bulb crops). However, the additional inclusion of spray drift mitigation has no effect on reducing the maximum PECsw for the 'R3 stream' and 'R4 stream' scenarios – due to these maximum PECs arising solely from run-off contamination. Therefore, with respect to the aquatic plant FOCUS Step 4 refined risk assessment, it is only necessary to consider the risk when run-off mitigation measures are included – as presented for each Step 4 scenario and crop use in the following table.

Table B.9.2.20 Asulam TER estimates for higher aquatic plants using maximum FOCUS Step 4 surface water PECs with 80% run-off mitigation (i.e. surrounding vegetative strip)

Scenario type	**Exposure from spray drift plus drain flow #**				**Exposure from spray drift plus run-off #**				
	D3	**D4**	**D4**	**D6**	**R1**	**R1**	**R2**	**R3**	**R4**
Water body	**ditch**	**pond**	**stream**	**ditch**	**pond**	**stream**	**stream**	**stream**	**stream**
Use in spinach crops:									
PEC_{sw} * [µg asulam /L]	15.253	0.527	12.102	15.350	0.525	10.032	13.492	14.187	14.946
72 h ErC_{50} [µg asulam /L]	270								
TER	17.7	512.3	22.3	17.6	514.3	26.9	20.0	19.0	18.1
Use in flower bulb crops:									
PEC_{sw} * [µg asulam /L]	15.199	0.527	11.827	15.350	0.525	10.025	13.151	14.185	14.514
72 h ErC_{50} [µg asulam /L]	270								
TER	17.8	512.3	22.8	17.6	514.3	26.9	20.5	19.0	18.6

* In scenarios where it is possible to grow a second crop, the maximum PECsw value for the crop which gives the higher exposure value is included.
# Maximum (peak) FOCUS Step 4 PECsw values estimated in Section 8.6 (Tables B.8.166 and B.8.167 for spinach and flower bulb crops respectively).

With a vegetative field strip of sufficient width to reduce run-off by 80%, the derived higher aquatic plant TERs for both crop uses are all within the Uniform Principles trigger value of 10 – indicating an acceptable risk to high aquatic plants (and other aquatic life) when 80% run-off mitigation measures are included.

B.9.2.5.5 Aquatic life risk assessment for asulam's metabolites

An aquatic life risk assessment is required in relation to exposure to sulphanilamide (a minor metabolite in water-sediment degradation studies but major soil metabolite) and MBSC (a potential major metabolite in water-sediment degradation studies but minor soil metabolite). Additional, an aquatic life risk assessment is required for three further major metabolites identified in aqueous photolysis studies i.e. sulphanilic acid, AP formamide and MCAPAP carbamic acid. Each is considered in turn below.

i) Aquatic life risk assessment for sulphanilamide

Aquatic toxicity data for asulam indicates it toxicity to algae and aquatic plants to be approximately at least 100 fold higher than its toxicity to fish or aquatic invertebrates (the latter including *Daphnia magna* and the sediment dwelling midge *Chironomus riparius*). Taking this into account, in line with earlier SANCO aquatic ecotoxicology guidance (Section 6.6 of SANCO/3268/2001, October 2002), the Notifier has argued that it is acceptable to restrict toxicity testing on asulam's structural related metabolite 'sulphanilamide' to algae / higher aquatic plants, with no specific toxicity testing or risk assessment required for fish and aquatic invertebrates (including sediment dwellers). This argument was previously accepted in the earlier Annex I evaluation of asulam (ref. EFSA Conclusion report, 2010) and on this basis is also considered acceptable by the RMS in relation to the current submission.

In support of the current submission, details have been provided for a previously evaluated sulphanilamide algal growth inhibition study conducted with *Pseudokirchneriella subcapitata* (study details summarised in Section B.9.2.3.2 of this DAR) - for which a 72h, 96h and 120h specific growth rate ErC50 of >21.15 mg metabolite /L was concluded in the earlier Annex I evaluation (ref. EFSA Conclusion Report for asulam, 2010). Details have also been provided for a newly conducted sulphanilamide 'higher aquatic plant' (*Lemna*) growth inhibition study (see Section B.9.2.4.2 of this DAR for study details) for which the most relevant derived study endpoint for the regulatory risk assessment is considered to be a specific growth rate 7 day ErC50 of 5.82 mg sulphanilamide /L (based on changes in frond number).

FOCUS 'Step 1' risk assessment for sulphanilamide

The relevant endpoints for the toxicity of sulphanilamide to algae and higher plants have been compared against the 'Step 1' scenario maximum PEC_{sw} value - see Table B.9.2.21.

Table B.9.2.21 Sulphanilamide algae and higher aquatic plant TER estimates - using the FOCUS Step 1 maximum surface water PEC from proposed uses in spinach and flower bulb crops.

Test organism	Test type and duration	Toxicity endpoint (mg/L)	PEC_{sw} # (mg/L)	TER	TER trigger
Pseudokirchneriella subcapitata (algae)	Acute, 120 h static test	72 h E_rC_{50} > 21.15	0.08635	244.9	10
Lemna minor (higher aquatic plant)	Acute, 7 day static test	7 day ErC_{50} = 5.82		67.4	10

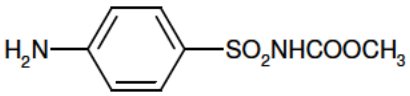
Note: Figures in bold indicate a breaching of the Annex VI trigger
Initial (maximum) Step 1 PECsw from Table B.8.160 of Section B.8.6 of Volume 3.

The derived TER values are greater than the trigger value of 10, indicating an acceptable risk from sulphanilamide exposure to algae and higher aquatic plants. Given the above concluded likelihood of a much lower toxicity to fish and aquatic invertebrates than to algae and higher aquatic plants, the risk to fish and aquatic invertebrates (including sediment dwellers) from sulphanilamide exposure will be covered by the risk assessment for algae and aquatic plants and on this basis a low and acceptable risk can also be concluded for these aquatic groups. No further evaluation is required.

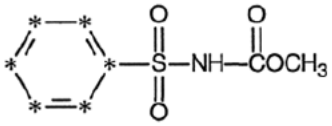
ii) Aquatic life risk assessment for the metabolite MBSC.

A comparison of the chemical structure of MBSC and asulam is included below.

Asulam:



MBSC:



The RMS notes that the chemical structure of MBSC is identical to asulam except for the lack of the NH_2 group. Given this small difference, the RMS considers that MBSC is likely to be of a similar or lower toxicity to aquatic life than asulam. However, no aquatic toxicity studies have been conducted using MBSC to confirm the relative toxicity of MBSC to aquatic life compared with that for asulam. Therefore a conservative assumption has been made in the aquatic risk assessment that MBSC is 10 times more toxic to aquatic life than asulam.

Taking into account that the toxicity of asulam to algae and higher aquatic plants is approximately an order of magnitude (or greater) higher than that to fish and aquatic invertebrates (including sediment dwellers), the same has been assumed for MBSC, with the following aquatic risk assessment only specifically including algae and higher aquatic plants (the risk to fish and aquatic invertebrates being covered by the assessment for these two most sensitive groups):

Table B.9.2.22 Algae and higher aquatic plants TERs for the metabolite 'MBSC' using FOCUS Step 3 maximum surface water PECs for the proposed crop uses.

Scenario type	**Exposure from spray drift plus drain flow #**				**Exposure from spray drift plus run-off #**				
	D3	**D4**	**D4**	**D6**	**R1**	**R1**	**R2**	**R3**	**R4**
Water body	**ditch**	**pond**	**stream**	**ditch**	**pond**	**stream**	**stream**	**stream**	**stream**
Assumed algal 72 h ErC_{50} [µg MBSC /L]##	66	66	66	66	66	66	66	66	66
Assumed *Lemna* 14 day EbC50 [µg MBSC /L]##	27	27	27	27	27	27	27	27	27
Use in spinach crops:									
PEC_{sw} * [µg MBSC. /L]	1.623	0.214	0.242	1.620	0.051	0.150	0.493	1.657	3.714
Algal TER:	40.7	308.4	272.7	40.7	1294.1	440.0	133.9	39.8	17.8
Lemna TER:	16.6	126.2	111.6	16.7	529.4	180.0	54.8	16.3	**7.3**
Use in flower bulb crops:									
PEC_{sw} * [µg MBSC /L]	1.476	0.455	1.178	1.479	0.051	0.746	0.462	0.525	1.518
Algal TER:	44.7	145.1	56.0	44.6	1294.1	88.5	142.9	125.7	43.5
Lemna TER:	18.3	59.3	22.9	18.3	529.4	36.2	58.4	51.4	17.8

* In scenarios where it is possible to grow a second crop, the maximum PECsw value for the crop which gives the higher exposure value is included.
[#] Maximum (peak) PECsw values taken from Table B.8.180 (spinach) and B.8.181 (flower bulbs) of Section B.8.6.

Based on extrapolation of asulam toxicity endpoints assuming a 10 fold higher toxicity of the MBSC metabolite.

For use in flower bulb crops, the algal and higher aquatic plant TERs are all in excess of the trigger value of 10 indicating an acceptable risk to aquatic life. This is also the case for use in spinach crops, with the exception of one out of nine of the *Lemna* TERs - with a TER of 7.3 for the 'R4 stream' scenario. However, given that the dominant route of entry route for this scenario is run-off (as indicated in Table B.8.168 of Section B.8.6), there is the potential to include run-off mitigation measures (vegetative strips) to reduce exposure to acceptable levels.

It is concluded that the proposed use in flower bulb crops poses a low risk to aquatic life from MBSC exposure. For the proposed use in spinach crops, there is a potential risk from MBSC exposure in one out of nine of the FOCUSsw scenarios, which will be adequately addressed by the inclusion of a vegetative strip of sufficient width to provide a 30% or greater reduction in run-off exposure.

iii) Aquatic life risk assessment for photolytic metabolites

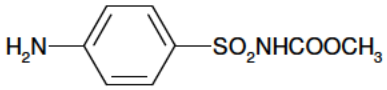
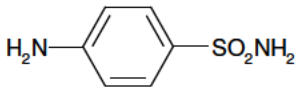
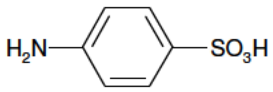
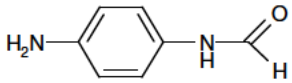
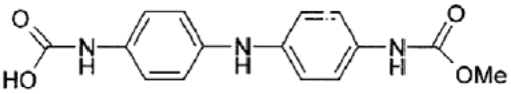
In an aqueous photolysis study using sterile aqueous buffer solutions at pH4 and pH9 several metabolites were formed in major amounts (i.e. >10% AR), although there were no metabolites formed in major amounts in another photolysis study using sterile natural surface water of pH7.8 taken from a UK reservoir (study details and summary in respectively Sections B.8.4.2 and B.8.4.6 of Volume 3 DAR). Major metabolites in the aqueous buffer solution studies included 'sulphanilic acid' (formed at pH9 at upto a maximum of 55.5% AR after 20 hours), 'AP formamide' (formed at pH4 at upto a maximum 24.2% AR) and 'MCAPAP carbamic acid' (formed at pH4 at upto a maximum 11.9% AR). Although the tested pHs in these studies are considered to be fairly extreme in relation to that likely to occur in natural surface waters in Europe, it cannot be excluded that these metabolites might be formed in 'major' amounts and therefore a risk assessment is required.

In addition to the above mentioned major metabolites, a number of minor metabolites (<10% AR) were also formed in both the 'buffered solution' and 'natural water' photolysis studies. However, given that these minor metabolites are unlikely to be greater than a 10 fold more toxic to aquatic life than the parent active substance asulam, any risk from exposure to these minor metabolites will be covered by the risk assessment conducted for asulam and therefore no specific 'minor metabolite' risk assessment is required.

No aquatic toxicity studies have been conducted with the three identified 'major' photodegradate metabolites. The Notifier has referred to the IUCLID dataset created by the European Chemicals Bureau of the European Commission which includes endpoints for the sulphanilic acid metabolite (ref. Substance 121-57-3, IUCLID Dataset, ECB, EC 2000) – with the specified endpoints indicating a low or moderate toxicity to fish (*Pimephales promelas* 96h LC50 = 100.4 mg/l), aquatic invertebrates (*Daphnia magna* 48h EC50 85.7 mg/l) and algae (*Scenedesmus subspicatus* 72h EbC50 = 91.0 mg/l and 72h ErC50 = 375 mg/l). However, the study details /reasoning supporting the referenced sulphanilic acid endpoints have not been provided and the position regarding data protection is also unclear.

The Notifier has argued given 'these metabolites do not contain any toxicological alerts on their structures', that they are consequently likely to be of lower toxicity than asulam and therefore the risk assessment for asulam (which will be present at a higher maximum concentrations in surface water) will also cover the risk from exposure to these photodegradates. A comparison of the structural formulas for the three 'major' photodegradates with that for asulam and its major soil metabolite sulphanilamide is included in Table B.9.2.23.

Table B.9.2.23 Comparison of the structural formula for asulam with that for its major soil metabolite sulphanilamide and for its three major aquatic photodegradates (sulphanilic acid, 'AP formamide' and 'MCAPAP carbamic acid').

Common name	Structural formula and chemical name
Asulam	 Methyl sulphanilylcarbamate
Sulphanilamide	 4-Aminobenzenesulphnamide
Sulphanilic acid	 4-Aminobenzene sulphonic acid
AP formamide	 N-(4-Aminophenyl)formamide
MCAPAP carbamic acid	 4(4-methoxycarbonylaminophenyl)aminophenyl carbamic acid

None of the three photodegradates, or the major soil metabolite sulphanilamide, contain the '$SO_2NHC00CH_3$' chemical group present in asulam - which appears to be the toxophore associated with the higher toxicity of asulam to algae (e.g. *Pseudokirchneriella subcapitata* 72h ErC50s of 1.73 mg /L for asulam compared with >21.15 mg /L for sulphanilamide) and higher aquatic plants (e.g. *Lemna gibba* 14 day frond number 'EbC50' of 0.27 mg /L for asulam compared with *Lemna minor* 7 day frond number 'EyC50' of 2.3 mg /L for sulphanilamide). Therefore, it is considered by the RMS that the aquatic toxicity of asulam's photodegradates is likely to be lower than that for asulam. However, given that the evidence to support a lower toxicity than asulam is limited, the RMS has conducted an aquatic life risk assessment in the following tables, based on use of FOCUS Step 3 and Step 4 maximum PECsw estimates for these photodegradates (ref. Tables B.8.174-B.8.177 of Section B.8.6) and

on the conservative assumption that these metabolites are ten times more toxic to aquatic life than asulam (this approach being broadly in line with that suggested in Section 6.6 of SANCO/3268/2001 Guidance Document on Aquatic Ecotoxicology).

Table B.9.2.24 Sulphanilic acid TERs for algae and higher aquatic plants based on use of maximum FOCUS Step 3 surface water exposure PEC estimates

Scenario type	**Exposure from spray drift plus drain flow #**				**Exposure from spray drift plus run-off #**				
	D3	**D4**	**D4**	**D6**	**R1**	**R1**	**R2**	**R3**	**R4**
Water body	**ditch**	**pond**	**stream**	**ditch**	**pond**	**stream**	**stream**	**stream**	**stream**
Assumed algal 72 h ErC_{50} [µg met./L] ##	66	66	66	66	66	66	66	66	66
Assumed *Lemna* 7 day ErC50 [µg met./L]##	27	27	27	27	27	27	27	27	27
Use in spinach crops:									
PEC_{sw} * [µg met./L]	6.367	0.22	5.052	6.408	0.219	4.188	9.121	15.145	26.287
Algal TER:	10.4	300.0	13.1	10.3	301.4	15.8	**7.2**	**4.4**	**2.5**
Lemna TER:	**4.2**	122.7	**5.3**	**4.2**	123.3	**6.4**	**3.0**	**1.8**	**1.0**
Use in flower bulb crops:									
PEC_{sw} * [µg met./L]	6.345	0.22	4.937	6.408	0.238	10.041	8.827	15.187	25.499
Algal TER:	10.4	300.0	13.4	10.3	277.3	**6.6**	**7.5**	**4.3**	**2.6**
Lemna TER:	**4.3**	122.7	**5.5**	**4.2**	113.4	**2.7**	**3.1**	**1.8**	**1.1**

* In scenarios where it is possible to grow a second crop, the maximum PECsw value for the crop which gives the higher exposure value is included.
# Maximum (peak) PECsw values taken from Table B.8.174 (spinach) and B.8.175 (flower bulbs) of Section B.6
µg met./L = µg metabolite/L. Concentration based on extrapolation of asulam toxicity endpoints assuming a 10 fold higher toxicity of the sulphanilic acid metabolite.

Table B.9.2.25 AP formamide TER estimates for algae and higher aquatic plants based on use of maximum FOCUS Step 3 surface water exposure PEC estimates

Scenario type	**Exposure from spray drift plus drain flow #**				**Exposure from spray drift plus run-off #**				
	D3	**D4**	**D4**	**D6**	**R1**	**R1**	**R2**	**R3**	**R4**
Water body	**ditch**	**pond**	**stream**	**ditch**	**pond**	**stream**	**stream**	**stream**	**stream**
Assumed algal 72 h ErC_{50} [µg met./L]##	66	66	66	66	66	66	66	66	66
Assumed *Lemna* 7 day ErC50 [µg met./L]##	27	27	27	27	27	27	27	27	27
Use in spinach crops:									
PEC_{sw} * [µg met. /L]	2.183	0.075	1.732	2.197	0.075	1.436	3.127	5.191	9.011
Algal TER:	30.2	880.0	38.1	30.0	880.0	46.0	21.1	12.7	**7.3**
Lemna TER:	12.4	360.0	15.6	12.3	360.0	18.8	**8.6**	**5.2**	**3.0**
Use in flower bulb crops:									
PEC_{sw} * [µg met. /L]	2.175	0.075	1.692	2.197	0.082	3.442	3.026	5.206	8.74
Algal TER:	30.3	880.0	39.0	30.0	804.9	19.2	21.8	12.7	**7.6**
Lemna TER:	12.4	360.0	16.0	12.3	329.3	**7.8**	**8.9**	**5.2**	**3.1**

* In scenarios where it is possible to grow a second crop, the maximum PECsw value for the crop which gives the higher exposure value is included.
# Maximum (peak) PECsw values taken from Table B.8.174 (spinach) and B.8.175 (flower bulbs) of Section B.6
µg met./L = µg metabolite/L. Concentration based on extrapolation of asulam toxicity endpoints assuming a 10 fold higher toxicity of the AP formamide metabolite.

Table B.9.2.26 MCAPAP carbamic acid TER estimates for algae and higher aquatic plants based on use of maximum FOCUS Step 3 surface water exposure PEC estimates

Scenario type	**Exposure from spray drift plus drain flow #**				**Exposure from spray drift plus run-off #**				
	D3	**D4**	**D4**	**D6**	**R1**	**R1**	**R2**	**R3**	**R4**
Water body	**ditch**	**pond**	**stream**	**ditch**	**pond**	**stream**	**stream**	**stream**	**stream**
Assumed algal 72 h ErC_{50} [µg met. /L]##	66	66	66	66	66	66	66	66	66
Assumed *Lemna* 7 day ErC50 [µg met. /L]##	27	27	27	27	27	27	27	27	27
Use in spinach crops:									
PEC_{sw} * [µg met. /L]	2.375	0.082	1.885	2.391	0.082	1.562	3.403	5.65	9.806
Algal TER:	27.8	804.9	35.0	27.6	804.9	42.3	19.4	11.7	**6.7**
Lemna TER:	11.4	329.3	14.3	11.3	329.3	17.3	**7.9**	**4.8**	**2.8**
Use in flower bulb crops:									
PEC_{sw} * [µg met. /L]	2.367	0.082	1.842	2.391	0.089	3.746	3.293	5.665	9.512
Algal TER:	27.9	804.9	35.8	27.6	741.6	17.6	20.0	11.7	**6.9**
Lemna TER:	11.4	329.3	14.7	11.3	303.4	**7.2**	**8.2**	**4.8**	**2.8**

* In scenarios where it is possible to grow a second crop, the maximum PECsw value for the crop which gives the higher exposure value is included.
# Maximum (peak) PECsw values taken from Table B.8.174 (spinach) and B.8.175 (flower bulbs) of Section B.6.
µg met./L = µg metabolite /L. Concentration based on extrapolation of asulam toxicity endpoints assuming a 10 fold higher toxicity of the MCAPAP carbamic acid metabolite.

For the proposed use in spinach crops, using 'Step 3' exposure values, the Uniform Principles TER trigger of 10 is breached for the three photodegradates in 1-3 FOCUS scenarios for algae and 3-7 FOCUS scenarios for higher aquatic plants (*Lemna*), out of a total of 9 FOCUS scenarios considered for this use. For the proposed use in flower bulb crops, using 'Step 3' exposure values, the Uniform Principles TER trigger of 10 is breached for all three photodegradates in 1-4 FOCUS scenarios for algae and 4-7 FOCUS scenarios for higher aquatic plants (*Lemna*), out of a total of 9 FOCUS scenarios considered for this use. It is concluded that use of the FOCUS Step 3 exposure values are insufficient to demonstrate an acceptable risk from the photolytic metabolites. A further risk assessment has therefore been conducted using FOCUS Step 4 exposure values, with inclusion of exposure mitigation measures - in the form of a 5 metre 'no spray' spray drift buffer zone plus vegetative field strip of sufficient width to reduce run-off by 80%.

Table B.9.2.27 Sulphanilic acid TER estimates for algae and higher aquatic plants based on use of maximum FOCUS Step 4 surface water exposure PEC values (including a 5 metre spray drift buffer plus 80% run-off mitigation)

Scenario type	**Exposure from spray drift plus drain flow #**				**Exposure from spray drift plus run-off #**				
	D3	**D4**	**D4**	**D6**	**R1**	**R1**	**R2**	**R3**	**R4**
Water body	**ditch**	**pond**	**stream**	**ditch**	**pond**	**stream**	**stream**	**stream**	**stream**
Assumed algal 72 h ErC_{50} [µg met./L]##	66	66	66	66	66	66	66	66	66
Assumed *Lemna* 7 day ErC50 [µg met./L]##	27	27	27	27	27	27	27	27	27
Use in spinach crops:									
PEC_{sw} * [µg met./L]	1.726	0.191	1.846	2.348	0.19	1.53	2.111	3.526	6.239
Algal TER:	38.2	345.5	35.8	28.1	347.4	43.1	31.3	18.7	10.6
Lemna TER:	15.6	141.4	14.6	11.5	142.1	17.6	12.8	**7.7**	**4.3**
Use in flower bulb crops:									
PEC_{sw} * [µg met./L]	1.72	0.191	1.804	1.786	0.19	2.161	2.044	3.542	6.059
Algal TER:	38.4	345.5	36.6	37.0	347.4	30.5	32.3	18.6	10.9
Lemna TER:	15.7	141.4	15.0	15.1	142.1	12.5	13.2	**7.6**	**4.5**

* In scenarios where it is possible to grow a second crop, the maximum PECsw value for the crop which gives the higher exposure value is included.
# Maximum (peak) PECsw values taken from Table B8.176 and Table B.8.177 of Section B.8.6 of Volume 3.
µg met./L = µg metabolite /L. Concentration based on extrapolation of asulam toxicity endpoints assuming a 10 fold higher toxicity of the sulphanilic acid metabolite.

Table B.9.2.28 AP formamide TER estimates for algae and higher aquatic plants based on use of maximum FOCUS Step 4 surface water exposure PEC values (including a 5 metre spray drift buffer plus 80% run-off mitigation)

Scenario type	**Exposure from spray drift plus drain flow #**				**Exposure from spray drift plus run-off #**				
	D3	**D4**	**D4**	**D6**	**R1**	**R1**	**R2**	**R3**	**R4**
Water body	**ditch**	**pond**	**stream**	**ditch**	**pond**	**stream**	**stream**	**stream**	**stream**
Assumed algal 72 h ErC_{50} [µg met. /L] ##	66	66	66	66	66	66	66	66	66
Assumed *Lemna* 7 day ErC50 [µg met. /L] ##	27	27	27	27	27	27	27	27	27
Use in spinach crops:									
PEC_{sw} * [µg met. /L]	0.592	0.065	0.633	0.805	0.065	0.524	0.724	1.209	2.139
Algal TER:	111.5	1015.4	104.3	82.0	1015.4	126.0	91.2	54.6	30.9
Lemna TER:	45.6	415.4	42.7	33.5	415.4	51.5	37.3	22.3	12.6
Use in flower bulb crops:									
PEC_{sw} * [µg met. /L]	0.589	0.065	0.618	0.612	0.065	0.741	0.701	1.214	2.077
Algal TER:	112.1	1015.4	106.8	107.8	1015.4	89.1	94.2	54.4	31.8
Lemna TER:	45.8	415.4	43.7	44.1	415.4	36.4	38.5	22.2	13.0

* In scenarios where it is possible to grow a second crop, the maximum PECsw value for the crop which gives the higher exposure value is included.
# Maximum (peak) PECsw values taken from Table B.8.176 and Table B.8.177 of Section B.8.6.
Base µg met./L = µg metabolite /L. Concentration based on extrapolation of asulam toxicity endpoints assuming a 10 fold higher toxicity of the AP formamide metabolite.

Table B.9.2.29 MCAPAP carbamic acid TER estimates for algae and higher aquatic plants based on use of maximum FOCUS Step 4 surface water exposure PEC values (including a 5 metre spray drift buffer plus 80% run-off mitigation).

Scenario type	Exposure from spray drift plus drain flow #				Exposure from spray drift plus run-off #				
	D3	D4	D4	D6	R1	R1	R2	R3	R4
Water body	ditch	pond	stream	ditch	pond	stream	stream	stream	stream
Assumed algal 72 h ErC_{50} [µg met./L] ##	66	66	66	66	66	66	66	66	66
Assumed *Lemna* 7 day ErC50 [µg a.s./L] ##	27	27	27	27	27	27	27	27	27
Use in spinach crops:									
PEC_{sw} * [µg met. /L]	0.644	0.071	0.689	0.876	0.071	0.571	0.788	1.315	2.328
Algal TER:	102.5	929.6	95.8	75.3	929.6	115.6	83.8	50.2	28.4
Lemna TER:	41.9	380.3	39.2	30.8	380.3	47.3	34.3	20.5	11.6
Use in flower bulb crops:									
PEC_{sw} * [µg met. /L]	0.641	0.071	0.673	0.666	0.071	0.806	0.762	1.321	2.26
Algal TER:	103.0	929.6	98.1	99.1	929.6	81.9	86.6	50.0	29.2
Lemna TER:	42.1	380.3	40.1	40.5	380.3	33.5	35.4	20.4	11.9

* In scenarios where it is possible to grow a second crop, the maximum PECsw value for the crop which gives the higher exposure value is included.
# Maximum (peak) PECsw values taken from Table B.8.176 and Table B.8.177 of Section B.8.6.
µg met./L = µg metabolite /L. Concentration based on extrapolation of asulam toxicity endpoints assuming a 10 fold higher toxicity of the MCAPAP carbamic acid metabolite.

Based on the FOCUS Step 4 exposure estimates, for both crop uses with the inclusion of 5 metre spray drift buffer plus 80% run-off mitigation, the algae and higher aquatic plant (*Lemna*) TERs are within the Uniform Principles trigger value of 10 for AP formamide and MCAPAP, indicating an acceptable risk. For sulphanilic acid, the TERs are within this trigger value for algae but for both crop uses in breach of it in two out of the nine scenarios for higher aquatic plants (*Lemna* TERs 7.7 and 4.3 for spinach and 7.6 and 4.5 for flower bulbs). However, the RMS considers the included assumption of a 10 fold higher toxicity of this metabolite than the parent active substance asulam is very conservative, particularly given the absence of the '$SO_2NHC00CH_3$' chemical group – which is a likely toxophore (see discussion above). Taking this into account, the RMS concludes that with the inclusion of 5 metre spray drift buffer plus 80% run-off mitigation, exposure to these photolytic metabolites poses a low and acceptable risk to aquatic life.

B.9.2.5.6 Conclusions regarding toxicity and risk to aquatic life

Asulox is of low to moderate toxicity to fish and aquatic invertebrates (including sediment dwellers) but of high toxicity to algae and higher aquatic plants. The log Pow values of asulam and its metabolites are below the bioaccumulation trigger value of log 3.0 (asulam log Pow = 0.15), indicating a lack of potential for bioaccumulation in fish and other aquatic organisms.

Based on asulam's high toxicity to algae and higher aquatic plants and lack of rapid degradability, both asulam and its formulated product 'Asulox' should be classified under the 'CLP Regulation' as 'acute category 1' and 'chronic category 1' (with H410 hazard statement 'Very toxic to aquatic life with long lasting effects') For further labelling details see Section B.9.5.2.

The conducted risk assessment indicates that for the proposed uses in spinach and flower bulb crops risk mitigation measures are required to protect aquatic life. The

conducted FOCUS Step 4 risk assessment is considered sufficient to indicates an acceptable risk to aquatic life with the inclusion of a 5 metre 'no spray' spray drift buffer zone plus a vegetative field strip of sufficient width to reduce run-off by 80%. However, individual Member States may choose to use other risk mitigation measures.

B.9.3 Effects on terrestrial vertebrates other than birds

B.9.3.1 Toxicity

Data on the mammalian toxicity of asulam are summarised in Section B.6 'Mammalian Toxicology' of the Volume 3 DAR. No additional data have been provided in this submission over that considered previously for the earlier Annex I evaluation of asulam. A summary of mammalian toxicity endpoints relevant to the 'other terrestrial vertebrate' wildlife risk assessment is presented below in Table B.9.3.1, with the previously agreed toxicity endpoints for use in the ecotoxicology risk assessment highlighted in bold.

Table B.9.3.1 Summary of mammalian toxicity endpoints relevant to the 'other terrestrial vertebrate' risk assessment.

Type of exposure	Test substance	Toxicity*	References Doc. No.
Acute oral toxicity on rat	'Asulox'	LD_{50} >2000 mg product /kg bw #	[redacted] (2001n)
Acute oral toxicity on rat	Asulam sodium Asulam sodium	**LD_{50}** > 5000 mg asulam sodium /kg bw ≡ **>4564 mg asulam/kg bw** As above.	[redacted] (1987) [redacted] (1988)
2-generation study on rat	Asulam sodium	NOAEL = **46 mg asulam/kg bw/day**	[redacted] (1981)

No mortality or clinical signs of toxicity observed in the study
* Values in bold = previously agreed regulatory endpoints (EFSA conclusion report) - used in the previous and current 'Other terrestrial vertebrate' risk assessment.

B.9.3.2 Risk assessment

The risk to wild mammals from the proposed crop uses of Asulox (containing 400g asulam/L) has been evaluated based on current EFSA (2009) risk assessment guidance. This differs from that used in the previous Ecotoxicology Volume 3 DAR (2006) - which was based on earlier SANCO/4145/2000 (2002) methodology.

Given that the formulation 'Asulam 400g/L SL' is a simple solution of asulam in water, it does not pose any additional risk over that from the active substance. Therefore, the risk from the formulation will be covered by the asulam risk assessment.

B.9.3.2.1 Risk assessment for wild mammals (from dietary route of exposure)– 'screening step'

The screening step crop groupings, critical use patterns and indicator species relevant to the uses of 'Asulox' are given in Table B.9.3.2.

Table B.9.3.2: Screening step crop groupings and critical use patterns relevant to the use of 'Asulox'.

Crop group	Critical GAP crop	Indicator species	Critical use pattern		
			Rate[a] (g asulam /ha)	No. of apps	App. Interval
'Bare soils ...'	Spinach (pre-emergence i.e. BBCH <10)	Small granivorous mammal	2400	1	-
'Bulbs and onion like crops …'	Flower bulbs (post-emergence, all growth stages)	Small herbivorous mammal	2400	1	-
'... leafy vegetables ...'	Spinach (post-emergence BBCH 12-14)	Small herbivorous mammal	2400	1	-

[a] Maximum application rate

Estimation of acute daily dietary dose:

The acute 'daily dietary dose' (DDD)for each indicator species is calculated by multiplying the 90th percentile shortcut value (SV_{90}) by the application rate in kg a.s. /ha.

DDD = application rate (kg **asulam** /ha) x SV_{90}

In the case of the use on spinach and flower bulbs there is only one application and so a MAF_{90} is not required. Details are included in Table B.9.3.3.

Table B.9.3.3: Screening step – estimation of acute exposure to asulam

Crop group	Indicator species	90th percentile shortcut value	App. rate (kg asulam /ha)	MAF_{90}	DDD (mg a.s./kg bw/ day)
'Bare soils ...'	Small granivorous mammal	14.4	2.4	1.0	34.6
'Bulbs and onion like crops'	Small herbivorous mammal	118.4	2.4	1.0	284.2
'... leafy vegetables ...'	Small herbivorous mammal	136.4	2.4	1	327.4

Estimation of long-term daily dietary dose:

The long-term 'daily dietary dose' (DDD) for each indicator species is calculated by multiplying the mean shortcut value (SV_{mean}) based on the mean residues by the application rate in kg a.s./ha.

DDD = application rate (kg **asulam** /ha) x SV_{mean} x f_{twa}

The 21-day f_{twa} based upon a default DT_{50} of 10 days is 0.53, as given in the EFSA Guidance Document.

In the case of the use on spinach and flower bulbs there is only one application and so a MAF_m is not required. Details are included in Table B.9.3.4.

Table B.9.3.4: Screening step – estimation of long-term exposure to asulam

Crop group	Indicator species	Mean shortcut value	App. rate (kg asulam /ha)	MAF_m	f_{twa}	DDD (mg asulam /kg bw/ day)
'Bare soils ...'	Small granivorous mammal	6.6	2.4	1.0	0.53	8.4
'Bulbs and onion like crops'	Small herbivorous mammal	48.3	2.4	1.0	0.53	61.4
'... leafy vegetables ...'	Small herbivorous mammal	72.3	2.4	1.0	0.53	92.0

Screening step acute toxicity exposure ratio (TER_A) for wild mammals:

The acute risk to wild mammals from asulam was assessed by calculating toxicity exposure ratios (TER_A) using the following equation:

$$TER_A = \frac{LD_{50}\ (mg/kg bw/day)}{Acute DDD (mg/kg bw/day)}$$

The resulting TER_A values are given in Table B.9.3.5. The acute TERs are all greater than the Uniform Principles acute TER trigger value of 10 indicating a low and acceptable acute risk to wild mammals from the proposed crop uses of asulam.

Table B.9.3.5 Screening step – acute risk to wild mammals from dietary exposure

Indicator species (and related crop uses)	LD_{50} (mg asulam /kg bw/day)	Acute DDD (mg asulam /kg bw/day)	TER_A	Uniform Principles trigger value
Small granivorous mammal (pre-crop emergence use in spinach)	>4564	34.6	>131.9	10
Small herbivorous mammal (post-crop emergence in flower bulbs)		284.2	>16.1	10
Small herbivorous mammal (post-crop emergence in spinach)		327.4	>13.9	10

Screening step long-term (reproductive) toxicity exposure ratio (TER_{LT}) for wild mammals:

The long-term toxicity exposure ratio (TER_{LT}) for asulam following application of Asulox is calculated using the following equation:

$$TER_{LT} = \frac{NOAEL(mg/kgbw/day)}{Long\text{-}term\ ETE(mg/kgbw/day)}$$

The resulting TER_{LT} values are given in Table B.9.3.6. The long-term TER for small granivorous mammals exposed to a pre-crop emergence treatment in spinach crops is in excess of the Uniform Principles trigger value of 5 indicating an acceptable risk. However the long-term TERs for small herbivorous mammals exposed to post-crop emergence treatment in flower bulb and spinach crops are both in breach of this trigger value - indicating the need for a 'Tier 1' long-term risk assessment for these post-crop emergence uses.

Table B.9.3.6 Screening step – long-term (reproductive) risk to wild mammals from dietary exposure

Indicator species (and related crop uses)	NOAEL (mg asulam /kg bw/day)	Long-term DDD (mg asulam /kg bw/day)	TER_{LT}	Uniform Principles trigger value
Small granivorous mammal (pre-crop emergence use in spinach)	46	8.4	5.5	5
Small herbivorous mammal (post-crop emergence in flower bulbs)		61.4	**0.7**	5
Small herbivorous mammal (post-crop emergence in spinach)		92.0	**0.5**	5

B.9.3.2.2 Tier 1 long-term (reproductive) risk assessment (from dietary exposure) for post-crop emergence use in spinach and flower bulb crops

The screening assessment indicates an acceptable acute and long-term risk to wild mammals from the proposed pre-crop emergence use in spinach crops and also an acceptable acute risk to wild mammals from post-crop emergence use in flower bulbs and spinach crops. However, a potential long-term risk to wild mammals from post-crop emergence use in flower bulbs and spinach crops has been identified - indicating the need for a more refined 'Tier 1' long-term risk assessment for these crop uses.

i) Long-term (reproductive) risk from post-crop emergence use in spinach BBCH 12-14 (Tier 1 'Leafy vegetables' use scenario):

Details of the relevant generic focal species, mean short-cut values and relevant crop stages are as follows:
Small insectivorous mammal ("shrew") – 4.2 (BBCH 10-19)
Large herbivorous mammal ("lagomorph") – 14.3 (all season)
Small omnivorous mammal ("mouse") – 7.8 (BBCH 10-49)

Daily dietary dose (DDD) = application rate (kg a.s./ha) x TWA (assuming effects caused by LTE) x shortcut value x MAF_m (single application so MAF not applicable).
= 2.4 x 0.53 x 4.2 (small insectivorous mammal) = 5.3 mg kg bw/day
= 2.4 x 0.53 x 14.3 (large herbivorous mammal) = 18.2 mg kg bw/day
= 2.4 x 0.53 x 7.8 (small omnivorous mammal) = 9.9 mg kg bw/day

Long-term TER = NOAEL / DDD
= 46 / 5.3 = 8.7 (small insectivorous mammal)
= 46 / 18.2 = **2.5** (large herbivorous mammal)
= 46 / 9.9 = **4.6** (small omnivorous mammal)

Long-term (reproductive) risk from post-crop emergence use in flower bulbs (Tier 1 'Bulbs and onion like crops' use scenario):
Details of the relevant generic focal species, mean short-cut values and relevant crop stages are as follows:
Small insectivorous mammal ("shrew") – 4.2 (BBCH 10-19)
Small herbivorous mammal ("vole") -43.4 (BBCH ≥ 40)
Small omnivorous mammal ("mouse") – 4.7 (BBCH 10-39)

Daily dietary dose (DDD) = application rate (kg a.s./ha) x TWA (assuming effects caused by LTE) x shortcut value x MAF_m (single application so MAF not applicable).
= 2.4 x 0.53 x 4.2 (small insectivorous mammal) = 5.3 mg kg bw/day
= 2.4 x 0.53 x 43.4 (small herbivorous mammal) = 55.2 mg kg bw/day
= 2.4 x 0.53 x 4.7 (small omnivorous mammal) = 6.0 mg kg bw/day

Long-term TER = NOAEL / DDD
= 46 / 5.3 = 8.7 (small insectivorous mammal)
= 46 / 55.2 = **0.8** (small herbivorous mammal)
= 46 / 6.0 = 7.7 (small omnivorous mammal)

ii) 'Tier 1' long-term (reproductive) risk assessment conclusions for post-emergence crop uses:

The 'Tier 1' risk assessment for post-crop emergence use of asulam indicates breaches in the 'Uniform Principles' long-term TER trigger value of 5 from the foraging of large herbivorous and small omnivorous mammals in treated spinach and from the foraging of small herbivorous mammals (voles) at later growth stages in flower bulb crops (i.e. BBCH ≥ 40). Therefore, further refinements to the long-term risk assessment are required to address the potential risk to these generic focal species from post-crop emergence use.

B.9.3.2.3 Refined long-term (reproductive) risk assessment for wild mammals

Breaches in the Uniform Principles long-term TER trigger values of 5 were obtained in the 'Tier 1' risk assessment for several generic focal species and each are considered further below:

i) Refined long-term risk assessment for large herbivorous mammals: post-crop emergence use in spinach BBCH 12-14 ('Tier 1' long-term TER = 2.5)

As agreed and presented in the EFSA 2010 Conclusion report, further refinement is possible based on foliar residue decline data evaluated in the previous Annex I evaluation of asulam. In the asulam 'Additional Report' to the DAR (November 2009), a 21 day time weighted average factor (21d TWA factor) of 0.0989 is calculated based on a DT_{50} value of 1.44 days (from three outdoor residue trials on spinach) and this value was then subsequently used to refine the long-term risk

assessment for herbivorous birds and mammals (as agreed and presented in the EFSA 2010 Conclusion report). It is therefore possible to refine the 'Tier 1' large herbivorous mammal risk assessment on this same basis:

Daily dietary dose (DDD)

= application rate (kg a.s./ha) x 21d TWA factor x mean shortcut value x MAF_m (single spray – therefore not applicable).

= 2.4 x 0.0989 x 14.3 = 3.39 mg **asulam** /kg bw /day.

Therefore, the refined long-term TER calculation for the large herbivorous mammal (consuming exposed foliage) becomes:

Long-term TER = NOAEL / DDD = 46 / 3.39 = 13.6.

This is above the trigger value of 5, indicating a low and acceptable risk to large herbivorous mammals feeding in post-crop emergence treated spinach crops.

ii) Refined long-term risk assessment for 'small omnivorous mammals': post-crop emergence use in spinach BBCH 12-14 ('Tier 1' long-term TER = 4.6)

The 'Tier 1' risk assessment results in a marginal breaching of the Uniform Principle trigger value of 5 for 'small omnivorous mammals feeding post-crop emergence in treated spinach crops. However, further refinement is possible by taking into account the reported rapid dissipation of asulam residues on foliage – asulam residues decline data indicating a foliar DT50 of 1.44 days and (by calculation) a 21 day TWA factor of 0.0989.

The RMS has refined the 'Tier 1' risk assessment for the 'small omnivorous mammal' taking into account the known relatively rapid dissipation of asulam residues on foliage and assuming a diet consisting of 25% weeds (foliage), 50% weed seeds and 25% ground arthropods (as detailed in EFSA 2009 guidance).

For seed and arthropods, in the absence of specific data, as for the 'Tier 1' risk assessment, the RMS's refined risk assessment includes use of a EFSA (2009) standard 21 day TWA factor of 0.53 (based on an active substance default DT50 = 10 days). However for weeds, based on the available foliar residue decline data for asulam, a refined 21 day TWA factor of 0.0989 may be used – based on an asulam foliar DT50 of 1.44 days indicated by previously evaluated asulam residue decline studies.

Food intake rates per unit body weight for each dietary food item may be calculated based on the 'Tier 1' defaults 'total diet' value of 0.27 (for small omnivorous mammals feeding in spinach crops) multiplied by the proportion that each item forms in the diet (i.e. x 0.25 for weeds, 0.5 for weed seeds, x 0.25 for ground arthropods), resulting in FIR/bw values of 0.0675 for weeds and arthropods and 0.135 for weed seeds.

Mean RUD values used in the refined long-term risk assessment are based on the maximum relevant 'Tier 1' values for the proposed post-crop emergence use in spinach crops at the proposed growth stages (BBCH 12-14).

In line with EFSA (2009) guidance, for each food item, the level of asulam exposure (or 'daily dietary dose') may be estimated using the following equation:

Daily dietary dose (mg /kg bw /day) = Food intake rate per unit body weight (FIR/bw) x mean residue per unit dose (mean RUD, mg /kg fresh weight diet) x Dose (kg a.s./ha) x 21 day TWA factor.

Daily dietary dose (DDD) from consumption of weeds
= FIR/bw (0.0675) x mean RUD (28.7) x dose (2.4 kg /ha) x 21 d TWA factor (0.0989)
= 0.4598 mg **asulam** /kg bw /day

Daily dietary dose (DDD) from consumption of weed seeds
= FIR/bw (0.135) x mean RUD (40.2) x dose (2.4 kg /ha) x 21 d TWA factor (0.53)
= 6.9031 mg **asulam** /kg bw /day

Daily dietary dose (DDD) from consumption of ground arthropods
= FIR/bw (0.0675) x mean RUD (7.5) x dose (2.4 kg /ha) x 21 d TWA factor (0.53)
= 0.6439 mg **asulam** /kg bw /day

Therefore total DDD (or exposure)
= 0.4598 (weeds) + 6.9031 (weed seeds) + 0.6439 (ground arthropods)
= 8.0068 mg **asulam** /kg bw /day

On this basis the refined long-term TER calculation for the small omnivorous mammal feeding in post-crop emergence treated spinach crops becomes:

Refined long-term TER = NOAEL / refined total DDD = 46 / 8.0068 = 5.7

The long-term TER of 5.7 is above the trigger value of 5, indicating a low and acceptable risk from long-term exposure to small omnivorous mammals foraging in spinach crops after post-crop emergence use of asulam.

iii) Refined long-term (reproductive) risk assessment for small herbivorous mammals (voles): post-emergence use in flower bulbs BBCH ≥ 40 ('Tier 1' long-term TER = 0.8)

Further refinement of asulam exposure estimates for herbivorous mammals is possible by taking into account the relatively rapid dissipation of asulam on foliage – as indicated by the foliar residue decline data evaluated in the previous Annex I evaluation of asulam. In the asulam 'Additional Report' to the DAR (November 2009), a 21 day time weighted average factor (21d TWA factor) of 0.0989 is calculated based on a DT_{50} value of 1.44 days (from three outdoor residue trials on spinach) and this value was then subsequently used to refine the long-term risk assessment for herbivorous birds and mammals (as agreed and presented in the EFSA 2010 Conclusion report). It is therefore possible to refine the 'Tier 1' small herbivorous mammal risk assessment on this same basis:

Daily dietary dose (DDD)

= application rate (kg a.s./ha) x 21d TWA factor x mean shortcut value x MAF_m (single spray – therefore not applicable).

= 2.4 x 0.0989 x 43.4 = 10.30 mg **asulam** /kg bw /day.

Therefore, the refined long-term TER calculation for the large herbivorous mammal (consuming exposed foliage) becomes:

Long-term TER = NOAEL / DDD = 46 / 10.3 = 4.5.

The long-term TER of 4.5 is in breach of the trigger value of 5 - indicating a potential risk to small herbivorous mammals (voles) foraging in flower bulb crops following post-crop emergence use of asulam (which requires further consideration – see Conclusion section below).

iv) Conclusion for refined long-term (reproductive) risk assessment:

The above refined long-term risk assessments (relating to the dietary route of exposure), indicate that the proposed post-crop emergence use of asulam in spinach crops poses an acceptable long-term risk to large herbivorous mammals (refined long-term TER = 13.6) and to small omnivorous mammals (refined long-term TER = 5.7).

The refined long-term risk assessment for small herbivorous mammals (voles) foraging in flower bulb crops following post-crop emergence use of asulam indicates a potential and currently unresolved risk (refined long-term TER of 4.5). However, the RMS notes that the refined risk assessment is conservative and may over-estimate the risk, particularly in relation to assuming that the diet of small herbivorous mammals consumed over the assumed 21 day time long-term exposure period will be obtained entirely from treated flower bulb crops (i.e. PT = 1) – which is considered unlikely. Also, taking into account that voles will only feed in habitats that provided good cover, the dietary spray deposition factor of 0.6 included in the EFSA guidance may be an over-estimate. The RMS considers that when these factors are taking into account ,the small herbivorous mammal long-term TER is likely to be greater than the trigger value of 5 – indicating an acceptable long-term risk to small herbivorous mammals.

B.9.3.2.4 Risk to wild mammals from exposure to asulam via contaminated drinking water

In line with EFSA's (2009) Guidance Document, a risk assessment is required for wild mammals drinking water from contaminated puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This is relevant for all of the uses of 'Asulox' and therefore needs to be assessed.

According to EFSA (2009) guidance (Section 5.5) due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of the effective application rate (in g/ha) to the acute and long-term relevant endpoints (in mg/kg bw/d) does not exceed 50 for 'less sorptive substances' with a Koc of <500 L/kg (which is the case for asulam – Koc = 25.4, ref. Section B.8.6 of current Volume 3 DAR). Therefore an initial risk assessment has been conducted on this basis:

Drinking water wild mammal acute risk assessment:

Application rate (g per ha) / acute LD50 (mg/kg bw) =

2400 / >4564 mg asulam/kg bw = < 0.53

Drinking water wild mammal long-term risk assessment:

Application rate (g per ha) / long-term NOAEL (mg/kg bw /day) = 2400/46 = 52

The acute ratio of 0.53 is within the risk ratio trigger of 50 indicating a low and acceptable risk from potential acute effects. However, the long-term ratio of 52 is above the trigger value of 50 - indicating the need for further refinement of the drinking water long-term risk assessment for wild mammals.

The current EFSA (2009) drinking water risk assessment guidance assesses the risk to small granivorous mammals - which based on the relatively low water content of their diet are considered to be a representative worst case 'generic focal species' for wild mammals. Using the EFSA (2009) Excel spreadsheet and based on a maximum dose of 2400g asulam /ha, a concentration in spray from the proposed crop uses of 12 grams /L (maximum dose divided by minimum spray volume of 200 L /ha), an asulam Koc value = 25.4 L/Kg and a long-term NOAEL of 46 mg asulam /kg bw /day, the long-term drinking water TER = 46.4. This long-term TER is above the long-term TER trigger value of 5 - indicating a low and acceptable long-term risk to wild mammals from the drinking of contaminated puddle water.

In conclusion, the proposed crop uses of asulam sodium pose a low and acceptable acute and long-term risk to wild mammals from the consumption of contaminated drinking water.

B.9.3.2.5 Risk to wild mammals from potential secondary poisoning via bio-accumulation in fish and earthworms

The log octanol water partition co-efficient of asulam (log Pow) is 0.15 which is below the trigger value of log 3.0 for which there is considered the potential for bio-accumulation. Also, there are no major soil or water metabolites with a log Pow which breach this trigger value. It is concluded that there is a lack of potential for bioaccumulation and therefore no further assessment in relation to the risk to fish-eating and earthworm-eating mammals is required.

B.9.3.2.6 Risk to wild mammals of endocrine effects

The results of mammalian toxicity studies conducted with asulam, including two generation and teratogenicity evaluations, do not indicate any endocrine disrupting effects (ref. Section B.6.10 of Volume 3 DAR). A low risk of endocrine effects to any exposed wild mammals can therefore be concluded and no further evaluation is required at this time.

B.9.3.2.7 Overall risk assessment conclusions for 'other terrestrial vertebrates'

The risk assessment indicates that the proposed crop uses of asulam in spinach and flower bulb crops pose a low and acceptable risk to wild mammals from consumption via their diet or from drinking water, with the exception of the diet related risk to small herbivorous mammals from late post-crop emergence use in flower bulbs (BBCH ≥ 40) – for which an acceptable long-term risk has not been (numerically) demonstrated (refined long-term TER = 4.5). However the risk assessment conducted for small herbivorous mammals is conservative in assuming that all of the diet of small herbivorous mammals will be obtained from within treated crops (i.e. PT = 1) and also in assuming for voles (the EFSA indicator species) a dietary spray deposition value of 0.6 – which is likely to be an over-estimate when it is taking into account that voles usually feed in habitats that provide good ground cover. Therefore, the RMS considers that when these factors are taking into account ,the small herbivorous mammal long-term TER is likely to be greater than the trigger value of 5 – indicating an acceptable risk to small herbivorous mammals.

There is a lack of potential for bioaccumulation in fish and earthworms (log Pow < 3.0) and therefore a low risk of secondary poisoning to fish-eating and earthworm-

eating birds. Mammalian toxicity data indicate a lack of endocrine effects and on this basis there is considered to be a low risk of such effects in exposed wild mammals.

B.9.4 Effects on bees

B.9.4.1 Toxicity to bees

B.9.4.1.1 Acute oral toxicity to bees:

Report: S. Schmitzer (1998) Laboratory testing for toxicity (acute contact and oral LD_{50}) of asulam on honey bees (*Apis mellifera L.*) (Hymenoptera, Apidae). United Phosphorus Limited, Unpublished report No.: C015416; CA 8.7.1/01.

This study has previously been evaluated at EU level.

Guidelines:

EPPO Bull 22, 203-215, 1992. Deviations: None which affected the study

GLP: Yes

Executive Summary:

An acute oral toxicity study was conducted with the honey bee, *Apis mellifera*. The results of did not reveal acute toxic effects of asulam to honey bees via oral exposure. Based on the results found here, the oral (24 h and 48 h) LD_{50} of asulam is >123.7 µg/bee.

Materials and Methods

Test Material:

Asulam, purity: 80.6% (w/w, analytical), Batch no.: PN 24004

Test Design:

4-6 week old female honeybees of species *Apis mellifera* were used in the study. Oral and contact tests were conducted in stainless steel chambers. In incubators the temperature was 27-28°C and the relative humidity 55-63%. Tests were performed in the dark.

Oral toxicity test: groups of 10 individuals (30 bees per test concentration) were exposed to an untreated control, 5 dosages of test substance (8.7, 14.7, 33.5, 67.0 and 123.7 µg/bee) and a toxic standard (0.2 µg dimethoate per bee). Before the 48 hours exposure period, the bees were starved for 90 minutes. The uptake duration of test substance, toxic standard and control was 4 hours.

The parameters assessed in both oral and contact tests were mortality and behaviour abnormalities (vomiting, apathy, intensive cleaning) after 30, 45, 60 minutes, 2, 4 hours (first day), 24 and 48 hours.

Results and Discussion:

Oral toxicity test - None of the 150 bees died after ingestion of asulam by the end of the experiment. No behavioural abnormalities occurred during the experimental time. The key biological information is given in Table B9.4.1.

Table B.9.4.1 Acute oral toxicity of asulam to the honey bee (*Apis mellifera*)

Oral toxicity test		
Concentration (µg/bee)	Mortality (%)	
	24 h	48 h
Untreated control	0	0
8.7	0	0

Oral toxicity test		
Concentration (µg/bee)	Mortality (%)	
	24 h	48 h
14.7	0	0
33.5	0	0
67	0	0
123.7	0	0
Toxic standard (dimethoate)	93.3	93.3

Conclusions:

The results of this study did not reveal acute toxic effects of asulam to honey bees in the oral toxicity test. Based on the results found here, the oral (24 h and 48 h) LD_{50} of asulam is >123.7 µg/bee.

RMS Comments:

This study has previously been evaluated at EU level. As part of the current evaluation, the RMS has re-checked the study summary against the full study report and is in agreement with it. The study is considered to be scientifically valid and suitable for use in the regulatory risk assessment. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level. In addition to a lack of any treatment related mortality at up to the highest test dose of 123.7 µg/bee (which related to a concentration in syrup consumed as food of 5 µg/mg food), there were no treatment related behavioural abnormalities. These test results therefore indicate that asulam is of low acute oral toxicity to bees.

B.9.4.1.2 Acute contact toxicity to bees:

Report: S. Schmitzer (1998) Laboratory testing for toxicity (acute contact and oral LD_{50}) of asulam on honey bees (*Apis mellifera L.*) (Hymenoptera, Apidae). United Phosphorus Limited, Unpublished report No.: C015416; CA 8.7.2/01.

This study has previously been evaluated at EU level.

Guidelines:

EPPO Bull 22, 203-215, 1992. Deviations: None which affected the study.

GLP: Yes.

Executive Summary:

An acute contact toxicity study was conducted with the honey bee, *Apis mellifera*. The results did not reveal any acute toxic effects of asulam to honey bees via contact exposure. Based on the results found here, the contact LD_{50} of asulam on honeybees is clearly in excess of 100 µg/bee (24 h and 48 h).

Materials and Methods

Test Material:

Asulam, purity: 80.6% (w/w, analytical), Batch no.: PN 24004

Test Design:

4-6 week old female honeybees of species *Apis mellifera* were used in the study. Oral and contact tests were conducted in stainless steel chambers. In incubators the temperature was 27-28°C and the relative humidity 55-63%. Tests were performed in the dark.

Contact toxicity test: groups of 10 individuals (30 bees per test concentration) were exposed to an untreated control (only anaesthetisation), a solvent control (water with 0.6% Adhäsit), 5 dosages of test substance (6.25, 12.5, 25, 50 and 100 µg/bee) and a toxic standard (0.2 µg dimethoate per bee) over a period of 48 hours. Before application of control and substance, the bees were anaesthetised during 115 to 240 seconds with CO_2.

The parameters assessed in both oral and contact tests were mortality and behaviour abnormalities (vomiting, apathy, intensive cleaning) after 30, 45, 60 minutes, 2, 4 hours (first day), 24 and 48 hours.

Results and Discussion:

Contact toxicity test: 2 of the 150 bees exposed to asulam died by the end of the experiment in the 50 µg/bee treatment group. No test substance related behavioural abnormalities occurred during the experimental time. No further mortality occurred in the other dosage groups.

The key biological information is given in Table B.9.4.2.

Table B.9.4.2 Acute contact toxicity of asulam to the honey bee (*Apis mellifera*)

Contact toxicity test		
Concentration (µg/bee)	Mortality (%)	
	24 h	48 h
Untreated control	0	0
Solvent control	0	0
6.3	0	0
12.5	0	0
25	0	0
50	6.7	6.7
100	0	0
Toxic standard (dimethoate)	46.7	53.3

Conclusions:

The results of this study did not reveal acute toxic effects of asulam to honey bees in the contact toxicity test. Based on the results found here, the contact LD_{50} of asulam on honeybees is clearly in excess of 100 µg/bee (24 h and 48 h).

RMS Comments:

This study has previously been evaluated at EU level. The study is scientifically valid and suitable for use in the regulatory risk assessment. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level.

This study has previously been evaluated at EU level. As part of the current evaluation, the RMS has re-checked the study summary against the full study report and is in agreement with it. The study is considered to be scientifically valid and suitable for use in the regulatory risk assessment. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level. In addition to a lack of any treatment related mortality at up to the highest test dose of 100 µg/bee, there were no treatment related behavioural abnormalities.

Summary of toxicity to honey bees (acute oral and contact toxicity tests):

There were no treatment related mortalities or behavioural abnormalities at up to the highest test dose in either the acute oral or contact toxicity tests. A summary of the honey bee toxicity data is presented in Table B.9.4.3.

Table B.9.4.3 The acute oral and contact toxicity of asulam to honeybees

Study type *	Results LD_{50} µg asulam /bee	Test guidelines	Reference
48-hr oral	> 123.7 #	EPPO Bull. 22, 203-215, 1992	Schmitzer S (1998)
48-hr contact	> 100 ##		

* GLP compliant
# Oral dose from consumption of asulam in food (syrup) – relating at the higher test dose of 123.7 µg asulam /bee to a concentration of 5 µg asulam /mg diet.
From topical application in solvent to ventral thorax.

B.9.4.2 Potential for contamination of bee hives from foraging in asulam treated crops

The Notifier has not conducted any studies in relation to the potential for bees to become contaminated with asulam or its major metabolite sulphanilamide from possible residues in nectar or pollen and this is not a standard requirement for this asulam submission - which although being considered under Regulation 1107/2009 is being evaluated under earlier Directive 91/414 ecotoxicology data requirements -as agreed as an interim measure for submissions received prior to 1st January 2015.

However, details have been provided as a result of the conducted 'Literature Review' of a publication (Kaufmann and Kaenzig 2004) reporting residues of both asulam and sulphanilamide (referred to as 'sulfanilamide') in honey collected from hives in Switzerland - with it being suggested that its presence may be as a result of bees foraging in meadows previously treated with asulam. The paper has been reviewed by the RMS (see Literature Review at Section B.9.12.) and is considered to be 'relevant' in relation to regulatory ecotoxicology data requirements. It also appears to be of a good scientific standard, although taking into account that only a summary of the asulam /sulphanilamide honey residue data and of the analytical methods is included in the publication, it cannot be regarded as fully reliable for regulatory use and as such has been categorised by the RMS as 'Reliable with restrictions' (Category 2). However, this classification is sufficient for it to be considered in the regulatory risk assessment.

Extracts from the Kaufmann and Kaenzig (2004) publication are included below, followed by the RMS's comments.

Study ref: Kaufmann A & Kaenzig A (2004) 'Contamination of honey by the herbicide asulam and its antibacterial active metabolite sulfanilamide'; Food Addit Contam 2004 Jun 21(6): pp564-571.

Published Abstract

A number of antibacterial drugs (antibiotics) like sulfonamides, tetracyclines and streptomycin are used for the treatment of bacterial diseases in beehives. Yet, the finding of sulfanilamide residues in some 15 Swiss honeys out of some 350 samples could not be explained by such apicultural practice. Bees occasionally collect nectar from meadows treated with the herbicide asulam. Such honey is not only contaminated by asulam, but also by its degradation product sulfanilamide. This is the first report that the use of a herbicide causes the appearance of residues of an antibacterial active metabolite belonging to the category of sulfonamide drugs in food. The relevance of this finding lies in the fact that the use of the herbicide asulam might cause unacceptable residue levels of sulfanilamide in a product for human consumption.

Results and Discussion (extracts from published paper)

Fifteen honey samples officially collected from the local market contained measurable concentrations of sulfanilamide in the range 3–227 $\mu g\,kg^{-1}$, whereby in four cases, the sulfanilamide content was above the maximum permitted residue level of 50 $\mu g\,kg^{-1}$, according to the ordinance on foreign substances and constituents in foods (Swiss Federal Office of Public Health 2002). Figure 2 shows a typical chromatogram of a honey containing sulfanilamide and asulam residues.

In the beginning, the positive sulfanilamide findings were not easy to explain. Initially, sulfanilamide was the first discovered drug belonging to the group of sulfonamides. Chemical modifications of the side chain of the molecule produced a large number of derivatives exhibiting clearly stronger antibacterial activities. Hence, it was not evident why an 'old', not very potent drug, which is therefore not anymore easily obtainable, should be used in apiculture.

If sulfanilamide was used for the treatment of bees, honey samples from these producers should show very high antibacterially effective concentrations of sulfanilamide. However, unlike the mentioned cases concerning sulfathiazole with measured levels up to above 10 $mg\,kg^{-1}$ (Seiler and Kaufmann 2002), only small amounts of sulfanilamide in the $\mu g\,kg^{-1}$ range were found. Such levels are more easily explained by unintentional contamination than by a prohibited therapeutic use.

Further inquires by the Official Food Control Authority of the Canton of Aargau, focused on other possible sources of sulfanilamide, finally referred to the following theory as a 'working hypothesis' (Jahresbericht Kantonales Laboratorium Aargau 2000):

> Sulfanilamide contamination in honey might be closely interrelated to the agricultural use of the herbicide asulam, because the active agent is known to be degraded into sulfanilamide and asulam-containing products are usually applied in cultures and plantations foraged by honeybees.

Consequently, this hypothesis was tested by analysing sulfanilamide-positive honey samples for possible asulam residues. It was shown that the described sample preparation used for the determination of sulfonamides in honey could be used as well for the sample clean up of asulam. The substance survived the hydrolysis step and was also concentrated efficiently on the SPE material. Therefore, all samples containing sulfanilamide residues were (re)analysed with the modified method.

In all the 15 sulfanilamide-positive honey samples, asulam was detected in concentrations of 1–200 $\mu g\,kg^{-1}$ (table 1). One sample contained asulam residue levels reaching the provisional maximum residue level of 200 $\mu g\,kg^{-1}$, which was fixed by the Swiss Federal Office of Public Health (January 2002) after discovering the unexpected asulam contamination in honey. These results showed a clear correlation between sulfanilamide and asulam content (figure 3). Note that among the 350 honeys from the local market, there were no samples where either only asulam or only sulfanilamide was detected.

Table 1. Sulfanilamide and asulam content of some honey samples.

Sulfanilamide ($\mu g\,kg^{-1}$)	Asulam ($\mu g\,kg^{-1}$)
227	26
190	48
150	200
56	20
26	18
20	2
18	4
15	8
10	1
10	3
10	16
8	9
6	1
4	4
3	1

Table 2. Sulfanilamide and asulam content in honey harvested in different seasons.

Sample owner	Collection period	Sulfanilamide ($\mu g\,kg^{-1}$)	Asulam ($\mu g\,kg^{-1}$)
Beekeeper A	April/May	227	185
	June/July	59	20
Beekeeper B	April/May	125	115
	June/July	45	11
Beekeeper C	April/May	44	91
	June/July	39	18
Beekeeper D	April–June	20	7
	July	< 5	< 5

The results and findings of these investigations are in agreement with the present main hypothesis: that sulfanilamide contamination in honey might be closely related to the agricultural use of the herbicide asulam in springtime. This rather unexpected finding should be investigated and clarified in detail by means of further experimental studies and projects. In Switzerland, as a consequence of these findings has been that the approval of asulam for agricultural use, especially with regard to the treatment area, has to be modified by the authorizing administration. For instance, restricting the use of asulam to application in autumn would probably resolve the problem to do with the contamination of honey. However, asulam is used world wide in various geographical environments to treat different 'crops', ranging from potato and sugarcane to Christmas tree cultures. Therefore, different circumstances might require appropriate precautions and measures to prevent a contamination of the food chain by its metabolite sulfanilamide.

RMS comments:

Based on the details presented, the RMS is in agreement with the conclusion drawn in the paper that the identified residues of asulam and sulphanilamide in honey are likely to be as a result of bees foraging in asulam treated grassland. Maximum reported residues in honey were 200 µg asulam /Kg and 227 µg sulphanilamide /Kg.

However, the RMS notes that the publication does not include any direct evidence of exposure from observations of bees foraging in treated crops /meadows in which the presence of asulam and sulphanilamide residues in nectar (used by bees to produce honey) has been confirmed by chemical analysis. Also, the paper only includes a summary of the residue data and analytical methods used – which have not been validated for accuracy by the RMS. Taking these factors into account, as discussed in the 'Literature Review' in Section B.9.12. the publication is considered 'relevant' and 'Reliable with restrictions' (Category 2). The implications of the reported conclusions have been considered further in the bee regulatory risk assessment (Section B.9.4.3 below).

B.9.4.3 Bee risk assessment

In addition to potential foraging on the flowers of treated flower bulb crops, bees may forage on flowering weeds in treated spinach and flower bulb crops. Also, they may also forage on aphid honeydew produced by aphid present within the crop. Therefore, given that for both crop uses bees may be potentially exposed to asulam, a bee risk assessment is required.

There is no additional risk from the formulated product 'Asulam 400g/L SL' over that of the active substance asulam, as it is a simple dilution in water, therefore the conducted risk assessment relates to that from exposure to the active substance – which also covers the formulation.

The risk assessment has been conducted in line with current terrestrial ecotoxicology guidance detailed under SANCO/10329/2002 (October 2002), based on Directive 91/414's ecotoxicology data requirements – carried over as an interim measure to Regulation 1107/2009 applicable to submissions received prior to 1st January 2015. Although under these data requirements there is no specific requirement for the Notifier to investigate the potential risk to bees from contamination of nectar or pollen by the active substance, the conducted Literature review has identified the presence of asulam (and its metabolite sulphamilamide) in honey sampled from hives in Switzerland (ref. Kaufmann A & Kaenzig A (2004) – with the foraging of bees in treated crops being implicated as the potential source of exposure. Therefore, in addition to a standard 'hazard quotient' risk assessment, the risk to bees from this possible route of exposure is also considered (briefly) below.

'Hazard quotient' acute risk assessment:

Table B.9.4.4 Hazard quotients for honeybees based on laboratory toxicity studies

Application rate (g asulam/ha)	Exposure route	LD_{50} (µg asulam /bee)	Hazard quotient	Uniform Principles trigger
2400	Oral	> 123.7	< 19.4	50
	Contact	> 100	< 24	50

Comparing the application rate with the acute oral and contact toxicity of asulam therefore results in hazard quotients which are less than the Uniform Principles trigger value of 50, indicating a low risk to bees.

Risk from possible residues in hive honey:

Details in the literature review identified paper by Kaufmann and Kaenzig (2004) indicates maximum residues present in hive honey of 200 µg asulam /Kg and 227 µg sulphanilamide /Kg - with it being thought likely that these residues originated from bees foraging in treated grassland. This crop use differs from the proposed uses in spinach and flower bulb crops. There are also possible differences in the rate of application - no information being available to the RMS regarding the application rate of asulam used in Switz meadows prior to honey residue sampling from hives. Therefore, there is uncertainty regarding the applicability of the asulam and sulphanilamide honey residues data to the proposed crop uses, which needs to be taken account of in the risk assessment.

In the absence of an EU agreed risk assessment methodology to assess the risk from active substance residues in honey, no risk assessment has been included for this route of exposure in this current evaluation.

Bee risk assessment conclusion.

Asulam is of low toxicity to bees. The standard 'hazard quotient' acute risk assessment indicates a low risk to bees from the proposed use of asulam in spinach and flower bulb crops. No bee risk mitigation measures are required to be included based on this risk assessment.

Evidence obtained from the conducted literature review indicates the possibility of exposure to bees from foraging in treated crops and also from the possible consumption in the hive of contaminated honey. However, in the absence at this time of an EU agreed risk assessment methodology for this, no specific risk assessment for this route of exposure has been included.

B.9.5 Effects on other crop dwelling non-target arthropods

B.9.5.1 Toxicity to non-target arthropods

Details have been provided for studies conducted using artificial and natural test substance, for which studies summaries are included below. All of the studies were previously briefly summarised and fully evaluated in the earlier Annex I evaluation for asulam (EFSA Conclusion report, 2010).

B.9.5.1.1 Non-target arthropod studies using artificial substrates:

i) Report: M. Moll & R. Bützler (2001) Effects of EXP 04668 A on the parasitoid *Aphidius rhopalosiphi* in the laboratory - dose response test. United Phosphorus Limited, Unpublished report No.: C017898; [CP 10.5.1/01].

This study has previously been evaluated at EU level.

Guidelines:

IOBC WPRS 2000 (Mead-Briggs et al. 2000). Deviations: None which affected the study.

GLP: Yes

Executive Summary:

The effects of EXP 04668A (400 g/L SL) on the parasitoid *Aphidius rhopalosiphi* was measured in the laboratory in a Tier 1 dose response test (exposure on a glass substrate). Under these worst-case conditions, the LR_{50} of Asulam 400 g/L SL is 1210.6 g asulam/ha (95% confidence limits: 807.7 and 1814.6 g/ha), equivalent to 3088 mL product/ha. The reproductive capacity of *Aphidius* was not statistically significantly reduced at 92 g asulam/ha = 235mL Asulam 400 g/L SL /ha compared to the control but was statistically reduced at 230 and 576 g asulam/ha compared to the control.

Materials and Methods

Test Material:

EXP 04668A, purity 392 g/L, Batch no.: OP990533

Test Design:

The effects of EXP 04668A (400 g/L SL) applied at 5 dose rates (92, 230, 576, 1440 and 3600 g asulam/ha, equivalent to 235, 587, 1469, 3674 and 9184 mL product/ha) on the parasitoid *Aphidius rhopalosiphi* was measured in the laboratory by contacting substance treated glass surfaces. In the same way, parasitoids were exposed to a deionised water control (200 L/ha) and a Perfekthion EC (toxic standard) at the rate of 1.5 µL Perfekthion/L. Additionally, a check for significant sublethal treatment effects (parasitation activity) compared to a water control was performed in variants with <50% corrected mortality.

The test was performed in a controlled environment room. The temperature was 19-25°C during the exposure phase and 18-25°C during the post-exposure period. The relative humidity was 75-85% during the exposure phase and 74-85% during the post-exposure period. The light intensity was 1160-1340 lux during the exposure phase and 3090-13300 lux during the post-exposure period. The light regime was 16-hour light and 8 hour dark.

Results and Discussion:

The main biological information is summarised in Table B.9.5.1.

Table B.9.5.1 Toxicity of 'Asulam 400 g/L SL' to the parasitoid *Aphidius rhopalosiphi* (glassplate treated substrate)

Treatment group (g asulam/ha)	Mortality (%) *	Corrected mortality	Reproduction (mummies/female) **	Reduction of parasitation efficiency (%)
Control	2.5 ± 5.0	-	16.2 ± 10.1	-
EXP 04668A				
92 (235 mL product/ha)	2.5 ± 5.0	0.0	8.7 ± 8.2	46.3
230	12.5 ± 9.6	10.3	5.1 ± 4.6 ##	68.5
576	47.5 ± 15.0 #	46.2	4.9 ± 7.8 ##	69.8
1440	57.5 ± 29.9 #	56.4	-	-
3600	67.5 ± 12.6 #	66.7	-	-
Toxic standard	100.0 ± 0.0 #	100.0	-	-

* percentage values represent means and standard deviation from 4 replicates each with 7 female and 3 male parasitoids.
** mean and standard deviation from maximum 15 replicates each with 1 female parasitoid.
# significant difference compared to the control, Fisher Exact Test, $p<0.05$.
significant difference compared to the control, Bonferroni-U-Test, $p<0.05$.

Conclusions:

Under worst-case laboratory conditions the LR_{50} of Asulam 400 g/L SL is 1210.6 g asulam/ha (95% confidence limits: 807.7 and 1814.6 g asulam/ha), equivalent to 3088 mL product/ha.

The reproductive capacity of *Aphidius rhopalosiphi* was not statistically significantly reduced at 92 g asulam/ha = 235mL Asulam 400 g/L SL /ha compared to the control but was statistically reduced at 230 and 576 g asulam/ha compared to the control.

RMS Comments:

This study has previously been evaluated at EU level. The study is scientifically valid and suitable for use in the regulatory risk assessment. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level.

ii) Report: M. Moll (1999a) Effects of EXP04668A on the parasitoid *Aphidius rhopalosiphi* (Hymenoptera, Aphidiidae) in the laboratory. United Phosphorus Limited, Unpublished report No.: R006295; CP 10.5.1/02.

This study has previously been evaluated at EU level.

Guidelines:

IOBC/WPRS Polgar, (1988). Deviations: None which affected the study

GLP: Yes

Executive Summary:

The effects of EXP 04668A (400 g/L SL) on the parasitoid *Aphidius rhopalosiphi* was measured in the laboratory in a Tier 1 single rate test (exposure on a glass substrate). Under the conditions of the test, mortality was less than <50% mortality at the applied rate of 11 L product/ha (4301 g asulam/ha). Reproduction of *Aphidius* was not affected when exposed to Asulam 400 g/L SL at this rate.

Materials and Methods

Test Material:

EXP04668A (400 g/L SL), purity 391 g/L asulam, Batch no.: OP980096

Test Design:

Approximately 48 hours old adult *Aphidius rhopalosiphi* (5 females and 5 males per test cage) were exposed to dried spray deposits on glass plates under laboratory conditions. The units were sprayed with 11 L EXP04668A/ha in 200 L water/ha, 200 L tap water/ha (control) and 0.85 mL Perfekthion EC in 200 L water /ha (toxic standard). During the exposure period (48hours), each treatment group (control, test substance and toxic standard) consisted of 4 replicates, each containing 10 aphids (5 male and 5 female). During the post-exposure period (24 hours of parasitation followed by 12 days of post-parasitation), 20 replicates with 1 female per replicate for the control group and 13 replicates with 1 female per replicate for the test substance group were made.

Within the exposure period the temperature was 18.5-21.5°C, the relative humidity 70-90% and the light intensity of 700-810 lux. During the post-exposure period the temperature was 20-22°C, the relative humidity 79.2-82% and the light intensity of 2200-3050 lux. The light regime was 16-hour light and 8 hours darkness. The exposure cages were ventilated.

Endpoints were mortality (assessed after 1, 2, 24 and 48 hours) and reproduction efficiency of the survivors (number of mummies counted 12 days after the 24 hours parasitation period).

Results and Discussion:

The results are summarised in Table B.9.5.2.

Table B.9.5.2 Toxicity of Asulam 400 g/L SL to the parasitoid *Aphidius rhopalosiphi* (glassplate treated substrate)

Treatment	Mortality (%)	Corrected mortality (%)	Parasitation Efficiency (No. of aphid mummies per female)
Control (water 200 L/ha)	7.5	-	5.9
EXP04668A (11 L/ha)	42.5	37.8	5.5
Toxic standard (0.85 mL/ha)	100	100	-

Conclusions:

Under the conditions of the test mortality of the parasitoid *Aphidius rhopalosiphi* there was less than <50% mortality at the applied rate of 11 L product/ha (4301 g asulam/ha). Reproduction of the parasitoid *Aphidius rhopalosiphi* was not affected when exposed to Asulam 400 g/L SL at this rate.

RMS Comments:

This study has previously been evaluated at EU level. The study is scientifically valid and suitable for use in the regulatory risk assessment. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level.

iii) Report: A. Gossmann (2001) Effects of EXP 04668 A on the predatory mite *Typhlodromus pyri* in the laboratory - dose response test. United Phosphorus Limited, Unpublished report No.: C017897; CP 10.5.1/03.

This study has previously been evaluated at EU level.

Guidelines: IOBC WPRS 2000. Deviations: None

GLP: Yes

Executive Summary:

The effects of EXP 04668A (400 g/L SL) on the predatory mite *Typhlodromus pyri* was measured in the laboratory in a Tier 1 dose response test (exposure on a glass substrate). Under these worst-case conditions, there was significant mortality at test rates of 1440 and 3600 g asulam/ha and significant reproductive effects at all test rates (92 g asulam/ha and above). The LR_{50} was determined to be 2966 g asulam/ha (equivalent to 7566.3 mL Asulox/ha). The reproduction was reduced compared to the control by about 50% or more at 92 g asulam/ha and above.

Materials and Methods

Test Material: EXP 04668A (400 g/L SL), purity 392 g asulam/L, Batch no.: OP990533

Test Design:

Approximately 1 day old protonymphs of *Typhlodromus pyri* (20 individuals per test unit) were exposed to dried spray deposits of 92, 230, 576, 1440 and 3600 g as/ha (diluted in 200 L deionised water/ha) on glass plates (3 replicates per treatment group). Deionised water was used as a control treatment and Perfekthion EC (6 mL/ha in 200 L/ha) as a reference standard.

The duration of the mortality assessment period was 7 days. The reproductive performance was examined for another 7-day period in the control and in the test item rates where corrected mortality was ≤50%.

Results and Discussion:

The results are summarised in Table B.9.5.3.

Table B.9.5.3 Toxicity of 'Asulam 400 g/L SL' to the predatory mite *Typhlodromus pyri* (glassplate treated substrate)

Treatment group (g asulam/ha)	Mortality (%) 7day after application	Corrected mortality (%)	Reproduction rate (mean number of eggs per female)	Effect on reproduction (R)
Control	10.0	-	8.2	-
EXP 04668A				
92 (235 mL product/ha)	23.3	14.8	3.3 **	60.3
230	13.3	3.7	4.7 **	42.3
576	25.0	16.7	2.4 **	70.8
1440	33.3 *	25.9	1.2 **	85.7
3600	65.0 *	61.1	No reproduction evaluated	-
Perfekthion EC				
6 mL/ha	86.7 *	85.2	No reproduction evaluated	-

* significant difference compared to the control, Fisher-exact-test, α=0.05.
** significant difference compared to the control, Mann & Witney-U-Test, α=0.05Asulox® caused no statistically significant lethal effects on the predatory mite *Typhlodromus pyri* exposed up to 576 g asulam/ha (equivalent to 1469.4 mL Asulox®/ha).

Significant acute lethal effects were observed at dosages of 1440 g asulam/ha (equivalent to 3673.5 Asulam 400 g/L SL /ha) and higher.

Based on the results presented above, the LR_{50} value was determined to be 2966 g asulam/ha (equivalent to 7566.3 mL Asulox®/ha) with 95% confidence limits of 1824 g asulam/ha to 4823 g asulam/ha (equivalent to 4653.1 and 12303.6 mL Asulam 400 g/L SL /ha, respectively).

Conclusions:

Asulam 400 g/L SL (EXP 04668A) caused significant acute and sublethal effects on the predatory mite *Typhlodromus pyri* when applied under laboratory conditions on glass plates. The LR_{50} was determined to be 2966 g asulam/ha (equivalent to 7566.3 mL Asulam 400 g/L SL/ha). The reproduction was reduced compared to the control by about 50% or more at 92 g asulam/ha and above.

RMS Comments:

This study has previously been evaluated at EU level. The study is scientifically valid and suitable for use in the regulatory risk assessment. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level.

iv) Report:U. Luehrs (1999a) Effects of EXP04668A on the predatory mite *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) in the laboratory. United Phosphorus Limited, Unpublished report No.: R006339; CP 10.5.1/04.

This study has previously been evaluated at EU level.

Guidelines:

IOBC/WPRS 1988. Deviations: None.

GLP: Yes.

Executive Summary:

The effects of EXP 04668A (400 g/L SL) on the parasitoid *Aphidius rhopalosiphi* was measured in the laboratory in a Tier 1 single rate test (exposure on a glass substrate). Under the conditions of the test, mortality was less than <50% mortality at the applied rate of 11 L product/ha (4301 g asulam/ha). Reproduction of *Typhlodromus* was significantly affected when exposed to Asulam 400 g/L SL at this rate, with a reduction of more than 50% compared to the control.

Materials and Methods

Test Material:

EXP04668A (400 g/L SL), purity 391 g/L asulam, Batch no.: OP980096

Test Design:

Under laboratory conditions (ventilated climatic chamber, temperature of 24-26°C. relative humidity of 74-76%, 16h light-8h dark), approximately 2-3 day old individuals (protonymphs) of *Typhlodromus pyri* were exposed to dried residues of the test substance which was sprayed onto glass plates. The glass plates were sprayed with the field rate of 11 L of EXP04668A in 200 L deionised water/ha. The control was sprayed with deionised water and the toxic standard with 11 mL Perfekthion EC in 200 L deionised water. There were 5 replicates per treatment group, with 20 individuals per test unit (100 individuals per treatment group). For reproduction testing, the sex ratio of the individuals shall be 1 male: 5 females at the minimum. From day 7 onwards, the sex ratio was not sufficient in the test group and toxic standard.

The dead and escaped mites were counted at day 1, 3, 7, 9, 11 and 14 after test initiation. Number of eggs laid and number of live and dead juvenile stages per female were counted at day 7, 9, 11 and 14 after test initiation.

Results and Discussion:

The main biological information (mortality after 1 week and reproduction rate after 2 weeks) is summarised in Table B.9.5.4.

Table B.9.5.4 Toxicity of 'Asulam 400 g/L SL' to the predatory mite *Typhlodromus pyri* (glassplate treated substrate)

Treatment	Mortality (%)	Corrected mortality (%)	Reproduction rate (mean of total No. of eggs per female)	Quotient of treated and untreated Series (R)
Control (water 200 L/ha)	12	-	8.9	-
Asulox® EXP04668A (11 L/ha)	52	45.45	2.0	0.22
	41.3 *	33.3 *	2.5 *	0.28 *
Toxic standard (11 mL/ha)	73	-	0.3	-

* value was calculated based on 4 replicates as one was excluded as an outliner (according to Dixon) due to a high escape rate.

The mortality of *Typhlodromus pyri* exposed to residues of EXP04668A in the rate of 11 L/ha was increased (corrected mortality 45.45% or 33.3% if outliner is excluded from calculation) and

significantly different compared to the control (Dunnett-Test, α = 0.05). The reproduction rate in the test rate was reduced (R = 0.22 or 0.28 based on 4 replicates) and also significantly reduced compared to the control (Dunnett-Test, α = 0.05).The criteria of validity were met.

Conclusions:

Both mortality and reproduction rate of *Typhlodromus pyri* were affected (mortality <50%) when exposed to residues of Asulam 400 g/L SL (EXP04668A) at the rate of 11 L/ha.

RMS Comments:

This study has previously been evaluated at EU level. The study is scientifically valid and suitable for use in the regulatory risk assessment. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level.

v) Report: S. Schmitzer (2002) Effects of EXP04668A (AE F074383 00 SL33) on the wolf spider *Pardosa spec.* in the laboratory. United Phosphorus Limited, Unpublished report No.: C030817; CP 10.5.1/05.

This study has previously been evaluated at EU level.

Guidelines:

IOBC Heimbach et al., 2000, BBA draft guideline 1994. Deviations: None.

GLP: Yes

Executive Summary:

The effects of EXP 04668A (400 g/L SL) on the wolf spider *Pardosa sp.* was measured in the laboratory in a Tier 1 single rate test (exposure on an inert substrate). Under the conditions of the test, Asulam 400 g/L SL had no effects on mortality and food consumption of *Pardosa sp*. under these worst-case conditions if applied at a rate of 9.18 L product/ha (3.6 kg asulam/ha).

Materials and Methods

Test Material:

EXP04668A (400 g/L SL), purity 392 g/L asulam, Batch no.: OP990533

Test Design:

The species composition for the test was 72.5% *Pardosa amentata*, 15.7% *Pardosa proxima*, 1.0% *Pardosa pullata* and 10.8% subadults.

Under laboratory conditions, outdoor collected wolf spiders *Pardosa spec*., were exposed to three treatment groups: a water control, a test item group (at a rate of 3.6 kg asulam/ha in 400 L water/ha) and a toxic standard group (Perfekthion EC applied at the rate of 800 g as/ha in 400 L water/ha). Each group contained 34 replicates with 17 replicates containing 1adult/subadult male and 17 replicates containing 1 adult/subadult female.

The substances were sprayed upon the quartz sand and the spiders via laboratory spray applicator.

The spiders were exposed to deionised water, test item and reference item for 14 days. After 2 hours and on day 1, 2, 3, 4, 7, 8, 10, 11 and 14 the number of dead or damaged individuals was assessed. The food consumption was assessed on day 1, 2, 3, 4, 8 and 11.

Results and Discussion:

The effects of Asulam 400 g/L SL to wolf spiders *Pardosa spec*. are summarised in Table B.9.5.5.

Table B.9.5.5 Toxicity of Asulam 400 g/L SL to the wolf spider *Pardosa sp.* (overhead spray to spiders and quartz sand substrate)

Treatment	Adult mortality rate (%)	Corrected adult mortality rate (%)	Mean number of consumed flies per spiders	Reduction of feeding capacity #
Water control	0.0	-	3.4	-
EXP04668A (3.6 kg asulam/ha)	0.0	0.0	3.4	0.0
Perfekthion EC (0.8 kg as/ha)	82.4	82.4 *	4.4	-29

* significant different from the control, fisher exact-test, p=0.05, two sided.
# negative values means a higher food consumption compared to the control.

No test item related behavioural abnormalities occurred during the study.

Conclusions:

Asulam 400 g/L SL had no effects on mortality and food consumption of *Pardosa sp*. under worst-case laboratory conditions if applied at a rate of 9.18 L product/ha, equivalent to 3.6 kg asulam/ha.

RMS Comments:

This study has previously been evaluated at EU level. The study is scientifically valid and suitable for use in the regulatory risk assessment. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level.

vi) Report:M. Mead-Briggs (1991) An evaluation of the side-effects of Asulox 80 SG on lycosid spiders. United Phosphorus Limited, Unpublished report No.: R001634; CP 10.5.1/06.

This study has previously been evaluated at EU level.

Guidelines: Not reported. Deviations: not stated

GLP: Yes

Executive Summary:

The effects of Asulox 80 SG on the wolf spider *Pardosa sp.* was measured in the laboratory in a Tier 1 single rate test (exposure on an inert substrate). Under the conditions of the test, Asulox 80 SG had no effects on mortality and food consumption of *Pardosa sp*. under these worst-case conditions if applied at a rate of 2 kg product/ha, equivalent to 1.616 kg asulam/ha.

Materials and Methods

Test Material:

Asulam, purity 80.8%, Batch no.: FA 14252

Test Design:

In a first study, containers of moist sand were treated with asulam sodium dry technical applied at a rate of 2 kg product/ha (1.616 kg asulam/ha). A control was run in parallel.

In a second study, carried out in parallel, containers of moist sand were sprayed with spiders already present.

There were 20 test arenas with one spider per treatment group. 4 aphids were introduced into each arena for assessing the feeding capacities of spiders.

Immature lycosid spiders were then introduced and their condition and feeding activity were monitored over a 6-day period.

Results and Discussion:

In both tests, none of the treated spiders died during the 6-day study. There was no apparent change in their feeding capacity.

Conclusions:

The results demonstrate that Asulox 80 SG should not be harmful to spiders when applied at a rate of 2 kg product/ha, equivalent to 1.616 kg asulam/ha.

RMS Comments:

This study has previously been evaluated at EU level. Although not conducted to a specific guideline, the study methodology is considered acceptable. The study is scientifically valid and suitable for use in the regulatory risk assessment. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level.

vii) Report: A. Waltersdorfer (2002) Toxicity to the foliage dwelling predator *Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae) in the laboratory Asulam water-soluble concentrate. United Phosphorus Limited, Unpublished report No.: C028606; CP 10.5.1/07.

This study has previously been evaluated at EU level.

Guidelines: IOBC 2000; Deviations: None.

GLP: Yes.

Executive Summary:

The effects of 'AE F074383 00 SL33 A103' (equivalent to 'Asulam 400 g/L SL' or 'Asulox') on the green lacewing *Chrysoperla carnea* were measured in the laboratory in a Tier 1 multiple rate test (exposure on a glass substrate). Under these worst-case conditions, there was no treatment-related mortality at test rates of 1440 and up to and including 2000 g asulam/ha and no significant reproductive effects at all test rates. The LR_{50} was determined to be >2000 g asulam/ha (equivalent to 5.10 L 'Asulam 400 g/L SL' /ha) and the NOER (mortality and reproduction) was 2000 g asulam/ha.

Materials and Methods

Test Material: AE F074383 00 SL33 A103 purity 392 g/L, Batch no. OP990533

Test Design:

50 larvae of green lacewing *Chrysoperla carnea* were exposed individually to dry residues of AE F074383 00 SL33 A103 applied to glass petri dishes. The product was applied at rates of 20 (1% drift rate), 80 (4% drift rate) and 2000 g asulam/200 L water/ha. A toxic reference standard,

Dimethoate 40 EC, was applied at a rate of 45 mL product/200 L water/ha and a control was applied at rate of 200 L water/ha.

The test was performed in a controlled environment room at temperature of 23-25.5°C and relative humidity of 32-72%. The light/dark cycle was 16:8 hours. The pre-imaginal mortality was monitored over the duration of the study.

The reproduction phase started 8 days after emergence of adults. A record was kept of any animals dying during the 24-hour laying interval. For calculating total fecundity the total number of females was reduced by 0.5 for each dead female found. The hatching rate was reported as the number of fertile eggs per female per day expressed in percent.

Results and Discussion:

The toxicity of the test material residues to the larvae is summarised in Table B.9.5.6.

Table B.9.5.6 Toxicity of 'Asulam 400 g/L SL' to the green lacewing *Chrysoperla carnea* (glassplate treated substrate)

Treatment group (g asulam/ha)	Mortality (%)	Corrected preimaginal mortality (%)	Number of fertile eggs/female/day
Control	16	-	21.1 ± 0.99
AE F074383 00 SL33 A103			
20	20	4.8	24.8 ± 3.11
80	20	4.8	22.9 ± 6.86
2000	12	- 4.8	20.8 ± 0.21
Toxic standard 45 mL dimethoate/ha	62	54.8	n.d.

n.d.: not detected.

No significant effects were observed at any concentrations of AE F074383 00 SL33 A103.

Conclusions:

The dose rates of 20, 80 and 2000 g asulam/ha had no influence on mortality and reproduction.

RMS Comments:

This study has previously been evaluated at EU level. The study is scientifically valid and suitable for use in the regulatory risk assessment. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level.

B.9.5.1.2 Non-target arthropod 'extended laboratory' studies using natural (foliar or soil surface) test substrates:

i) Report: M. Moll (1999b) Effects of EXP04668A on the parasitoid *Aphidius rhopalosiphi* (Hymenoptera, Aphidiidae) - Extended Laboratory study. United Phosphorus Limited, Unpublished report No.: R007954 and C031135; CP 10.5.2/01.

This study has previously been evaluated at EU level.

Guidelines:

IOBC/WPRS Polgar, (1988). Deviations: None.

GLP: Yes.

Executive Summary:

The effects of EXP 04668A ('Asulam 400 g/L SL') on the parasitoid *Aphidius rhopalosiphi* was measured in the laboratory in an extended laboratory test (exposure on a plant substrate). Under the conditions of the test, mortality and reproduction of *Aphidius* were not affected when exposed to 440 mL/ha 172 g asulam/ha) and to 11 L/ha (4301 g asulam/ha) of Asulam 400 g/L SL (EXP04668A).

Materials and Methods

Test Material:

EXP04668A (400 g/L SL), purity 391 g/L asulam, Batch no.: OP980096

Test Design:

Under extended laboratory conditions, approximately 48 hours old adult *Aphidius rhopalosiphi* (5 females per test cage) were exposed to dried spray deposits on plant surfaces (barley seedlings) over a period of 48 hours. The plants were sprayed with 440 mL (drift rate) and 11 L (field rate) EXP04668A/ha in 200 L water/ha. The control was treated with tap water and the toxic standard with 8.5 mL Perfekthion EC in 200 L water/ha. The exposure period was followed by a post-exposure period (24hours parasitation and 12 day's post-parasitation). During the fecundity/parasitation phase aphid *Rhopalosiphum padi* was used as host.

During the exposure period (48 hours), each of the treatment groups (control, test substance and toxic standard) consisted of 4 replicates, each containing 10 aphids (5 female, except in the control group where 2 of 20 parasitoids were male). During the post-exposure period, 18 replicates with 1 female per replicate for the control group and 20 replicates with 1 female per replicate for the test substance group were made.

During the exposure period the temperature was 21-23.5°C, the relative humidity 57 - 82% and the light intensity of 1660 - 1780 lux. During the post-exposure period the temperature was 21-27°C, the relative humidity 45 - 78% and the light intensity of 1530-2270 lux. The light regime was 16-hour light and 8-hours darkness. The exposure cages were ventilated.

Number of living and dead parasitoids was counted 1, 2, 24 and 48 hours after test initiation. Number of aphid mummies was recorded 12 days after the 24 hours parasitation period.

Endpoints were mortality and reproduction efficiency of the survivors.

Results and Discussion:

The main biological information (mortality after 48 hours exposure and reproduction efficiency at day 12 after parasitation period) has been summarised in Table B.9.5.7.

Table B.9.5.7 Toxicity of 'Asulam 400 g/L SL' to the parasitoid *Aphidius rhopalosiphi* under extended laboratory conditions (with foliar test substrate)

Treatment		Mortality (%)	Corrected mortality (%)	Parasitation Efficiency (No. of aphid mummies per female)
Control (water 200 L/ha)		0	-	16.4
EXP04668A	(440 mL/ha)	0	0	11.9
	(11 L/ha)	0	0	13.0
Toxic standard	(8.5 mL/ha)	50	50	-

Conclusions:

Under the conditions of the test, mortality and reproduction of the parasitoid *Aphidius rhopalosiphi* were not affected when exposed to 440 mL/ha 172 g asulam/ha) and to 11 L/ha (4301 g asulam/ha) of 'Asulam 400 g/L SL' (EXP04668A).

RMS Comments:

This study has previously been evaluated at EU level. The study is scientifically valid and suitable for use in the regulatory risk assessment. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level.

ii) Report: U. Luehrs (1999b) Effects of EXP04668A on the predatory mite *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) - Extended laboratory study. United Phosphorus Limited, Unpublished report No.: R007956; 10.5.2/02.

This study has previously been evaluated at EU level.

Guidelines: IOBC/WPRS 1988. Deviations: None

GLP: Yes.

Executive Summary:

The effects of EXP 04668A (400 g/L SL) on the predatory mite *Typhlodromus pyri* was measured in the laboratory in an extended laboratory test (exposure on a plant substrate). Mortality was affected by <50% and reproduction rate of *Typhlodromus* was significantly (compared to control) affected when exposed to residues of Asulam 400 g/L SL (EXP04668A) at rates of 440 mL/ha (172 g asulam/ha) and 11 L/ha (4301 g asulam/ha).

Materials and Methods

Test Material:

EXP04668A (400 g/L SL), purity 391 g/L asulam, Batch no.: OP980096

Test Design:

Under laboratory conditions, approximately 2.5 days old individuals (protonymphs) of *Typhlodromus pyri* were exposed to dried residues of EXP04668A which were sprayed onto detached primary leaves of *Phaseolus vulgaris*. The leaves were sprayed with the 4% drift rate: 440 mL in 200 L deionised water/ha and the maximum field rate of 11 L in 200 L deionised water/ha. The control was sprayed with 200 L deionised water/ha and the toxic standard with 38 mL Perfekthion EC in 200 L deionised water/ha. There were 5 replicates per treatment group with 20 individuals per unit (100 individuals per treatment group). During the study, the sex ratio shall be 1 male: 5 females at the minimum. As on day 7, the sex ratio was greater than this value in the control and the maximum field rate group, males originating from same treatment but another replicate were added to meet the recommended sex ratio.

The test was performed in a ventilated climatic chamber under the following environmental conditions (during exposure period): temperature of 24-26.5°C, relative humidity of 45-93%, light intensity 950-1450 lux and photoperiod of 16h light / 8h dark.

Number of dead and escaped mites counted at day 1, 3, 7, 9, 11 and 14 after test initiation. Number of eggs laid and number of live and dead juvenile stages per female counted at day 7, 9, 11 and 14 after test initiation. Endpoints were mortality after one week and reproduction rate after 2 weeks.

Results and Discussion:

The results (mortality after one week and reproduction rate after 2 weeks) are summarised in Table B.9.5.8.

Table B.9.5.8 Toxicity of Asulam 400 g/L SL to the predatory mite *Typhlodromus pyri* under extended laboratory conditions (with foliar test substrate).

Treatment	Mortality (%)	Corrected mortality (%)	Reproduction rate (mean of total No. of eggs per female)	Quotient of treated and untreated Series (R)
Control (water 200 L/ha)	9.0	-	9.3	-
EXP04668A				
4% drift rate (440 mL/ha)	24.0	16.48	4.6	0.49
max field rate (11 L/ha)	44.0	38.46	2.3	0.25
Toxic standard (11 mL/ha)	100	-	No reproduction evaluated	-

The mortalities of *Typhlodromus pyri* exposed to residues of EXP04668A in the 4% drift rate (440 mL in 200 L deionised water/ha) and the maximum field rate (11 L in 200 L deionised water/ha) was increased (corrected mortality 16.5% and 38.5%, respectively) and both were significantly different compared to the control (Dunnett-Test, $\alpha = 0.05$). The reproduction in the tested rates was reduced (R = 0.49 at 4% drift rate and 0.25 at maximum field rate) and both were significantly different compared to the control (Dunnett-Test, $\alpha = 0.05$).

Conclusions:

Mortality was affected by <50% and reproduction rate of *Typhlodromus pyri* was significantly (compared to control) affected when exposed to residues of Asulam 400 g/L SL (EXP04668A) at rates of 440 mL/ha (172 g asulam/ha) and 11 L/ha (4301 g asulam/ha).

RMS Comments:

This study has previously been evaluated at EU level. The study is scientifically valid and suitable for use in the regulatory risk assessment. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level.

iii) Report: A. Drexler (2002) Effects of EXP04668A on the reproduction of rove beetles *Aleochara bilineata* - extended laboratory study. United Phosphorus Limited, Unpublished report No.: C030818; 10.5.2/03.

This study has previously been evaluated at EU level.

Guidelines:

IOBC Grimm et al., 2000. Deviations: None

GLP: Yes.

Executive Summary:

The effects of Asulam 400 g/L SL (EXP04668A, 400 g/L SL) on the rove beetles *Aleochara bilineata* was measured in the laboratory in an extended laboratory test (exposure on a realistic substrate). No statistically significant effects on reproduction capacity of *Aleochara* occurred after exposure to residues of up to a rate of 3600 g asulam/ha in 400 L water/ha.

Materials and Methods

Test Material:

EXP04668A (400 g/L SL), purity 392 g asulam/L, Batch no.: OP990533

Test Design:

Under extended laboratory conditions, 2-5 days old adult *Aleochara bilineata* (4 replicates with 10 female and 10 male beetles each) were exposed in test units (18.3 cm x 13.6 cm x 6.0 cm) to spray deposits of 36 g asulam/ha (108.1 g product/ha), 144 g asulam/ha (432.4 g product/ha) and 3600 g asulam/ha (10.809 kg product/ha) diluted in 400L deionised water/ha on natural soil (LUFA 2.1). Deionised water was used as a control treatment and Perfekthion (4400 mL/ha) as a reference treatment.

The beetles were exposed to control, test and reference item for 28 days. On day 7, 14 and 21 ca. 500 puparia of *Delia antiqua* were dug into the soil of each replicate to be parasitised by the larvae of the beetles.

On day 28 the adults were separated from the soil and the soil with the puparia was allowed to dry for seven days. On day 35 the puparia were washed out of the natural soil and transferred into an emergence container. The emergence of the beetles was observed from day 35-104.

Results and Discussion:

The main biological results are summarised in Table B.9.5.9.

Table B.9.5.9 Toxicity of 'Asulam 400 g/L SL' to the rove beetle *Aleochara bilineata* under extended laboratory conditions (oversprayed natural soil test substrate).

Treatment group (g asulam/ha)	Mean number of emerged beetles per replicate	Reduction of reproduction efficiency
control (deionised water)	947	-
EXP04668A		
36	862	8.9
144	847	10.6
3600	843	10.9
Perfekthion EC (4400 mL/ha)	144 *	84.8 *

- not applicable.
* significant difference compared to control (Student-T-test, one sided smaller, α=0.05).

Conclusions:

No statistically significant effects on reproduction capacity of rove beetles *Aleochara bilineata* occur after exposure to residues of Asulam 400 g/L SL (EXP04668A) up to a rate of 3600 g asulam/ha in 400 L water/ha in this extended laboratory study.

RMS Comments:

This study has previously been evaluated at EU level. The study is scientifically valid and suitable for use in the regulatory risk assessment. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level.

B.9.5.1.3 Summary of non-target arthropod toxicity data

Standard laboratory and 'extended laboratory' toxicity tests have been reported for the two 'ESCORT 2' indicator species *Typhlodromus pyri* and *Aphidius rhopalosiphi*.

Further studies have also been conducted on additional ground/leaf dwelling arthropods, as represented by *Aleochara bilineata*, *Chrysoperla carnea*, *Pardosa* and *Hypoaspis aculeifer*.

The non-target arthropod toxicity studies are considered scientifically valid and suitable for use in the regulatory risk assessment. The studies were mostly carried out with the representative 40% w/v asulam soluble concentrate formulation 'Asulox' – also referred to as 'Asulam 400 g/L SL' (code named 'EXP 04668A') and as such are fully applicable. In addition, one study on the wolf spider (*Pardosa*) was conducted with an 80% soluble granule (SG) formulation of asulam. However, given the lack of co-formulants in both formulations which are likely to affect toxicity, the test results with the SG formulation are also considered applicable to 'Asulox'.

All of the studies were previously briefly summarised and fully evaluated in the earlier Annex I evaluation for asulam (EFSA Conclusion report, 2010) – with the representative formulation being referred to at this time as either 'Asulox' or 'EXP04668A' (also referred to as 'Asulam 400g/L SL' in this submission). There are no changes to the conclusions reached in this current updated evaluation. The studies were previously briefly summarised by the RMS in Section B.9.5 of the earlier March 2006 DAR and for completeness these tables are included below (with a few minor amendments /clarifications) as Tables B.9.5.10 to B.9.5.12.

Table B.9.5.10 Effects of 'Asulam 400g/L SL' ('EXP04668A') on non-target arthropods in glass plate laboratory toxicity studies with *A. rhopalosiphi* and *T. pyri*

Species	Test substrate & description	Applica-tion rate	Results (control corrected mortality in brackets)	Test guide-lines*	Refer-ences
A. rhopal-osiphi (cereal aphid parasitoid wasp)	Glass plate dose response study, 48 h exposure to freshly dried residue. Exposed females transferred (for 1 day) to aphid infested cereal plants, level of parasitism assessed 12 days later.	11 L Asulox/ha (containing 391 g/L asulam)	% Mortality (48 h) Control: 7.5 11 L Asulox/ha: 42.5# *(37.8)* **LR_{50} > 11 L Asulox/ha** Parasitism (no. aphid mummies/female) Control: 5.9 11 L Asulox/ha: 5.5 **No statistically significant effects on parasitism.**	IOBC/WPRS, Polgar 1988	Moll M (1999)
A. rhopal-osiphi (cereal aphid parasitoid wasp)	Glass plate dose response study, 48 h exposure to freshly dried residue. Exposed females transferred (for 1 day) to aphid infested cereal plants, level of parasitism assessed 11-12 days later.	235, 587, 1469, 3674 & 9184 ml Asulox/ha (containing 392 g/L asulam)	% Mortality (48 h) Control: 2.5 235 ml Asulox/ha: 2.5 *(0)* 587 ml Asulox/ha: 12.5 *(10.3)* 1469 ml Asulox/ha: 47.5# *(46.2)* 3674 ml Asulox/ha: 57.5# *(56.4)* 9184 ml Asulox/ha: 67.5# *(66.7)* **LR_{50} = 3088 ml Asulox/ha** Parasitism (no. aphid mummies/female) Control: 16.2 235 ml Asulox/ha: 8.7 (46.3% reduction) 587 ml Asulox/ha: 5.1# (68.5 % reduction) 1469 ml Asulox/ha: 4.9# (69.8% reduction) **Statistically significant effects on parasitism.**	IOBC WPRS, Mead-Briggs *et al.* 2000	Moll M & Buetz-ler R (2001)

Typhlod-romus pyri (predatory mite)	Glass plate, 14 day exposure period. Protonymphs exposed to a dried deposit on freshly sprayed glass slides. Assessments made for mortality 7 day after introduction & for fecundity of surviving adult females 7-14 days after introduction.	11 L Asulox/ha (containing 391 g/L asulam)	% Mortality (7 day) Control: 12 11 L Asulox/ha: 41.3# *(33.3)* **LR_{50} >11 L Asulox/ha** Fecundity (no. eggs per female during days 7-14) Control: 8.9 Test substance: 2.5# **Statistically significant effects on mortality and fecundity.**	IOBC/WPRS 1988	Lührs U (1999)
Typhlod-romus pyri (predatory mite)	Glass plate dose response study, 14 day exposure period. Protonymphs exposed to a dried deposit on freshly sprayed glass slides. Assessments made for mortality 7 day after introduction & for fecundity of surviving adult females 7-14 days after introduction.	235, 587, 1469, 3674 & 9184 ml Asulox/ha (containing 392 g asulam/L)	% Mortality (7 day) Control: 10 235 ml Asulox/ha: 23.3 *(14.8)* 587 ml Asulox/ha: 13.3 *(3.7)* 1469 ml Asulox/ha: 25.0 *(16.7)* 3674 ml Asulox/ha: 33.3# *(25.9)* 9184 ml Asulox/ha: 65.0# *(61.1)* **LR_{50} = 7566 ml Asulox/ha** Fecundity (no. of eggs per female during days 7-14) Control: 8.2 235 ml Asulox/ha: 3.3# (60.3% reduction) 587 ml Asulox/ha: 4.7# (42.3% reduction) 1469 ml Asulox/ha: 2.4# (70.8% reduction) 3674 ml Asulox/ha: 1.2# (85.7% reduction) **Statistically significant effects on mortality and fecundity.**	IOBC WPRS Blümel *et al.* 2000	Goss-mann A (2001a)

* Tests conducted without significant deviation from guideline and in accordance with GLP
# Significant difference compared to the control ($P < 0.05$)
Note: values in italics are control corrected

Table B.9.5.11 Effects of 'Asulam 400g/L SL' ('EXP04668A') on non-target arthropods in extended laboratory toxicity studies with *A. rhopalosiphi* and *T. pyri*

Species	Test substrate & description	Applica-tion rate	Result	Test guide-lines*	Refer-ences
A. rhopa-losiphi (cereal aphid parasitoid wasp)	Barley seedlings, 48 h exposure of female wasps to freshly sprayed seedlings. Behaviour and mortality assessed. Fecundity of surviving wasps also examined by transferring (for 1 day) to aphid-infested barley & assessing parasitism after 12 days.	440 ml & 11 L Asulox/ha (containing 391 g asulam/L)	% Mortality (48 h) Control: 0 440 ml Asulox/ha: 0 11 L Asulox/ha: 0 **LR_{50} > 11 L Asulox/ha** Parasitism (no. aphid mummies/female) Control: 16.4 440 ml Asulox/ha: 11.9 11 L Asulox/ha: 13.0 **No statistically significant effects on mortality parasitism.**	IOBC/WPRS Polgar 1988	Moll M (1999q)
Typhlod-	Bean leaves, 14 day	440 ml &	% Mortality (7 day)	IOBC/W	Lueh-rs

romus pyri (predatory mite)	exposure period. Protonymphs exposed to a dried deposit on freshly sprayed detached leaves. Assessments made for mortality 7 day after introduction & for fecundity of surviving adult females 7-14 days after introduction.	11 L (field rate) Asulox/ha (containing 391 g asulam/L)	Control: 9.0 440 ml Asulox/ha 24.0# *(16.48)* 11 L Asulox/ha: 44.0# *(38.46)* **LR_{50} >11 L Asulox/ha** Fecundity (no. eggs per female during days 7-14) Control: 9.3 440 ml Asulox/ha: 4.6# 11 L Asulox/ha: 2.3# **Statistically significant effects on mortality and fecundity.**	PRS 1988	U (1999m)

* Tests conducted without significant deviation from guideline and in accordance with GLP
# Significant difference compared to the control (P < 0.05)
Note: values in italics are control corrected

Table B.9.5.12 Effects of 'Asulam 400g/L SL' ('EXP04668A') /Asulox 80SG on non-target arthropods in laboratory toxicity studies with additional species

Species	Test substrate & description	Application rate	Result	Test guide-lines*	References
Aleochara bilineata (rove beetle)	Adult beetles exposed to spray deposits on natural substrate for 28 days. *Delia antique* (onion fly) larvae introduced on days 7, 14 & 21. *Delia* pupae removed on day 35, with levels of beetle parasitism subsequently assessed based on counts of F1 adult beetle emergence.	0.276, 1.10 & 27.6 L Asulox/ha (equivalent to 108, 432 & 10809 g asulam/ha respect-ively) Asulox contained 392 g asulam/L	Adult beetle mortality not specifically measured, although a lack of any effect on numbers of emerging next generation beetles suggests no effects. Mean number of emerged beetles per replicate Control: 947 0.276 L Asulox/ha: 862 1.10 L Asulox/ha: 847 27.6 L Asulox/ha: 843 **No statistically significant effects on fecundity.**	IOBC Grimm *et al.* 2000	Drexler A (2002)
Chrysope-rla carnea (lacewing)	Larvae exposed to dry residues on glass petridishes. Once larvae had pupated they were transferred to untreated chambers. Preimaginal mortality, egg laying, egg hatching & adult mortality were assessed during the study.	0.051, 0.204 & 5.10 L Asulox/ha (equivalent to 20, 80 & 2000 g asulam/ha) Asulox contained 392 g asulam/L	Pre-imaginal mortality (%) Control: 16 0.051 L Asulox/ha: 20 *(4.8)* 0.204 L Asulox/ha: 20 *(4.8)* 5.10 L Asulox/ha: 12 *(-4.8)* **LR_{50} > 5.10 L Asulox/ha** No. of fertile eggs/female/day Control: 21.1 0.051 L Asulox/ha: 24.8 0.204 L Asulox/ha: 22.9 5.10 L Asulox/ha: 20.8 **No statistically significant effects on mortality or reproduction.**	IOBC Vogt *et al.* 2000	Walters-dorfer A (2002)
Pardosa (Wolf Spider)	Immature spiders introduced to containers of treated moist sand. Moist sand subsequently sprayed with spiders	2 kg 'Asulox 80SG' /ha (equivalent to 1.616 kg asulam/ha)	% Mortality (6 day) Control: 0 1.67 L Asulox 80SG/ha: 0 **LR_{50} > 2 kg 'Asulox 80SG' /ha** Food consumption	Internal company protocol	Mead-Briggs M (1991)

	present. Mortality and food consumption were monitored over 6 days.	Asulox 80SG contained 808 g asulam/ha	Spiders in all groups were feeding actively. **No statistically significant effects on mortality.**		

* Tests conducted without significant deviation from guideline and in accordance with GLP
Note: values in italics are control corrected

Table B.9.5.12 cont: Effects of 'Asulam 400g/L SL' ('EXP04668A') /Asulox 80SG on non-target arthropods in laboratory toxicity studies with additional species

Species	Test substrate & description	Application rate	Result	Test guidelines*	References
Pardosa (Wolf Spider)	Container of moist sand and subadult/adult spiders sprayed. Mortality and food consumption were monitored over 14 days.	9.18 L Asulox/ha (equivalent to 3.6 kg asulam/ha) Asulox contained 392 g asulam/L	% Mortality (14 day) Control: 0 9.18 L Asulox/ha: 0 **LR_{50} > 9.18 L Asulox/ha** Food consumption (mean number of flies per spider) Control: 3.4 9.18 L Asulox/ha: 3.4 **No statistically significant effects on mortality and food consumption.**	IOBC Heimbach *et al.* 2000, BBA draft guideline 1994	Schmitzer S (2002a)
Hypoaspis aculeifer (predaceous mite)	Protonymphs exposed to treated soil. Mortality assessed after 14 days. Surviving mites transferred to untreated mating units for 7 days, then reproductive units for oviposition assessment.	400 L Asulox/ha (equivalent to 156.8 kg asulam/ha or 4370 mg asulam/kg soil #) Asulox contained 392 g asulam/L	% Mortality (14 day) Control: 11 400 L Asulox/ha: 7 **LR_{50} > 400 L Asulox/ha** Reproduction (fertile eggs/female/7 days) Control: 24.6 400 L Asulox/ha: 24.8 **No statistically significant effect on mortality or reproduction.**	SETAC Candolfi 2001, EPPO 142 1989, Bakker *et al.* in preparation	Feije R (2002a)

* Tests conducted without significant deviation from guideline and in accordance with GLP
\# Calculated from using a surface area of 12.57 cm^2 and mean soil weight of 4.51g.

B.9.5.2 Non-target arthropod risk assessment

B.9.5.2.1 Introduction

The submitted studies (summarised in Tables B.9.5.10 to B.9.5.12) are suitable for use in a regulatory risk assessment, with the derived study endpoints being unchanged from that previously concluded (EFSA Conclusion Report, 2010).

The representative formulation 'Asulam 400g/L SL' is identical to that previously evaluated (under its UK product name 'Asulox') with the proposed pre and early post-emergence use in spinach also being identical. In addition to use in spinach crops, post-emergence use in flower bulb crops is also proposed at identical rates to that in spinach crops. However, the risk assessment is not affected by this additional crop use – the risk from post crop emergence use in spinach crops also covering that from the similar proposed use in flower bulb crops.

No additional toxicity studies or other data have been included in this current submission over that previously evaluated in the earlier Annex I evaluation (EFSA Conclusion Report 2010) – in relation to which a low risk to non-target arthropods was concluded. Also, there has been no change in the currently used non-target arthropod risk assessment methodology since this time. Therefore, with a few minor amendments, the originally risk assessment is still relevant and has been included largely unchanged below, with just a few minor amendments /clarifications.

B.9.5.2.2 Non-target arthropod 'ESCORT 2' risk assessment

In line with ESCORT 2 guidance and the current Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) details have been supplied for glass plate residue toxicity tests conducted with the indicator species *A. rhopalosiphi* and *T. pyri*. The results of these studies have been used to conduct an in-field and off-field 'Tier 1' ESCORT2 risk assessment. Given that for both crop uses (i.e. spinach and flower bulbs) only one application is applied per crop, where is no need to include a multiple application factor in these calculations.

ESCORT 2: 'Tier 1' risk assessment Hazard Quotient (HQ) calculations:

The following equations were used to calculate the HQ values with both indicator species, for in-field and off-field exposure scenarios, respectively:

In-field HQ = Application rate ÷ LR_{50}

Off-field HQ = Application rate x (drift factor ÷ VDF) x CF ÷ LR_{50}

- Vegetation distribution factor (VDF) = 10 (assumed standard value to adapt the overestimated exposure given by the 90th percentile drift values to a more realistic deposit estimation for off-field habitats).
- Correction factor (CF) = 10 ('Tier 1' uncertainty factor for the extrapolation from indicator species to all off-field non-target arthropods).
- The drift value is set at 2.77% for 1 application in field crops at 1m distance.

In-field and off-field hazard quotients for *A. rhopalosiphi* and *T. pyri*, calculated as above, are given in Table B.9.5.13.

Table B.9.5.13 Hazard quotients (HQ) for in-field and off-field exposure scenarios from the proposed crop uses of 'Asulam 400g/L SL'

Species	Application rate: litres 'Asulam 400g/L SL' /ha	LR_{50}: litres 'Asulam 400g/L SL' /ha	In-field HQ	Off-field HQ
A. rhopalosiphi	6.0	3.088	1.94	0.05
T. pyri	6.0	7.566	0.79	0.02

As the hazard quotients are less than the trigger value of 2 this indicates under the ESCORT 2 risk assessment methodology an acceptable risk to non-target terrestrial arthropods present in 'in-field' and 'off-field' habitats from the recommended application rate of Asulox (6.0 L/ha). This is further supported by the lack of adverse effects from exposure to asulam reported in laboratory toxicity studies with other crop relevant foliar and soil dwelling predators.

However, the above 'Tier 1' ESCORT 2 risk assessment is based only on mortality effects. Given the significant effects on *A. rhopalosiphi* and *T. pyri* reproduction

reported in glass plate residue tests, the significance of this in relation to the safety of the proposed use needs further consideration.

Assessment of fecundity effects on *A. rhopalosiphi* and *T. pyri* in extended lab studies

In an extended laboratory study with *A. rhopalosiphi* on treated barley seedlings, reproduction was not significantly reduced compared to control at 11 litres 'Asulam 400g/L SL' /ha. Therefore the results of the study conducted under more realistic conditions than the worst case glass plate studies indicate that under field conditions there is not likely to be adverse effects on *A. rhopalosiphi* fecundity.

In an extended laboratory study on bean leaves, reproduction of *T. pyri* was reduced significantly compared to control at both rates tested, 11 L product/ha and 440 ml product/ha, with 75% and 51% reductions in numbers of eggs laid per female, respectively. Therefore, there is a possible potential risk of adverse reproductive effects to non-target arthropods situated in 'in-field' habitats from the proposed use at 6 litres product /ha and further evaluation is required.

In relation to effects on 'off-field' non-target arthropods, based on the standard assumption (based on 90th percentile values) of 2.77% spray drift at 1 metre, levels of off-field exposure from use at the maximum proposed dose will be 6000 x 0.0277 = 166.2 ml product /ha. Although under ESCORT2 guidance, in relation to the 'Tier 2' risk assessment, off-field non-target arthropods should be assumed to be five times more sensitive than the standard indicator species (by inclusion of 'higher tier' 'correction factor' of 5), this increased sensitivity is more than off-set by the inclusion of a 'vegetation distribution factor' of 10. Therefore, when these two factors are taken into account, the effective dose for comparison with the toxicity of indicator species is 166.2 x 5/10 = 83.1 ml product /ha –which is much lower than the 440ml product /ha reported to result in 51% reductions in fecundity in the T. pyri extended lab (bean leaf substrate) toxicity studies. Any potential effects on the fecundity of non-target arthropods present in off-field habitats can therefore be predicted to less than 50% - which based on 'ESCORT 2' effects criteria for 'Tier 2' studies is deemed acceptable. An acceptable risk to off-field NTAs may therefore be concluded and no further assessment is required for this.

Further refined risk assessment in relation to potential adverse fecundity effects from in-field exposure to non-target arthropods

The potential for adverse fecundity effects have been indicated in laboratory glass plate residues toxicity studies with the two sensitive indicator species *T. pyri* and *A. rhopalosiphi*. However under the more realistic conditions of extended laboratory studies with these species where the effects of foliar residues were assessed, such adverse effects only occurred with *T. pyri*. Although use at 11 L/ha (equivalent to 1.8 times the proposed total dose per crop) resulted in 75% reductions in egg laying, use at 440ml/ha (equivalent to 176g asulam /ha) resulted in only 51% reductions, this latter figure being just above the ESCORT 2 maximum acceptable effect criteria of 50% for 'extended lab' higher tier studies.

The results of foliar residue studies conducted in spinach indicate a foliar half-life of 1.44 days (as supported in Section B.7.6 of the Volume 3 DAR). Based on this degradation rate and the maximum proposed crop dose in spinach and flower bulbs of 6 L/ha (equivalent to 2.4 kg asulam/ha), foliar residues will be reduce to levels equivalent to that initially obtained from a foliar application of 150g asulam/ha some 7.2 days after application and to that initially obtained from a foliar application of 4.7g

asulam/ha after 14.4 days. Based on the lack of adverse effects on fecundity from use up to 2.4 kg asulam/ha in the *A. rhopalosiphi* extended lab toxicity study and just 51% reductions in fecundity from use at 176g asulam/ha in the T. pyri extended lab toxicity study, adverse effects on fecundity to 'in-field' non-target arthropods from the much reduced foliar residues present 14 days after application (equivalent to 4.7g asulam/ha) would appear very unlikely.

Therefore, the above refined risk assessment based on the sensitivity of the indicator species T. pyri to effects on fecundity and likely rapid declines in foliar residues (DT50 = 1.44 days) indicates a likely lack of significant adverse fecundity effects on in-field non-target arthropods within 2 weeks of application. This is also further supported by the lack of adverse effects from exposure to freshly dried asulam residues in the other reported lab studies conducted with a relevant foliar dwelling predator (*Chrysoperla*), at up to 5.1 litres Asulox/ha and with three relevant ground dwelling predators (*Aleochara, Pardosa and Hypoaspis*) at doses of 9 litres product /ha or greater.

B.9.5.2.3 Non-target arthropod risk assessment conclusions

The risk assessment indicates that the proposed use of asulam in spinach and flower bulb crops is not likely to result in adverse mortality effects to non-target terrestrial arthropods. Based on the results of extended laboratory studies with *T. pyri* there may possibly be some initial adverse effects on fecundity. However, given the short foliar half-life of asulam, any adverse fecundity effects are likely to be short lived. It is concluded that the proposed use of 'Asulam 400g/L SL' in spinach and flower bulb crops poses an acceptable risk to non-target arthropods.

B.9.6 Effects on earthworms

B.9.6.1 Toxicity to earthworms

Details have been provided for earthworm acute and chronic toxicity studies conducted with asulam and its major soil metabolite sulphanilamide. These toxicity studies were previously evaluated (with no issues identified) in the Annex I evaluation for asulam (EFSA Conclusion Report 2010) - with the exception of a newly reported sulphanilamide earthworm chronic toxicity study (McCormac 2011) - which has been evaluated under the current submission. Study endpoints based on asulam sodium have been converted to asulam equivalents using a conversion factor of 0.9128 based on molecular weight differences of asulam (230.2) and asulam sodium (252.2). Details for these studies are included below, followed by a regulatory risk assessment.

B.9.6.1.1 Acute toxicity of asulam and sulphanilamide to earthworms

i) Report: J.W. Handley & P.M. Wetton (1992) The acute toxicity of asulam sodium to earthworms (*Eisenia foetida*). United Phosphorus Limited, Unpublished report No.: R001746; CA 8.9.1/01.

This study has previously been evaluated at EU level.

Guidelines:

OECD No 207 (1984) and EEC Commission Directive 87/302/EEC (1988). Deviations: None

GLP: Yes

Executive Summary:

In an acute toxicity study on asulam sodium conducted with the earthworm, *Eisenia fetida*, the 7-

day and 14-day LC_{50} was calculated to be 1460 mg asulam/kg (95% confidence limits: 1278-1735) and 1004 mg asulam/kg (95 confidence limits: 822-1187), respectively. No significant effects of asulam sodium were observed on the weight of earthworms after 14 days exposure.

Materials and Methods

Test Material:

Asulam sodium, purity 90.7%, Batch no.: EN 50005

Test Design:

40 earthworms of species *Eisenia foetida* (4 replicates of 10 earthworms per treatment level) were exposed to 5 test concentrations (180, 320, 560, 1000 and 1800 mg asulam sodium/kg soil), and a control. Earthworms were also exposed to chloroacetamide at the following concentrations: 5.6, 10, 18, 32 and 56 mg/kg soil. The test was performed in 1 litre glass beakers covered by plastic film with ventilation holes, under continuous illumination at 750 lux, a temperature of 21°C. Chloroacetamide was used as toxic standard.

Mortality after 7 and 14 days and body weight at test initiation and test termination was assessed.

Results and Discussion:

A dose-response increase in mortality was seen at test concentrations of 320 to 1800 mg asulam sodium/kg. No significant effects of asulam sodium were observed on the weight of earthworms after 14 days exposure. The results are summarised in Table B.9.6.1.

Table B.9.6.1 Acute toxicity of asulam to the earthworm *Eisenia foetida*

Nominal concentration (mg/kg)	% mortality (initial population: 40 ind./concentration)		Worm weights (mean ± standard deviation)	
Asulam	Day 7	Day 14	Day 0	Day 14
Control	0	0	0.43 ± 0.08	0.38 ± 0.06
180	0	0	0.39 ± 0.07	0.36 ± 0.07
320	0	3	0.39 ± 0.07	0.37 ± 0.06
560	3	15	0.39 ± 0.07	-
1000	5	43	0.38 ± 0.06	-
1800	60	88	0.38 ± 0.07	-
Chloracetamide				
Control	0	0		
5.6	0	0		
10	0	0		
18	73	80		
32	100	100		
56	100	100		

The 7-day and 14-day LC_{50} of asulam sodium was calculated to be 1600 mg/kg (95% confidence limits: 1400-1900) and 1100 mg/kg (95 confidence limits: 900-1300) respectively.

Based on mortality and sublethal effects on weight or behaviour, the NOEC was 180 mg asulam sodium/kg.

The artificial soil contained 10% sphagnum moss peat and as the log P_{ow} for asulam is <1, the endpoints need not to be corrected.

The 14-day LC_{50} of chloroacetamide was found to be 15 mg/kg (95% confidence limits: 14-16 mg/kg), what was within the normal range for this compound.

Conclusions:

The 14-day LC_{50} of asulam sodium to earthworms was 1100 mg/kg, equivalent to 1004 mg asulam/kg (95% confidence limits: 822-1187 mg asulam/kg). No effects on earthworm weight were observed at 180 mg/kg, equivalent to 164 mg asulam/kg.

RMS Comments:

This study has previously been evaluated at EU level. The study is scientifically valid and suitable for use in the regulatory risk assessment. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level.

ii) Report: P. Sowig (2002) Acute toxicity to earthworms (*Eisenia fetida*) Sulfanilamide substance, pure, Code: AE C473799 00 1B99 0001. United Phosphorus Limited, Unpublished report No.: C023318; CA 8.9.1/02.

This study has previously been evaluated at EU level.

Guidelines:

OECD 207, 1984, EU (=EEC) 92/69/EWG (1988). Deviations: None.

GLP: Yes

Executive Summary:

Based on these results of an acute toxicity study on sulphanilamide conducted with the earthworm, *Eisenia fetida*, the LC_{50} was greater than 1000 mg/kg dry artificial soil and the NOEC with no mortality, no intoxication signs and no significant weight decrease compared to the control were 560 mg/kg dry artificial soil.

Materials and Methods

Test Material:

Sulphanilamide, purity 99.9% w/w, Batch no.: AE C473799

Test Design:

Earthworms of species *Eisenia foetida* were used as test organisms.

The worms were exposed in the artificial soil to five nominal treatment levels 100, 180, 320, 560 and 1000 mg/kg dry artificial soil. Four replicates with 10 worms were tested for each treatment and the untreated control.

Mortality and intoxication symptoms were assessed at day 7 and 14 after application. Weight of worms was determined at start and end of testing. Weight changes were compared with the untreated control.

Results and Discussion:

At day 7 and 14, no mortality and no intoxication symptoms were observed in the control and all treatment levels. The main results of weight assessment are summarised in Table B.9.6.2.

Table B.9.6.2 Acute toxicity of sulphanilamide to the earthworm *Eisenia foetida*

Treatment group	Mean start weight of 10 worms (g)	Mean individual weight difference between start and end (g/worm)	Mean percent weight change/worm
Control	4.55500	0.02325	5.064
100 mg/kg	4.56250	0.03225	7.031
180 mg/kg	4.56500	0.04325	9.466
320 mg/kg	4.56750	0.02625	5.691
560 mg/kg	4.54250	0.03825	8.440
1000 mg/kg	4.55250	0.04850 *	10.648 *

* significantly different from the control (DUNCAN's Multiple Range Test, α=0.05).

At the start of the test there was no statistically significant difference of worm weights between the treated groups and the control.

At test termination there was a statistically significant weight change from the control at 1000 mg/kg only (alpha = 0.05).

Conclusions:

Based on these results, the LC_{50} of sulphanilamide to earthworms *Eisenia foetida* was greater than 1000 mg/kg dry artificial soil and the NOEC with no mortality, no intoxication signs and no significant weight decrease compared to the control was 560 mg/kg dry artificial soil.

RMS Comments:

This study has previously been evaluated at EU level. The study is scientifically valid and suitable for use in the regulatory risk assessment. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level.

B.9.6.1.2 Chronic toxicity of asulam and sulphanilamide to earthworms

Report: U. Luehrs (2000) Effects of ASULAM sodium salt on reproduction and growth of earthworms *Eisenia fetida* (Savigny 1826) in artificial soil. United Phosphorus Limited, Unpublished report No.: C010993; CA 8.9.2/01.

This study has previously been evaluated at EU level.

Guidelines:

BBA VI 2-2, 1994, ISO 11268-2, 1998
Deviations: Water content at test end was determined of 2 test containers per treatment group instead of all containers (according to BBA guideline) or one container (according to ISO guideline). This was not considered to affect the results of the study.

GLP: Yes

Executive Summary:

In a chronic toxicity study conducted with the earthworm, *Eisenia fetida*, to assess the effects of asulam sodium on reproduction and growth, the NOEC for asulam sodium salt found in this study was 198.0 mg/kg soil, equivalent to 180.7 mg asulam/kg, the highest concentration tested.

Materials and Methods

Test Material:

Asulam sodium salt, purity 896 g asulam sodium salt/kg, Batch no.: PN 24049

Test Design:

Under laboratory conditions, *Eisenia fetida* (40 worms per treatment group, 4 replicates of 10) were exposed to the following concentrations of asulam sodium salt which was mixed into the soil: 12.54, 25.09, 49.28, 99.46 and 198.0 mg as/kg artificial soil. The control was treated with deionised water. Derosal SC (8.0 mg/kg artificial soil) was used as toxic standard. The test duration was 8 weeks (4 weeks of adult worms' exposure and 4 weeks of offspring exposure).

The number of dead and damaged (e.g. apathy, rigidity) adult was recorded at day 28 after application. The cumulative amount of food added to each test container within the test period was also recorded. The mean body weights were assessed at day 0 and day 28. The number of young worms 8 weeks after application was recorded. pH and water content were measured at the start of each treatment group and 8 weeks after application.

Results and Discussion:

The pH was ranged from 6.1 to 6.3 at the beginning of the test and from 6.2 to 6.3 at the end of the test. Water content was ranged from 27.0 to 28.7% at the beginning of the test and from 26.2 to 29.5% at the end of the test.

The key biological information is summarised in Table B.9.6.3.

Table B.9.6.3 Chronic toxicity of asulam to the earthworm *Eisenia foetida*.

Concentration (mg asulam/kg)	Mortality (%) at test termination *	Bodyweight change (%) *	Reproduction Number of juveniles *	Amount of added (g) *
Control	0.0 ± 0.0	-0.4 ± 4.4	273 ± 19	20.0 ± 0
12.54	0.0 ± 0.0	5.4 ± 4.4	283 ± 22	19.3 ± 1
25.09	2.5 ± 5.0	-1.3 ± 3.2	199 ± 105	19.0 ± 0
49.28	5.0 ± 5.8	-2.2 ± 3.3	260 ± 82	18.8 ± 1
99.46	0.0 ± 0.0	2.7 ± 2.4	313 ± 28	18.8 ± 1
198.0	0.0 ± 0.0	6.8 ± 3.4 ***	317 ± 23	19.0 ± 0
Toxic standard Derosal DC, 8 mg/kg	15.0 ± 5.8 **	-21.4 ± 3.6 ***	0 ± 1 **	12.0 ± 0

* mean ± standard deviation from 4 replicates
** significant difference compared to the control (Mann-Withney-U-Test, α = 0.05)
*** significant difference compared to the control (Dunnett-Test, α = 0.05)

The results showed neither lethal nor sublethal effects of asulam sodium salt on E*isenia foetida*. Mortality, growth, reproduction and feeding activity of the earthworms were not affected by exposing them to concentrations ranged from 12.54 to 198.0 mg asulam sodium salt/kg artificial soil.

Conclusions:

The NOEC for asulam sodium salt found in this study was 198.0 mg/kg soil, equivalent to 180.7 mg asulam/kg, the highest concentration tested.

RMS Comments:

This study has previously been evaluated at EU level. The study is scientifically valid and suitable for use in the regulatory risk assessment. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level.

Report: A. McCormac (2011) Sulfanilamide – Determination of Chronic (sub-lethal) toxicity to the earthworm *Eisenia fetida* in artificial soil substrate. United Phosphorus Limited, Unpublished report No.: UP-11-10; CA 8.9.2/02.

This study has not previously been evaluated at EU level. Sulphanilamide is a potentially relevant soil metabolite and although long-term earthworm toxicity testing was not considered necessary in the DAR (March 2006) or EFSA Conclusion Report (2010), a new earthworm reproduction study has been conducted to address possible future concerns in this area.

Guidelines:

OECD 222 (2004). Deviations: None

GLP: Yes

Executive Summary:

Under laboratory conditions, *Eisenia fetida* were exposed to sulphanilamide mixed into artificial soil at concentrations of 1.88, 3.75, 7.5, 15.0 and 30.0 mg as/kg soil dry weight. The test duration was 8 weeks (28 days of adult worms' exposure and 28 days of offspring exposure). The results showed that sulphanilamide did not result in significant mortality, biomass changes or observed behaviour changes of E*isenia foetida* at treatment concentrations up to and including 30.0 mg/kg, the maximum tested. For reproduction, the numbers of juveniles produced were not affected up to and including 3.75 mg /kg soil dry weight and so this was determined as the NOEC.

Materials and Methods

Test Material:

Sulfanilamide, purity 100% w/w nominal, 101.4% w/w measured, Lot no.: L010098499

Test Design:

Under laboratory conditions, *Eisenia fetida* (40 worms per treatment group, 4 replicates of 10; 80 worms in the control, 8 replicates of 10) were exposed to the following concentrations of sulfanilamide which was mixed into the soil: 1.88, 3.75, 7.5, 15.0 and 30.0 mg as/kg artificial soil dry weight (containing 10% peat). The control was treated with purified water. Delsene 50 Flo (500 g/L suspension concentrate formulation of carbendazim) was used as a toxic standard in a separate bioassay. The test duration was 8 weeks (28 days of exposure to adult worms - before their removal and assessment - and a further 28 days of offspring exposure).

The number of dead and damaged (e.g. apathy, rigidity) adults was recorded at day 28 after application. The cumulative amount of food added to each test container within the test period was also recorded. The mean body weights were assessed at day 0 and day 28. The number of young worms 8 weeks after application was recorded. pH and water content were measured at the start of each treatment group and 8 weeks after application.

Results and Discussion:

The pH was ranged from 6.1 to 6.3 at the beginning of the test and from 4.8 to 5.6 at the end of the test. Water content was 50% water-holding capacity (WHC) at the beginning of the test and from 53 to 57% WHC at the end of the test.

The key biological information is summarised in Table B.9.6.4.

Table B.9.6.4 Chronic toxicity of sulphanilamide to the earthworm *Eisenia foetida*

Concentration (mg sulphanilamide/kg soil dry weight)	Mortality (%) at 28 DAT *	Corrected mortality (%) **	Change in worm fresh weight (%) ***	Mean number (Coeff. Var.) of juveniles per replicate ****	Decrease in numbers of juveniles relative to control (%) *****
Control	3	-	54 (15.8)	223 (18.9)	-
1.88	0	0	51 (15.9)	213 (19.2)	4
3.75	8	5	46 (12.7)	224 (10.4)	-1
7.5	8	5	38 (10.5)	136 (27.8) [1]	39
15.0	0	0	39 (13.9)	162 (25.2)	28
30.0	5	3	40 (14.9)	141 (20.7) [1]	37

* Mortality after 28 days. The 28 DAT results for individual treatments were compared to the control using Fisher's Exact Test ($\alpha = 0.05$) but none differed significantly. There were no signs of skin lesions or altered behaviour in the adult worms at 28 DAT for any of the treatment concentrations
** Data corrected for the mean control mortality using Abbott's formula
*** The mean (and standard deviation) for percentage change in worm weights in replicate arena between 0 and 28 DAT. A positive value indicates an increase in fresh weight. The test item and control were compared by one-way ANOVA ($\alpha = 0.05$), but there was no significant difference.
**** The mean number of juveniles produced per replicate. Values for the Coefficient of Variation are given in parentheses. The individual test item treatment results for reproduction were compared to control by one-way ANOVA and Dunnett's test ($\alpha = 0.05$), and [1] indicate where there was a significant difference ([1]$P < 0.001$)
***** A positive value indicates a decrease and a negative value an increase in reproduction, relative to the control

The results showed that sulphanilamide did not result in significant mortality, biomass changes or behaviour of adult earthworms (E*isenia foetida*) at treatment concentrations up to and including 30.0 mg sulphanilamide/kg soil dry weight, the maximum tested. For reproduction, the number of juveniles produced were not affected at a treatment concentration up to and including 3.75 mg sulphanilamide/kg soil dry weight.

Conclusions:

The NOEC for sulphanilamide found in this study was 3.75 mg/kg soil dry weight.

RMS Comments:

This study has not previously been evaluated at EU level. The study is GLP compliant and has been conducted to a standard agreed (OECD 222) test guideline. As a 'new' study to the RMS, the three required study guideline validity criteria (relating to the control results) have been checked. The rate of reproduction in the control (including 10 adults per replicate) at 223 per replicate is greater than the minimum required of 30 per replicate; the co-efficient of variation in the control of 18.9% is within the maximum considered valid of 30%; also the control mortality at 3% is within the maximum permitted of 10%. Therefore, all of the OECD 222 test guideline validity criteria have been met. It is concluded that the study results, indicating a long-term reproductive NOEC = 3.75 mg sulphanilamide /kg dw soil, are scientifically valid and suitable for use in the regulatory risk assessment.

B.9.6.2 Earthworm risk assessment

This risk assessment has been conducted in line with current terrestrial ecotoxicology guidance detailed under SANCO/10329/2002 (October 2002), based on Directive

91/414's ecotoxicology data requirements – carried over as an interim measure to Regulation 1107/2009 and applicable to submissions received prior to 1st January 2015.

A summary of the acute and long-term effects to earthworms of asulam and its one major (i.e. > 10% AR) soil metabolite sulfanilamide is given in Table B.9.6.5. A second soil metabolite 'methyl benzene sulphonyl carbamate' (MBSC) has been identified as being formed at upto 6% A.R. in soil. However, given the close structural similarity of this metabolite to asulam (differing only in the loss of a 'NH2 group – see 'Background information' Section B.9.0 for further details), the RMS considers that MBSC is not likely to be more toxicity to soil organisms than asulam and therefore any risk from its presence will be covered by the risk assessment conducted for asulam. According to EPPO 1992 environmental risk assessment guidance (Chap 8, Note 6), where the organic matter in test soils is 10% or greater (as in the reported studies) and the octanol/water partition co-efficient (log K_{ow}) is > 2, a correction factor of 2 should be applied to the LC_{50}. This is to take into account the likely greater biological availability in an average agricultural soil (which typically contains not > 5% organic matter). However, given that asulam and its major soil metabolite, sulphanilamide, both have a log K_{ow} of less than 2, this correction factor is not required.

Table B.9.6.5 Summary of acute and long-term effects on earthworms from asulam/sulphanilamide

Test substance	Study type & time scale	Toxicity endpoint	Test guideline*	References
Asulam sodium	Acute toxicity, 14 days	LC_{50} = 1004 mg Asulam /kg soil	OECD 207 (1984), EEC 87/302/EEC (1988)	Handley JW & Wetton PM (1992)
	Reproductive toxicity, 8 weeks	NOEC = 180.7 mg Asulam /kg soil	BBA VI 2-2, 1994, ISO 11268-2, 1998	Luehrs U (2000)
Sulphanil-amide	Acute toxicity, 14 days	LC_{50} > 1000 mg metabolite /kg soil	OECD 207 (1984), EEC 92/69/EWG (1988)	Sowig P (2002)
	Reproductive toxicity, 8 weeks	NOEC = 3.75 mg Metabolite /kg soil	OECD 222 (2004).	McCormac A (2011)

* Tests conducted without significant deviation from guideline and in accordance with GLP

There is no additional risk from the formulated product 'Asulam 400g/L SL' over that of the active substance, asulam, as it is a simple dilution in water. Therefore, studies on the effects of asulam are fully representative of that which would be obtained with Asulox.

Assessment of exposure and risk was conducted according to the principles as laid down in the current guidance document SANCO/10329/2002 rev 2 final, dated 17 October 2002. Based on the above toxicity endpoints and worst case exposure assumptions, the acute and long-term TERs for earthworms are determined in Table B.9.6.2.

Table B.9.6.2 Risk to earthworms from exposure to asulam and sulphanilamide

Compound	Toxicity endpoint mg/kg soil	PEC mg/kg soil#	TER	Trigger
Asulam	Acute: 14 d LC_{50} = 1004	3.2	313.7	10
	Long-term: 8 week NOEC = 180.7	3.2	56.5	5
Sulphanilamide	Acute: 14 d LC_{50} >1000	0.41	> 2439	10
	Long-term: 8 week NOEC = 3.75	0.41	9.1	5

See Section B.8.3 and Table B.8.108 for full details of these values

Conclusions:

The acute and long-term TERs for asulam and sulphanilamide are within the Uniform Principles trigger values, indicating a low and acceptable risk to earthworms from these substances.

B.9.7 Effects on soil macro-organisms

B.9.7.1 Toxicity to other soil macro-organisms

Details have been provided for an asulam chronic toxicity study conducted with the soil mite *Hypoaspis aculeifer* (Feije R 2002) which was considered in the previous Annex I evaluation for asulam (with no issues identified), together with details for a newly reported sulphanilamide chronic toxicity with the collembola *Folsomia candida* (Vinall S 2011). Study summaries for each are included below.

i) Report: R. Feije (2002) Asulam (EXP 04668 A = AE F074383): An extended laboratory single-dose test to evaluate the effects on survival and reproduction of the predaceous mite *Hypoaspis aculeifer Canestrini* (Acari: Laelapidae) in standard soil (LUFA 2.1) United Phosphorus Limited, Unpublished report No.: C030819 and C038575; 10.6.6/01

This study has previously been evaluated at EU level.

Guidelines:

SETAC Candolfi (2001), EPPO 142 (1989) Bakker *et al*., in preparation
Deviations: None

GLP: Yes

Executive Summary:

The effects of EXP 04668A ('Asulam 400 g/L SL') on the predaceous soil mite *Hypoaspis aculeifer* was measured in the laboratory in an extended laboratory test (exposure in a standard soil). No statistically significant effects on mortality and reproduction capacity of *Hypoaspis* occurred after exposure to Asulam 400 g/L SL at a rate of 156.8 kg asulam/ha in 400 L water/ha (corresponding to 400 L AE F074383 00SL33 A/ha), equivalent to 4370 mg asulam/kg soil, in this extended laboratory study.

Materials and Methods

Test Material**:**

EXP04668A or AE F074383 00SL33 A1 (400 g/L SL), purity 392 g asulam/L, Batch no.: OP990533

Test Design:

Under extended laboratory conditions, the test substance was applied to standard soil (LUFA 2.1) at one nominal rate: 156.8 kg asulam/ha diluted in 400L deionised water/ha (corresponding to 400 L AE F074383 00SL33 A/ha). The control was treated with deionised water. Dimethoate at a rate of 96 g a.s./ha (24% of the highest recommended field rate) was used as toxic reference.

The bioassay was initiated within 1 hour after application by confining 20 protonymphs of *Hypoaspis aculeifer* per Munger unit (inert glass material). Five units were prepared for the water control, 5 units for the test rate of asulam (EXP04668A = AE F074383 00SL33 A1) and 4 units for the toxic reference. 14 days after initiation mortality was assessed.

Reproductive success was determined for mites of the deionised water control and the 156800 g as/ha test substance rate. Hereto all surviving mites of these treatments were transferred to untreated mating units (keeping replicate groups together). After a 7-day mating period, 20 females of the 156800 g asulam/ha treatment and the water treatment were transferred to reproduction units (1 mite/unit) to determine egg production. After 3 days all females were transferred to a second series of identical reproduction units and 4 days later the females were removed. In this way there were two oviposition assessments in a 7-day period. Reproduction units were kept for egg hatch determination for an additional 5-6 days.

Mortality in the treatment groups was compared pair-wise to the water control group using Fisher's Exact Test. Egg production (fertile eggs/female/7 days) was compared to the water control group using ANOVA techniques.

Results and Discussion:

The main biological information is summarised in Table B.9.7.1.

Table B.9.7.1 Chronic toxicity of Asulam 400 g/L SL to the predaceous soil mite *Hypoaspis aculeifer*

Treatment group	Mortality after 14 days	Reproduction (fertile eggs/female/7 days)
Control	11%	24.6
	Corrected mortality after 14 days	Reproduction after 7 days (% reduction relative to control)
EXP 04668A (156.8 kg asulam/ha)	-4%	24.8 (-1%)
Toxic reference (dimethoate)	100%	Not assessed

Conclusions:

No statistically significant effects on mortality and reproduction capacity of predaceous mites *Hypoaspis aculeifer* occur after exposure to Asulam 400 g/L SL at a rate of 156.8 kg asulam/ha in 400 L water/ha (corresponding to 400 L AE F074383 00SL33 A/ha), in this extended laboratory study. This is equivalent to 4370 mg asulam/kg soil, using a surface area of 12.57 cm^2 and mean soil weight of 4.51 g.

RMS Comments:

This study has previously been evaluated at EU level, with it being considered to be scientifically valid and suitable for use in the regulatory risk assessment. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level.

ii) Report: S. Vinall (2011) Sulfanilamide – Laboratory determination of toxicity to the springtail *Folsomia candida* (Collembola, Isotomidae) in an artificial soil substrate. United Phosphorus Limited, Unpublished report No.: UP-11-11; CA 8.8.2.5/02

This study has not previously been evaluated at EU level.

Guidelines:

OECD No 232 (2009). Deviations: None

GLP: Yes.

Executive Summary:

Springtails (*Folsomia candida*) were exposed to 6 test concentrations of sulphanilamide (1.0, 3.125, 6.25, 12.5, 25 and 50 mg/kg dry soil weight). The number of surviving adults and F1 progeny in each test arena were recorded at 28 DAT. The 28-day EC_{50} for sulphanilamide with respect to the springtail *Folsomia candida*, was greater than 50 mg test item/kg soil dry weight, the highest concentration tested. In addition, no adverse effect on reproduction was seen at 50 mg/kg soil dry weight and so this was considered to be the NOEC.

Materials and Methods

Test Material:

Sulphanilamide, Measured purity 101.4%, Lot no.: L010098499

Test Design:

40 springtails of species *Folsomia candida* (4 replicates of 10 springtails per test item treatment level) were exposed to 6 test concentrations (1.0, 3.125, 6.25, 12.5, 25 and 50 mg test item/kg dry soil weight), and a control. *Folsomia candida* were also exposed to a toxic reference, phenmedipham at the concentration: 200 mg/kg soil dry weight. The test was performed in 125 mL glass jars covered by a lid, but were opened for brief periods every 1-4 days to allow fresh air into the arenas. The bioassay conditions were under a 16 h photoperiod of 490-640 lux, a temperature of 19.8-21.8°C and 74-81% relative humidity.

The number of surviving adults and F1 progeny in each test arena were recorded at 28 DAT.

Results and Discussion:

The main biological information is summarised in Table B.9.7.2.

Table B.9.7.2 Chronic toxicity of sulphanilamide to the springtail *Folsomia candida*

Treatment	Nominal concentration (mg sulphanilamide/ kg soil)	% mortality at 28 DAT [a)]	Corrected % mortality at 28 DAT [b)]	Mean no. progeny per replicate [c)]	% change in numbers of progeny, relative to control [d)]
Control	-	13.8	-	257#	-
Sulphanilamide	1.0	15.0	1.0	451*	-75.4
	3.125	35.0**	25.0	278	-8.3
	6.25	27.5	16.0	284	-10.5
	12.5	27.5	16.0	414	-61.0
	25	20.0	7.0	484**	-88.3
	50	7.5	0.0	512**	-99.2
Toxic reference	-	88.0***	86.0	6***	97.5

Control standard deviation = 39.4 and coefficient of variation 15.3 (checked by RMS based on control replicate data included in Appendix III of study report)
[a)] Mortality in individual test item treatments compared to the control using Fisher's Exact Test. Treatment means that differed significantly from the control are indicated with asterisks (*** P < 0.001, ** P < 0.01).
[b)] Calculated using Abbott's formula.
[c)] Fecundity assessment data from individual treatments were compared to the data from the control by one-way ANOVA and Dunnett's t-test. Values marked with asterisks differed significantly from the control (*** P < 0.001, ** P < 0.01, * P < 0.05). Note: significance observed for 50, 25 and 1.0 mg test item/kg treatment rates was due to an increase in juveniles.
[d)] A positive value indicates a decrease and a negative value an increase in reproduction, relative to the control.

No treatment-related mortality of adult springtails was seen up to and including 50 mg test item/kg dry soil. The median effect concentration (EC_{50}) for sulphanilamide after 28 days was found to be greater than 50 mg of test item/kg dry soil, the highest concentration tested. The NOEC for reproduction was 50 mg test item/kg soil dry weight.

Conclusions:

The 28-day EC_{50} for sulphanilamide with respect to the springtail *Folsomia candida*, was greater than 50 mg test item/kg soil dry weight, the highest concentration tested. In addition, no adverse effect on reproduction was seen at 50 mg test item/kg soil dry weight and so this was considered to be the NOEC.

RMS Comments:

This study has not previously been evaluated at EU level. The study is GLP compliant and has been conducted to a standard agreed (OECD) test guideline. The results of the untreated control pass the three specified validity criteria for OECD 232 (2009) i.e. mean adult mortality not exceeding 20% (actual 13.8%), mean number of juveniles per vessel (replicate) at least 100 (actual 257), and coefficient of variation not greater than 30% (actual 15.3%). The study results, indicates a statistically significant increase in progeny numbers at 1, 25 and 50 mg sulphanilamide /kg dw soil – although these effects are not considered 'adverse'. The RMS concludes that the study results indicate a long-term reproductive NOAEC = 50 mg sulphanilamide /kg dw soil – which has been used in the regulatory risk assessment.

B.9.7.2 Soil macro-organism risk assessment:

An assessment is required for asulam and also its major soil metabolite sulphanilamide. A second soil metabolite 'methyl benzene sulphonyl carbamate' (MBSC) has been identified as being formed at upto 6% A.R. in soil. However, given the close structural similarity of this metabolite to asulam (differing only in the loss of a 'NH2 group – see 'Background information' Section B.9.0 for further details), the RMS considers that MBSC is not likely to be more toxicity to soil organisms than asulam. Additionally MBSC is only of moderate persistence in soil (lab DT90 27.6-122 days). It is concluded that any risk from the presence of MBSC will be covered by the risk assessment conducted for asulam and therefore a specific risk assessment for this metabolite is not required.Based on current terrestrial ecotoxicology risk assessment guidance (SANCO/10329/2002 dated October 2002) and 'Annex II' data requirements (carried over as an interim measure to Regulation 1107/2009 from Dir. 91/414), toxicity data on soil macro-organisms (other than earthworms) that contribute to organic matter breakdown are required for more persistent active substances /metabolites (field DT50 ≥ 100 days) that fail the first tier 'ESCORT 2' hazard quotient risk assessment.

SANCO/10329 (2002) guidance also advises that where the field DT90 is >365 days, a litter bag study is required. Table B.8.92 of Section B.8.1.4 reports laboratory study aerobic soil first order DT_{90}s of 7.1-37.2 days for asulam and 10.3-148.7 days for sulphanilamide. Therefore, based on these levels of persistence a litter bag study is not required for either asulam or its major soil metabolite sulphanilamide.

Details have been provided for an asulam chronic toxicity study conducted with the soil mite *Hypoaspis aculeifer* which was considered in the previous Annex I evaluation for asulam (with no issues identified) and for a new study on sulphanilamide's chronic toxicity to the collembola *Folsomia candida*. Both are considered to be scientifically valid studies, suitable for use in a regulatory risk assessment. Both studies include an assessment of adverse reproductive effects on soil macro-organisms and as such address the possible potential for such effects reported in some of the crop dwelling non-target arthropod toxicity studies (Section B.9.5.1).

Soil macro-organism long-term toxicity exposure values (TERs) are presented for asulam and its metabolite sulphanilamide in Table B.9.7.3, based on a comparison of the concluded soil macro-organism long-term endpoint (NOEC /NOAEC) with the maximum derived soil PEC for each.

Table B.9.7.3 Soil macro-organism risk assessment

Test material	Organism	Endpoint	(mg asulam or sulphanil-amide /kg dw soil	Maximum PEC (mg asulam or sulphanil-amide /kg dw soil) #	TER	Trigger value
'Asulam 400 g/L SL'	*Hypoaspis*	NOEC	4370	3.20	1366	5
Sulphanilamide (major soil metabolite)	*Folsomia*	NOAEC	50	0.41	122	5

Estimated by RMS in Section B.8.3 of Volume 3 DAR

The long-term TERs for asulam and its major soil metabolite sulphanilamide are all in excess of the trigger value of 5, indicating a low and acceptable risk to soil macro-organisms from exposure to asulam and sulphanilamide following the proposed use of 'Asulam 400 g/L SL' ('Asulox') in spinach and flower bulb crops.

B.9.8 Effects on soil micro-organisms

B.9.8.1 Effects on soil microbial nitrogen transformation processes

i) Report: B.M. Luscombe (1981a) Asulam - Effect on soil micro-organisms: II. Studies on soil processes - carbon cycle, mobilisation of organic nitrogen, denitrification and enzyme activity. United Phosphorus Limited, Unpublished report No.: R000731; CA 8.10.1/01

This study has previously been evaluated at EU level.

Guidelines: Not specified. Deviations: Not specified.

GLP: No

Executive Summary:

Asulam when present in soil at a concentration of 16 mg asulam/kg (equivalent to an application of 4.5 kg/ha when distributed in a 25 mm layer of soil) did not cause any significant effects on any of the soil processes studied. However at a concentration of 160 mg asulam/kg in soil (equivalent to an application of 45 kg/ha) production of nitrate was 26% lower than in untreated soil after 56 days (end of study), and after 14 days nitrate loss from soil by denitrification was slightly, but significantly different from the controls. The activity of the soil enzymes dehydrogenase and phosphatase were not significantly affected by asulam at 16 ppm or 160 mg asulam/kg in the soil.

Materials and Methods

Test Material:

Asulox®, purity 40%, Batch no.: NE 281

Test Design:

Asulox® was distributed to a 25 mm layer of clay-loam soil at concentrations of 16 and 160 mg asulam/kg (equivalent to field rate of 4.5 and 45 kg/ha). After addition of asulam and amendments, soils were made up to 70% WHC with distilled water and incubated at 20°C in the dark. Untreated controls (no asulam) were run in a similar way.

Mobilisation of organic nitrogen: samples of soil were amended with nitrogen, using uric acid as source of nitrogen and energy. Rates of ammonification of organic nitrogen and nitrification were assessed.

Denitrification: To investigate the effect of incorporating asulam to give 16 or 160 ppm w/w on denitrification samples of soil were amended with nitrogen in the form of sodium nitrate, and sodium acetate as a non-fermentable source of energy.

Soil enzyme activity: The activities of dehydrogenase and phosphatase enzymes were studied in samples taken from the same batches of soil treated to give 16 and 160 ppm w/w asulam.

Results and Discussion:

Asulam at a concentration of 160 mg asulam/kg in soil caused a significant (25%) reduction in the formation of inorganic nitrogen during the first week after treatment but within 28 days there was no longer any difference between the organic nitrogen content of treated and untreated soils. Nitrate production was also reduced by asulam but it was not possible to determine how much

nitrification was affected by the reduction in the formation of ammonium. The effects of asulam seemed largely to have disappeared within 56 days of treatment although nitrate production was still 26% lower than untreated soil. After treatment with asulam to give 16 mg asulam/kg production of nitrate was significantly lower than in untreated soil for 28 days, but by 56 days the levels were not significantly different. The results are shown in Table B.9.8.1.

Table B.9.8.1 Effects of Asulox (asulam) on denitrification processes in soil

Group	Mean inorganic nitrogen (mg) produced by mineralization of uric acid				Mean nitrate-nitrogen (mg) produced following mineralization of uric acid				Reduction in nitrate-nitrogen (mg)			
	7 d	14 d	28 d	56 d	7 d	14 d	28 d	56 d	3 d	7 d	10 d	14 d
Untreated	12.8	17.7	20.6	16.1	4.9	14.7	17.6	16.1	8.6	21.3	23.0	24.4
16 mg/kg	9.3	14.7	23.0	19.1 [C]	1.3	5.0 [A]	12.4 [A]	12.0	2.7	14.8 [A]	16.5 [A]	19.3
160 mg/kg	8.5 [C]	12.5 [C]	21.0	19.7 [C]	0.1	2.8 [A]	10.3 [A]	11.9 [B]	3.3	7.1 [A]	14.7	20.2 [A]

[A] significantly different from the untreated control at the 1% level of probability (student's-t-test)
[B] significantly different from the untreated control at the 2% level of probability (student's-t-test)
[C] significantly different from the untreated control at the 5% level of probability (student's-t-test)

The activity of the soil enzymes dehydrogenase and phosphatase was not significantly affected by asulam at 16 ppm or 160 mg asulam/kg in the soil (see Table B.9.8.2)

Table B.9.8.2 Effects of Asulox (asulam) on soil enzyme activity

Group	Dehydrogenase activity			Phosphatase activity		
	µg Fromazan produced/5g/24 hours (mean value)			mg phenol formed/5g/2 hour (mean value)		
	6d	13 d	20 d	6d	13 d	20 d
Untreated	96.0	65.3	56.3	5.0	5.8	1.5
16 ppm	99.6	65.7	63.9	5.0	5.8	1.4
160 ppm	84.7	56.3	95.4	5.6	6.4	1.6

Conclusions:

Asulam, even when present in soil at a concentration of 160 mg asulam/kg, equivalent to an application of 45 kg/ha distributed in a 25 mm layer of soil, did not stop any of the soil processes studied.

In those processes, which were initially slowed by asulam treatment, rapid recovery was apparent. This indicates that asulam would not have any lasting effect on the soil micro-organism responsible for carrying out the processes.

RMS Comments:

This study has previously been evaluated at EU level. Both nitrogen and carbon transformation was assessed in the study, with the above details relating to nitrogen transformation. It is noted that the study was conducted in 1981, prior to the agreement of a standard soil microbial test guideline and also prior to the implementation of GLP. However, the study protocol is considered to be of an acceptable standard, with the study results suitable for use in the regulatory risk assessment. The tested formulation 'Asulox' is identical to the representative product 'Asulam 400g/L SL' and as a simple dilution of asulam in water will not affect the active

substance's toxicity to soil micro-organisms. The presented conclusions are consistent with the EFSA conclusion report (2010) and no concerns were previously raised at EU level regarding this study.

ii) Report: B.M. Luscombe (1981b) Asulam: Effects on soil micro-organisms - III. Studies on soil processes - nitrogen fixation. United Phosphorus Limited, Unpublished report No.: R000733; CA 8.10.1/02.

This study has previously been evaluated at EU level.

Guidelines:

Not specified. Deviations: Not specified

GLP: No

Executive Summary:

The effect of asulam on nitrogenase activity (nitrogen fixation) was assessed in root nodules of alfalfa, *Medicago sativa*, and in non-symbiotic nitrogen fixing soil bacteria. Nitrogen fixation in root nodules of alfalfa was not adversely affected at 8.93 mg asulam/kg soil (2.2 kg/ha) but was reduced at 89.3 mg asulam/kg soil (22 kg/ha) which was attributed to phytotoxic affects at this dose affecting the energy supply to the nodules. However even at the rate of 89.3 mg asulam/kg soil, asulam had no significant effect on the survival of soil rhizobia or on their ability to infect the roots of alfalfa and initiate nodule formation.

Materials and Methods

Test Material:

Asulox®, purity 40% w/v, Batch no.: NE 281

Test Design:

The acetylene reduction method was used to determine the effect of asulam on nitrogenase activity (nitrogen fixation) in root nodules of alfalfa, *Medicago sativa*, and in non-symbiotic nitrogen fixing soil bacteria. The soil was sprayed in a 25 mm thick layer at 530L/ha with 2.2 and 22 kg/ha asulam using dilution of Asulox®, equivalent to 8.93 and 89.3 mg asulam/kg soil respectively. Soils were incubated during three months at 20°C before introduction of alfalfa seedlings. The untreated group was prepared in the same conditions. After incubation, soils were distributed into pots with 18 pots per treatment. Each pot contained 3 alfalfa plants.

At daily intervals triplicate bottles of each soil were assayed for nitrogenase activity.

At the end of exposure, counts of nodules forming on alfalfa plants grown in soil treated with asulam were compared with nodule numbers in plants grown untreated soil to determine effects of asulam on survival of soil microbia, and on their ability to infect alfalfa roots and initiate nodule formation.

Results and Discussion:

Over a period of 56 days no significant difference occurred in rates of nitrogen fixation in untreated alfalfa plants and in plants treated with 2.2 kg/ha asulam. However, asulam at 22 kg/ha proved phytotoxic to alfalfa and by 35 days after treatment the effect on the plant had caused a significant reduction in the rates of nitrogen fixation due to the dependence of the bacteria on a supply of energy from the plant (see Table B.9.7.3).

Nitrogenase activity was very low in the study on non-symbiotic nitrogen fixation and became undetectable within four days after amending the soil with garden compost as source of energy.

During this time, 2.2 kg/ha asulam distributed in 25 mm layer of soil had no significant effect on the enzyme activity. Three days after distributing 22 kg/ha asulam in 25 mm layer of soil a stimulation in enzyme activity occurred which proved significant at the 5% level of probability.

Counts of nodules developing on alfalfa plants grown in untreated soil and asulam treated soils indicated that asulam, even when 22 kg/ha were distributed in a 25 mm layer of soil, had no significant effect on the survival of soil rhizobia or on their ability to infect the roots of alfalfa and initiate nodule formation.

Table B.9.8.3 Effects of Asulox (asulam) on denitrification processes in soil

Asulam mg/kg	Mean ethylene production (µmol/h) at day										Number of nodules per replicate pot
	1	4	7	11	14	25	28	35	43	56	
Control	0.4776	0.4272	0.5003	0.3229	0.2970	0.2420	0.1704	0.2220	0.2667	0.1310	3501
2.2	0.3908	0.3769	0.3605	0.1814	0.2954	0.1673	0.1392	0.1820	0.1811	0.1307	3584
22	0.4326	0.3881	0.3768	0.2495	0.2098	0.1555	0.0946	0.0689 *	0.0686	0.0548 *	2930

* significantly lower than the control at the 5% level of probability.

Conclusions:

Nitrogen fixation in root nodules of alfalfa was not adversely affected at 8.93 mg asulam/kg soil (2.2 kg/ha) but was reduced at 89.3 mg asulam/kg soil (22 kg/ha) which was attributed to phytotoxic affects at this dose affecting the energy supply to the nodules. However even at the rate of 89.3 mg asulam/kg soil, asulam had no significant effect on the survival of soil rhizobia or on their ability to infect the roots of alfalfa and initiate nodule formation.

RMS Comments:

This study has previously been evaluated at EU level. It is noted that it was conducted in 1981, prior to the agreement of a standard soil microbial test guideline and also prior to the implementation of GLP. However, the study protocol is considered to be of an acceptable standard, with the study results suitable for use in the regulatory risk assessment. The tested formulation 'Asulox' is identical to the representative product 'Asulam 400g/L SL' and as a simple dilution of asulam in water will not affect the active substance's toxicity to soil micro-organisms. The presented conclusions are consistent with the EFSA conclusion report (2010) and no concerns were previously raised at EU level regarding this study.

iii) Report: U. Kölzer (2002) Assessment of the side effects of Asulam sodium salt on the activity of the soil microflora. United Phosphorus Limited, Unpublished report No.: C021214; CA 8.10.1/03.

This study has previously been evaluated at EU level.

Guidelines:

SETAC 1995, EPPO 1994. Deviations: None

GLP: Yes (certified laboratory)

Executive Summary:

The effects of asulam on soil non-target micro-organisms were assessed in a study that measured nitrogen mineralization. The test soil (loamy sand) was treated with asulam (purity of 82.2%) at 7.2 kg/ha (equivalent to 9.60 mg asulam/kg soil) and incubated for a total of 28 days. The deviation between the treated soil and the control soil was lower than 25% at the end of the 28-

day evaluation for nitrogen turnover. Therefore the impact on soil was considered negligible an application rate of 7.2 kg asulam/ha (equivalent to 9.60 mg asulam/kg soil).

Materials and Methods

Test Material:

Asulam, purity 822 g/kg, Batch no. OP210109

Test Design:

The test soil (loamy sand) was treated with asulam (purity of 82.2%) at 7.2 kg asulam/ha (equivalent to 9.60 mg asulam/kg soil) and incubated for a total of 28 days. The control consisted of soil treated with deionised water. A toxic standard treatment group with a formulation of Dinoterb was also tested under identical conditions to validate the test methods. Each treatment group contained 3 replicates.

The effect of asulam sodium on nitrogen turnover was assessed by monitoring the concentrations of ammonium, nitrite and nitrate in soil after amending it with ground Lucerne.

Measuring the short-term respiration rate of soil after amending the soil with glucose assessed the effect of asulam sodium salt on carbon mineralization.

Samples of soils were taken after 6 hours, 14 and 28 days of exposure.

Results and Discussion:

The results are summarised in Table B.9.7.4. There was a statistically significant difference (-6.71%) in the rate of nitrogen turnover in soil treated with 2-fold asulam sodium salt and control soil at the end of the 28-day evaluation. Since the deviation between the treated soil and the control soil was below the threshold value (25%) provided by the SETAC guideline, the impact of asulam sodium salt on the nitrogen turnover was considered to be negligible.

The deviation between the toxic standard and the control for nitrogen turnover were above 25% and thereby validating the test system.

Table B.9.8.4 Effects of asulam sodium on denitrification processes in soil

Treatment group	$N\text{-}NH_4^+$-N per mg/100g dry weight (deviation from the control)		
	after 6 hours	after 14 days	after 28 days
Control	0.22	0.33	0.13
Asulam sodium salt	0.29 (31.82%)	0.39 (18.18%)	0.16 (23.08%)
Toxic standard	0.23 (4.55%)	1.53 (363.64%)	0.15 (15.38%)
	$N\text{-}NO_3$--N per mg/100g dry weight (deviation from the control)		
	after 6 hours	after 14 days	after 28 days
Control	1.57	1.63	2.63
Asulam sodium salt	1.56 (-0.64%)	1.07 (-34.36%)	2.39 (-9.13%) *
Toxic standard	1.58 (0.64%)	1.16 (-28.83%)	4.52 (71.86%) *
	$N\text{-}NO_2^-$-N per mg/100g dry weight (deviation from the control)		
	after 6 hours	after 14 days	after 28 days
Control	0.11	0.07	0.07
Asulam sodium salt	0.10 (-9.09%)	0.10 (42.86%)	0.09 (28.57%)
Toxic standard	0.09 (-18.18%)	3.57 (5000.00)	0.52 (642.86%)

Treatment group	N-NH_4^+-N per mg/100g dry weight (deviation from the control)		
	after 6 hours	after 14 days	after 28 days
	Nmin (sum of NO3--N, NH4+-N, NO2--N) per mg/100g dry weight (deviation from the control)		
	after 6 hours	after 14 days	after 28 days
Control	1.90	2.03	2.83
Asulam sodium salt	1.95 (2.63%)	1.56 (-23.15%)	2.64 (-6.71%)
Toxic standard	1.90 (0.00%)	6.26 (208.37%)	5.19 (83.39%)

* significant differences to the control (Dunnett, Multiple-t-test, two-sided, α=0.05).

Conclusions:

The deviation between the treated soil and the control soil was lower than 25% at the end of the 28-day evaluation for nitrogen turnover. Therefore the impact on soil was considered negligible at the applied dose of 7.2 kg asulam/ha (equivalent to 9.60 mg asulam/kg soil).

RMS Comments:

This study has previously been evaluated at EU level. Both nitrogen and carbon transformation was assessed in the study, with the above details relating to nitrogen transformation. The study is GLP compliant and has been conducted to a standard agreed test guideline. It is considered scientifically valid and suitable for use in the regulatory risk assessment. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level.

iv) Report: L. Schulz (2011) Sulfanilamide – Effects on the activity of soil microflora (Nitrogen transformation test). United Phosphorus Limited, Unpublished report No.: 11 10 48 048 N; CA 8.10.1/04.

This study has not previously been evaluated at EU level. Sulphanilamide is a potentially relevant soil metabolite. Although testing the effects of this metabolite on soil microbial activity was not considered necessary in the original DAR (March 2006), a new soil nitrification study has been conducted to address any possible future concerns in this area.

Guidelines:

OECD 216, 2000. Deviations: None

GLP: Yes (certified laboratory).

Executive Summary:

The effect of sulfanilamide at two concentrations (0.33 mg test item/kg dry soil & 3.3 mg test item/kg dry soil) on nitrogen turnover was assessed by monitoring the concentrations of ammonium, nitrite and nitrate in soil after amending it with ground lucerne. Samples of soils were taken at 0, 7, 14 and 28 days of exposure. The deviation between the treated soil and the control soil was lower than 25% at the end of the 28-day evaluation at the highest concentration tested (3.3 mg/kg soil).

Materials and Methods

Test Material:

Sulfanilamide, analysed purity 101.4%, Lot no. L010098499

Test Design:

The effect of sulfanilamide at two concentrations (0.33 mg/kg dry soil & 3.3 mg/kg dry soil) on nitrogen turnover was assessed by monitoring the concentrations of ammonium, nitrite and nitrate in soil after amending it with ground lucerne. Three replicates were included in the control and in each of the two test concentrations.

An agricultural sandy loam German field soil (54.8 sand, 36.5 silt and 8.7% clay) mixed with 5g/kg soil dw of lucerne meal was used in the study. The soil pH was 6.3 and an organic carbon content of 1.36% - with microbial biomass forming 2.55% of the total soil organic carbon content. These soil characturistics are within that specified as required in OECD 216.

Samples of soils were taken for analysis in the study at 0, 7, 14 and 28 days of exposure.

Results and Discussion:

Effects on concentrations of nitrate in the test soil are presented in the following table:

Table B.9.8.5 Results of sulfanilamide soil microbial nitrogen transformation study

Days after application	Control		0.33 mg/kg soil dry weight			3.3 mg/kg soil dry weight		
	NO_3-N [mg/kg soil d.w.]	mg NO_3-N/ kg soil d.w./ day	NO_3-N (mg/kg soil d.w.)	mg NO_3-N/ kg soil d.w./ day	Deviation from control (%)[1]	NO_3-N [mg/kg soil d.w.]	mg NO_3-N/ kg soil d.w./ day	Deviation from control (%)[1]
0	19.7	-	19.4	-	-1.5	19.3	-	-2.0
7	33.7	4.8	32.7	4.7	-2.9	34.9	5.0	+3.7
14	40.1	2.9	38.3	2.7	-4.7	40.8	2.9	+1.7
28	53.6	1.9	51.7	1.8	-3.5	51.7	1.8	-3.4

[1] based on NO3-nitrogen production
- = inhibition
+ = stimulation

No adverse effects of the test item on nitrogen transformation in soil were observed at either test concentration of sulphanilamide (0.33 mg/kg dry soil and 3.3 mg/kg dry soil) after 28 days. No significant differences between the control and the test item treatments were calculated.

In a separate study the reference item Dinoterb caused a stimulation of nitrogen transformation of +42.0 %, +68.1% and +92.3% at 6.80 mg, 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application.

Conclusions:

The deviation between the treated soil and the control soil nitrogen transformation rate (based on nitrate formation) was lower than 25% at the end of the 28-day evaluation at the highest concentration tested (3.3 mg/kg soil).

RMS Comments:

This study has not previously been evaluated at EU level. The study is GLP compliant and has been conducted to a standard agreed (OECD) test guideline. The coefficient of variation for the rate of nitrogen transformation in the control group was within the required 'validity criteria'

value of ≤ 15% (actual maximum 2.7%). The study design and test results are considered scientifically valid and suitable for use in the regulatory risk assessment. Based on a lack of any adverse treatment related effects on soil nitrification (deviation from control N transformation rates of < 25%) , the study NOEC is 3.3 mg sulphanilamide /kg dw soil (the highest test dose).

B.9.8.2 Effects on soil microbial carbon transformation processes

i) Report: B.M. Luscombe (1981a) Asulam - Effect on soil micro-organisms: II. Studies on soil processes - carbon cycle, mobilization of organic nitrogen, denitrification and enzyme activity. United Phosphorus Limited, Unpublished report No.: R000731; CA 8.10.2/01.

This study has previously been evaluated at EU level.

Guidelines:

Not specified. Deviations: Not specified

GLP: No

Executive Summary:

Asulam when present in soil at a concentration of 16 mg asulam/kg (equivalent to an application of 4.5 kg/ha when distributed in a 25 mm layer of soil) did not cause any significant effects on any of the soil processes studied. At concentrations of 16 and 160 mg asulam/kg in the soil did not have a significant effect on the metabolism of glucose, dried plant material, chitin, cellulose and gelatine, by the end of the studies (maximum of 28 days).

Materials and Methods

Test Material:

Asulox®, purity 40%, Batch no.: NE 281

Test Design:

Asulox® was distributed to a 25 mm layer of clay-loam soil at concentrations of 16 and 160 ppm (equivalent to field rate of 4.5 and 45 kg/ha). After addition of asulam and amendments, soils were made up to 70% WHC with distilled water and incubated at 20°C in the dark. Untreated controls (no asulam) were run in a similar way.

Carbon cycle

For both concentrations, there were 5 replicates of soils amended with glucose or dried wheat leaves and 2 replicates of soils (only for 16 ppm) amended with chitin, cellulose or gelatine.

Effects of asulam on processes in the carbon cycle were assessed by measuring the amount of carbon dioxide released after amending soil with specific carbon sources and from untreated soil samples.

Results and Discussion:

There was a general trend for asulam initially to reduce the carbon dioxide production from the amendments. Either the reduction was not large enough to be statistically significant, or recovery took place within 28 days of treatment. It was concluded that asulam at concentrations of 16 and 160 mg/kg in the soil did not have a significant effect on the metabolism of glucose, dried plant material, chitin, cellulose and gelatine, by the end of the studies (maximum of 28 days).

The results are shown in Table B.9.8.6.

Table B.9.8.6 Effects of Asulox (asulam) on carbon dioxide production in soil

Group	Mean carbon dioxide release (µmol) from soil amended with											
	Glucose			Plant material								
	1 d	3 d	6 d	1 d	2 d	4 d	7 d	9 d	14 d	18 d	21 d	28 d
Untreated	244	280	285	75	208	494	673	739	820	843	867	895
16 mg/kg	228	272	264	75	117 [A]	296 [A]	489 [A]	561 [A]	631 [A]	664 [B]	680 [B]	700 [B]
160 mg/kg	215 [C]	266	265	90	127 [A]	232 [A]	479 [A]	596 [B]	707 [D]	735	756	776
					Chitin							
					3 d	7 d	10 d	14 d	17 d	21 d	24 d	28 d
Untreated					115	340	396	430	450	463	470	485
16 mg/kg					-28 *	143	217	282 [D]	302	330	337	345
					Cellulose							
Untreated					-13	-25	-6	45	107	150	170	193
16 mg/kg					-66	-53	-51	-36	2	97	175	250
					Gelatin							
Untreated					220	268	290	295	297	285	282	265
16 mg/kg					172	265	285	290	285	280	270	252

[A] significantly different from the untreated control at the 0.1% level of probability (student's-t-test).
[B] significantly different from the untreated control at the 1% level of probability (student's-t-test).
[C] significantly different from the untreated control at the 2% level of probability (student's-t-test).
[D] significantly different from the untreated control at the 5% level of probability (student's-t-test).
* minus figures represent a depression in carbon dioxide release compared to the unamended control.

Conclusions:

Asulam at concentrations of 16 and 160 mg/kg in the soil did not have a significant effect on the metabolism of glucose, chitin, cellulose and gelatine, by the end of the studies (maximum of 28 days). There was a small (22%) but statistical significant reduction (at P = 0.05) in metabolism in 'plant material' amended soils containing 16 mg asulam /kg dw but no statistically significant effects at 160 mg asulam /kg dw.

RMS Comments:

This study has previously been evaluated at EU level. Both nitrogen and carbon transformation (i.e. carbon dioxide production) was assessed in the study, with the above details relating to carbon transformation. It is noted that the study was conducted in 1981, prior to the agreement of a standard soil microbial test guideline and also prior to the implementation of GLP. However, the study protocol is considered to be of an acceptable standard, with the study results suitable for use in the regulatory risk assessment. The tested formulation 'Asulox' is identical to the representative product 'Asulam 400g/L SL' and as a simple dilution of asulam in water will not affect the active substance's toxicity to soil micro-organisms. The presented conclusions are consistent with the EFSA conclusion report (2010) and no concerns were previously raised at EU level regarding this study. Althuogh there was a small 22% reduction in carbon dioxide release on day 28 in 'plant material' amended soils containing 16 mg asulam /kg dw, there was no

statistically significant effects on day 28 at 160 mg asulam /kg dw – suggesting a lack of treatment related effects. Also, all effects on day 28 were less than 25% - which is currently the usual 'trigger' value.

ii) Report: U. Kölzer (2002) Assessment of the side effects of ASULAM sodium salt on the activity of the soil microflora. United Phosphorus Limited, Unpublished report No.: C021214; CA 8.10.2/02.

This study has previously been evaluated at EU level.

Guidelines:

SETAC 1995, EPPO 1994. Deviations: None

GLP: Yes

Executive Summary:

The effects of asulam on soil non-target micro-organisms were assessed in a study that measured carbon mineralization. The test soil (loamy sand) was treated with asulam (purity of 82.2%) at 7.2 kg/ha (equivalent to 9.60 mg asulam/kg soil) and incubated for a total of 28 days. The deviation in the short-term respiration rate between the treated soil and the control soil was lower than 25% at the end of the 28-day evaluation. Therefore the impact on soil was considered negligible at an application rate of 7.2 kg asulam/ha (equivalent to 9.60 mg asulam/kg soil).

Materials and Methods

Test Material:

Asulam, purity of 822 g/kg, Batch no.: OP210109

Test Design:

The test soil (classified as a loamy sand) was treated with asulam sodium salt at 2-fold the maximum anticipated field application rate (2 x 4.38 kg asulam sodium/ha, equivalent to 2 x 3.6 kg as/ha) and incubated for a total of 28 days. The control consisted of soil treated with deionised water. A toxic standard treatment group with a formulation of dinoterb was also tested under identical conditions to validate the test methods. Each treatment group contained 3 replicates.

The effect of asulam sodium salt on nitrogen turnover was assessed by monitoring the concentrations of ammonium, nitrite and nitrate in soil after amending it with ground lucerne [with test results for this included separately under B.9.7.1iii) above].

Measuring the short-term respiration rate of soil after amending the soil with glucose assessed the effect of asulam sodium salt on carbon mineralization.

Samples of soils were taken after 6 hours, 14 and 28 days of exposure.

Results and Discussion:

Table B.9.8.7 summarises the results of the short-term respiration experiment.

Table B.9.8.7 Effects of asulam sodium on soil respiration

Treatment group	mg CO_2/h/100g dry weight * (deviation from the control)		
	after 6 hours	after 14 days	after 28 days
Control	0.63	0.60	0.55
Asulam sodium salt	0.72 (14.3%)	0.66 (10.0%)	0.57 (3.64%)
Toxic standard	0.71 (12.7%)	0.71 (18.3%)	0.39 (-29.1%)

* results are the mean of 3 replicates.

For the short-term respiration no statistically significant effect was observed. At the end of the study, the deviation between the short-term respiration rate in soil treated with test substance asulam sodium salt and control soil was + 3.64%.

The deviation between the toxic standard and the control for short-term respiration were above 25% and thereby validating the test system.

Conclusions:

The deviation in the short-term respiration rate between the treated soil and the control soil was lower than 25% at the end of the 28-day evaluation. Therefore the impact on soil was considered negligible at the application rate of 7.2 kg asulam/ha (equivalent to 9.60 mg asulam/kg soil).

RMS Comments:

This study has previously been evaluated at EU level. Both nitrogen and carbon transformation (i.e. carbon dioxide production) was assessed in the study, with the above details relating to carbon transformation. The study is GLP compliant and has been conducted to a standard agreed test guideline. It is considered scientifically valid and suitable for use in the regulatory risk assessment. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level.

B.9.8.3 Soil micro-organism risk assessment.

An assessment is required for asulam and also its major soil metabolite sulphanilamide. A second soil metabolite 'methyl benzene sulphonyl carbamate' (MBSC) has been identified as being formed at upto 6% A.R. in soil. However, given the close structural similarity of this metabolite to asulam (differing only in the loss of a 'NH2 group – see 'Background information' Section B.9.0 for further details), the RMS considers that MBSC is not likely to be more toxicity to soil micro-organisms than asulam. Additionally MBSC is only of moderate persistence in soil (lab DT90 27.6-122 days). It is concluded that any risk from the presence of MBSC will be covered by the risk assessment conducted for asulam and therefore a specific risk assessment for this metabolite is not required.The influence of asulam sodium on soil microflora was determined using short term respiration experiments and by monitoring nitrogen transformation. These studies were performed with the technical concentrate of asulam sodium containing 822g asulam /kg or with the formulated product 'Asulam 400 g/L SL' (referred to as 'Asulox'). All of these studies have been previously assessed at EU level for the Annex I evaluation of asulam, without any concerns being identified, and are considered suitable for use in the current regulatory risk assessment.

In addition, details have been provided for a newly reported GLP compliant nitrogen transformation study conducted to a standard recognised test guideline using the major soil metabolite sulphanilamide (Schulz L 2011). The study has been evaluated by the RMS and is considered scientifically valid and suitable for use in the regulatory risk assessment. A summary of the main effects in the reported soil micro-organism studies is presented in the following table.

Table B.9.8.8 Summary of effects of asulam and its major soil metabolite sulphanilamide on soil microbial nitrogen and carbon transformation

Test substance and what assessed	Time scale	Effect	Reference
'Asulam 400 g/L SL': soil microbial carbon and nitrogen transformation.	56-day	No effects on N-cycle at 16 mg asulam /kg dw soil throughout the study. An 81% reduction in nitrate production over the first 14 days at 160 mg asulam /kg soil, with such effects declining to a 26% reduction at day 56. Some initial reductions in carbon dioxide production (C-cycle) at both 16 and 160 mg asulam/kg dw soil, but with such effects declining to < 25% by day 28.	Luscombe (1981a)
'Asulam 400 g/L SL': nitrogen fixation in alfalfa root nodules.	56-day	Nitrogen fixation in root nodules of alfalfa plants grown in treated soil was not adversely affected at 8.93 mg asulam /kg dw soil but was significantly reduced at 89.3 mg asulam /kg dw soil – attributed to phytotoxic effects at this dose.	Luscombe (1981b)
Technical asulam (purity 822g/kg): soil microbial carbon and nitrogen transformation.	28-day	Compared with the control, treatment at 9.6 mg asulam /kg dw soil reduced nitrate formation by 35% after 14 days but by only 9% after 28 days. No statistically significant effects on C-cycle at 9.6 mg asulam /kg dw soil (with increases of 14% after 6 hours and 3.6% after 28 days).	Kölzer (2002)
Sulphanilamide: soil microbial nitrogen transformation.	28-day	Treatments at 0.33 and 3.3 mg metabolite /kg dw soil resulted in no significant adverse effects on nitrate formation throughout the study duration (nitrate levels < 5% different from untreated control).	Schulz L (2011)

The 'Asulam 400 g/L SL' soil microbial studies (ref. Luscombe 1981a and 1981b) were conducted at asulam soil exposure concentrations well in excess of the maximum PECsoil of 3.2 mg asulam /kg dw soil estimated by the RMS for the proposed crop uses in spinach and flower bulbs in Section B.8.3 of the Volume 3 DAR. Given the much greater exposure levels (x 50) in the higher of the two test doses (i.e. 160mg asulam /kg dw soil), adverse effects from use at these high test doses are not considered relevant to the risk assessment for these uses.

Effects on soil microbial carbon and nitrogen transformation are reported from two studies: use in one study (Luscombe 1981a) at 16 mg asulam /kg dw soil resulting in no effects on nitrate formation and reductions in carbon dioxide production by day 28 of less than 25%; and use in the other study (Kölzer 2002) at 9.6 mg asulam /kg dw

soil resulting in a day 28 reduction in nitrate formation of 9% and day 28 increase in carbon dioxide production of 3.6%. Additionally, in a third study (Luscombe 1981b), at the relevant test dose of 8.93 mg asulam /kg dw soil, there were no adverse effects on nitrogen fixation in the root nodules of alfalfa plants – providing some further supporting evidence although not strictly required. Therefore, it is concluded, the study results indicate effects on nitrogen and carbon transformation of well within the Uniform Principles trigger value of '>25% after 100 days' from soil exposure levels of between 3-5 times the maximum soil PECs from the proposed use in spinach and flower bulb crops. Therefore, a low and acceptable risk from asulam exposure to soil micro-organisms can be concluded.

With respect to effects of the major soil metabolite sulphanilamide, the RMS in Section B.8.3 of this DAR estimates a maximum soil PEC of 0.41 mg metabolite /kg dw soil from the proposed use in spinach and flower bulb crops. Given the reported lack of adverse effects of sulphanilamide on soil nitrate formulation at exposure concentrates of up to 3.3 mg metabolite /kg dw soil (Schulz L 2011) – equivalent to 8 times the maximum soil PEC – it is concluded that the risk of adverse effects on soil nitrogen transformation processes is low. Although, no specific studies on the effects on soil respiration /carbon transformation have been provided, paragraph 4 of the OECD 216 'nitrogen transformation test' guideline states '*The test method also allows estimation of the effects of substances on carbon transformation by the soil microflora. Nitrate formation takes place subsequent to the degradation of carbon-nitrogen bonds. Therefore, if equal rates of nitrate production are found in treated and control soils, it is highly probable that the major carbon degradation pathways are intact and functional*. Therefore a lack of reported significant adverse effects on nitrogen transformation also indicates a lack of adverse effects on carbon transformation. This is also supported by the non-requirement for such a carbon transformation study under Regulation 1107/2009.

A low and acceptable risk to soil micro-organisms from both asulam and sulphanilamide exposure can therefore be concluded.

B.9.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (IIA 8.6)

Effects on non-target fauna and aquatic plants have been addressed in other sections of this report. Therefore, this section refers only to non-target terrestrial plants.

B.9.9.1 Toxicity to non-target terrestrial plants

Report: K.P. Christensen (1992) Asulam Sodium - Determination of effects on seed germination, seedling emergence and vegetative vigour of ten plant species. United Phosphorus Limited, Unpublished report No.: R003652; CA 8.12/01.

This study has previously been evaluated at EU level.

Guidelines:

USEPA (= EPA) Subdivision J, 122-1 & 123-1 (1982). Deviations: None which affected the results of the study.

GLP: Yes

Executive Summary:

The toxicity of asulam sodium to ten plant species during seed germination, seedling emergence and vegetative growth was investigated. Toxic effects were quantified and are reported as ER_{25} and ER_{50} values, including a No Observed Effect Rate (NOER) for each species. The minimum

ER_{50} during the seedling emergence test was 250 g asulam sodium/ha (228 g asulam/ha) for lettuce shoot length. The minimum ER_{50} during the vegetative vigour test was 12 g asulam sodium/ha for cucumber root weight (11 g asulam/ha).

Materials and Methods

Test Material:

Asulam sodium, purity: 89.5%, Batch no.: EN50005

Test Design:

10 plant species were treated via water (germination), silica sand (emergence) and foliar (vegetative vigour) routes of administration: 6 dicot species and 4 monocot species (see Table 8.12/01-1).

Seedling Emergence: The test was conducted at measured concentrations ranging from 7.4 kg as/ha to 0.025 kg as/ha, depending on plant species. The test material solution concentration was verified analytically. Test endpoints: % emergence and shoot length. Three replicate pots were maintained for each treatment levels and control for each species tested. Ten seeds were planted at a depth of 1 cm in each replicate plot (30 seeds per treatment level). Each pot was filled with silica sand. The pots were grouped by species and randomly placed in an environmental growth chamber. Temperature, light intensity, carbon dioxide and relative humidity were monitored and recorded daily. A photoperiod of 16-hrs light: 8-hrs dark was maintained. A nutrient solution containing necessary minerals and trace elements for plant growth was continuously provided by sub irrigation on a daily basis. Percentage emergence and shoot length were measured after a 14 day exposure period to asulam sodium.

Vegetative Vigour: The test was conducted at measured concentrations ranging from 7.4 kg as/ha to 0.05 kg as/ha, depending on plant species. The test material solution concentration was verified analytically. Test endpoints: shoot length, shoot weight and root weight. Test system was the same as in the seedling emergence test. Approximately 500-800 seedlings per species were germinated to supply plants for the test. Healthy seedlings of uniform root and shoot development were selected and randomly transplanted. Five seedlings per species were planted in each pot. Three replicate pots were maintained for each treatment levels and control for each species tested. 7 days after the seedlings had been planted they were sprayed with 30 mL of the appropriate treatment solution. Effects on shoot length, shoot weight and root weight were evaluated 14 days after the application of asulam sodium to the plant foliage.

Statistical analyses were performed with help of Dunnett's test, Kruskal-Wallis test or Wilcoxon Signed Ranks test.

Results and Discussion:

The critical endpoint values of the study were observed in the seedling emergence and the vegetative vigour test. Therefore the results of the seed germination test are not reported in detail here. Seed germination EC50's for the ten tested species ranged from 0.82 kg asulam sodium /ha (for cabbage) to 12 kg asulam sodium /ha (for maize) and for all test species were less sensitive than EC_{50} values for seedling emergence and vegetative vigour. All concentrations in the study report were expressed in lb as/A and have been converted into g as/ha (lb/A x 1.121 = kg/ha).

Seedling Emergence: Summary of ER_{50} and NOER values conducted with asulam sodium based on Percent Emergence and Shoot Length are shown in Table B.9.9.1.

Table B.9.9.1 Effects of asulam on seedling emergence

Species	Family	Percent Emergence		Shoot Length	
		ER_{50} (g/ha)	NOER (g/ha)	ER_{50} (g/ha)	NOER (g/ha)
Brassica oleracea (Cabbage)	Brassicaceae	2020	820	430	110
Zea mays (Corn)	Gramineae	8970[a]	3400	2900	1900
Cucumis sativus (Cucumber)	Cucurbitaceae	>7400	7400	53800[a]	3400
Lactuca sativa (Lettuce)	Asteraceae	>930	930	250	110
Avena sativa (Oat)	Gramineae	>1900	1900	560	110
Allium cepa (Onion)	Liliaceae	>3360	3400	1500	210
Lolium perenne (Per.Ryegrass)	Gramineae	>820	820	710	110
Rhaphanus sativus (Radish)	Brassicaceae	>1900	1900	430	210
Glycine max (Soybean)	Leguminosae	>7400	7400	3300	820
Lycopersicon esculentum (Tomato)	Solanaceae	>7400	7400	5000	820

a: value was extrapolated beyond the treatment range.

The most sensitive species identified was *Lolium perenne* with regard to percent emergence (ER_{50} of >818.3 g as/ha) and *Lactuca sativa* with regard to shoot length (ER_{50} of 246.6 g as/ha).

Vegetative Vigour: Summary of ER_{50} and NOER Values conducted with asulam sodium based on Shoot Weight and Shoot Length are shown in Table B.9.9.2.

Table B.9.9.2 Effects of asulam on vegetative vigour

Species	Family	Shoot Weight		Shoot Length	
		ER_{50} (g/ha)	NOER (g/ha)	ER_{50} (g/ha)	NOER (g/ha)
Brassica oleracea (Cabbage)	Brassicaceae	390	<210	81800[a]	870
Zea mays (Corn)	Gramineae	>7400	7400	>7400	7400
Cucumis sativus (Cucumber)	Cucurbitaceae	12[b]	220	>3700	3700
Lactuca sativa (Lettuce)	Asteraceae	260	49	380	49
Avena sativa (Oat)	Gramineae	7060	870	14570[a]	870
Allium cepa (Onion)	Liliaceae	>7400	<460	33630[a]	<460
Lolium perenne (Per.Ryegrass)	Gramineae	7960[a]	3400	>6950	6950
Rhaphanus sativus (Radish)	Brassicaceae	590[b]	460[b]	>1910	200
Glycine max (Soybean)	Leguminosae	5500	430	8070	870
Lycopersicon esculentum (Tomato)	Solanaceae	1900[b]	870[b]	5270	1680

N/A: not applicable, due to a lack of significant slope (i.e., dose response) using any of the available regressions.
a: value was extrapolated beyond the treatment range.
b: root weight (most sensitive endpoint)

The most sensitive species identified was *Cucumis sativus* (Cucumber) with regards to root weight (ER_{50} of 12 g asulam sodium/ha). For all species, shoot or root weight were the most sensitive parameters.

Conclusions:

The toxicity of asulam sodium to ten plant species during seed germination, seedling emergence and vegetative growth was investigated. Toxic effects were quantified and are reported as ER_{25} and ER_{50} values, including a No Observed Effect Rate (NOER) for each species.

The minimum ER_{50} during the seedling emergence test was 250 g asulam sodium/ha (228 g asulam/ha) for lettuce shoot length. The minimum ER_{50} during the vegetative vigour test was 12 g asulam sodium/ha for cucumber shoot weight (11 g asulam/ha).

RMS Comments:

This study has previously been evaluated at EU level and was considered to be scientifically valid and suitable for use in the regulatory risk assessment. The conclusions presented are consistent with the EFSA conclusion report (2010) and no concerns were raised at EU level regarding the quality of the study.

B.9.9.2 Non-target terrestrial plant risk assessment

The non-target plant risk assessment has been conducted by the RMS according to current SANCO/10329 guidance (October 2002) – which defines non-target plants as 'non-crop plants located outside of the treatment area'.

'Asulox' ('Asulam 400 g/L SL') is a simple dilution in water of asulam sodium and as such effects from the testing of asulam sodium in the reported laboratory non-target plant phytotoxicity study may be extrapolated directly to this product. In line with other areas of the risk assessment, toxicity endpoints expressed in the study report in terms of amounts of asulam sodium have been converted to equivalent amounts of asulam based on molecular weight differences using a conversion factor of 0.9128 - details for which are included in the following table.

Table B.9.9.3 Summary of non-target plant toxicity study endpoints expressed in terms of equivalent amounts of asulam.

Parameter	Toxicity endpoint*	Reference
Seed germination	EC_{50} = 748 g asulam/ha (cabbage)	Christensen KP (1992)
Seedling emergence	EC_{50} = 228 g asulam/ha (lettuce)	
Vegetative vigour	EC_{50} = 11 g asulam/ha (cucumber)	

* Most sensitive value for the ten tested crop species.

Asulox is to be applied as a single application in spinach and flower bulb crops at a maximum dose of 240g asulam /ha. Spray drift is considered to be the principle exposure route for non-target terrestrial plants located in the vicinity of the treated area and has been estimated using Rautmann et al (2001) 90th percentile spray drift data.

Based on use of the maximum application rate for asulam and the lowest (most sensitive) derived seedling emergence (pre-emergence) and vegetative vigour (post-emergence) EC_{50} values, a standard SANCO/10329 'Tier 2' deterministic risk assessment has been conducted in the following table.

Table B.9.9.4 Non-target plant deterministic risk assessment - using the most sensitive seedling emergence and vegetative vigour toxicity EC50 values.

Distance m	Drift rate %	PEC g asulam/ha	Seedling emergence (pre-emergence) TER values (based on an EC_{50} of 228 g asulam/ha)	Vegetative vigour (post emergence) TER values (based on an EC_{50} of 11 g asulam/ha)
0	100	2400	**0.095**	**0.0046**
1	2.77	66.48	**3.43**	**0.17**
5	0.57	13.68	16.67	**0.80**
10	0.29	6.96	32.76	**1.58**
20	0.15	3.60	63.33	**3.06**
30	0.10	2.40	95.00	**4.58**
40	0.07	1.68	135.71	6.55

Note: figures in bold indicate areas of concern

Therefore, based on the use of the most sensitive reported EC_{50} values and the need for a TER of greater than 5 for effects on non-target plants to be deemed acceptable, with respect to pre-emergence seedling emergence effects a 5 metre buffer zone would be required and respect to post-emergence (vegetative vigour) effects a 40 metre buffer zone would be required in order to provide an acceptable risk.

Need for further 'higher' tier risk assessment

When considered previously by EFSA /Member States (EFSA Conclusion Report 2010), the largest permissible no-spray buffer zone was considered to be 30 metres. Given that the above deterministic risk assessment indicated the need for a buffer zone of 40 metres, this was not considered acceptable. Consequently, a data gap was set to address the risk to non-target plants – with this being specified as a 'critical area of concern'. The Notifier has addressed this data gap by conducting a probabilistic risk assessment, which takes into account the likely distribution of sensitive of non-target plants - as outlined in Section 7.3 of SANCO/10329 (October 2002) terrestrial ecotoxicology guidance – details for which are presented in italics below.

Notifier's probabilistic vegetative vigour risk assessment for non-target plants

A statistical analysis was carried out using the probabilistic HC_{05} approach (ETX 2.0[21]). This method has been developed by RIVM (Van Vlaardingen et al., 2004). This probabilistic assessment follows the approach outlined in the EU Terrestrial Ecotoxicology guidance document (SANCO/10329/2002). If the ER_{50} for less than 5% of the species is below the highest predicted exposure level, the risk to terrestrial plants is assumed to be acceptable.

The vegetative vigour input data are as shown in Table B.9.9.5 and the resulting species sensitive distribution is shown in Figure B.9.9.1.

[21] Van Vlaardingen PLA, Traas TP, Wintersen AM, Aldenberg T. 2004. *ETX* 2.0. A program to calculate hazardous concentrations and fraction affected, based on normally distributed toxicity data. Bilthoven, the Netherlands: National Institute for Public Health and the Environment (RIVM). Report no. 601501028/2004, 68 pp.

Table B.9.9.5 *Vegetative vigour ER_{50} values for asulam sodium on non-target plants*

Plant species	*ER_{50} (vegetative vigour) – g asulam sodium/ha*
Cabbage	*390*
Maize	*>7400*
Cucumber	*12*
Lettuce	*260*
Oat	*7060*
Onion	*>7400*
Ryegrass	*>7400*
Radish	*590*
Soya bean	*5500*
Tomato	*1900*

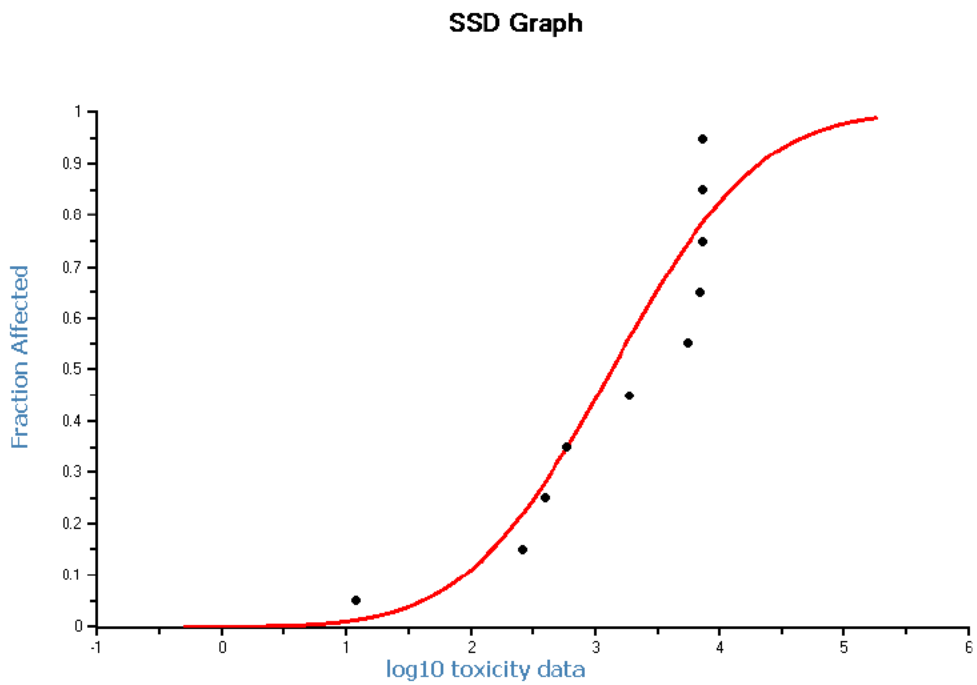


Figure B.9.9.1 *Species sensitivity distribution for asulam sodium on non-target plants (all ER_{50} values)*

The resultant median HC_{05} for vegetative vigour using ER_{50} data is calculated to be 36.35 g asulam sodium/ha (CL = 2.77 – 155.95 g/ha), equivalent to 33.19 g asulam/ha.

***Goodness of fit** – Three tests for normality were conducted. For the Anderson-Darling test acceptability was achieved for (p) levels of 0.025 and 0.01 (but not 0.05). For the Kolmogorov-Smirnov and Cramer von Mises tests acceptability was achieved for (p) levels of 0.05, 0.025 and 0.01.*

The risk to off-field non-target plants is assessed using the following ratio:

$$\text{TER} = \frac{\text{HC}_{05}\ \text{(g asulam/ha)}}{\text{PER by spray drift (g asulam/ha)}}$$

Using this approach, the risk to non-target plants is considered to be acceptable if the HC_{05} is greater than the PER (see Table B.9.9.6).

Table B.9.9.6 *Summary of probabilistic risk assessment for vegetative vigour for asulam (all ER_{50} values).*

Distance from edge of the crop (m)	*% drift*	*g asulam sodium/ha*	*HC_{05} (g asulam /ha)*	*Acceptable risk?*
1	*2.77*	*66.48*	*33.19*	*No*
5	*0.57*	*13.68*		*Yes*

The HC_{05} value for vegetative vigour data is above the exposure level (g asulam/ha) that would occur at 5 m from the edge of the crop. However, given that there was not universal acceptability of the normality of the distribution of the data the risk assessment has been re-run having omitted the toxicity endpoints for maize, onion and ryegrass which were all >7,400 g asulam sodium/ha.

Risk assessment excluding greater than values

If the greater than toxicity endpoints are omitted then all of the tests for normality are acceptable for all probability levels. The resulting species sensitive distribution is shown in Figure B.9.9.2.

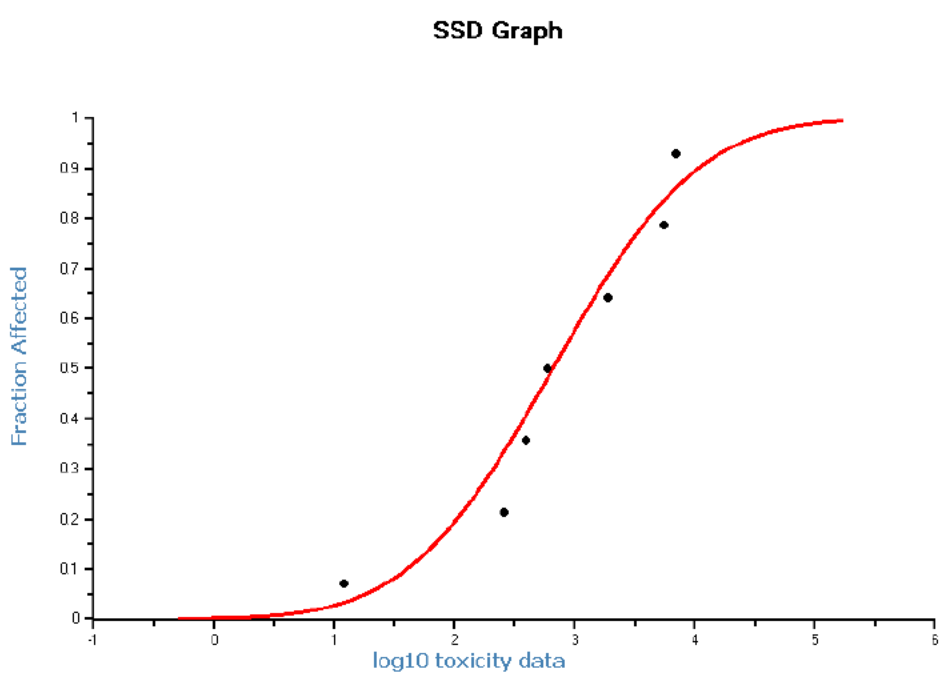


Figure B.9.9.2 *Species sensitivity distribution for asulam sodium on non-target plants (excluding > ER_{50} values)*

The resultant median HC_{05} for vegetative vigour using ER_{50} data = 15.14 g asulam sodium/ha (CL = 0.40 – 88.59 g/ha), equivalent to 13.82 g asulam/ha. Again, the HC_{05} value for vegetative vigour data is above the exposure level (g asulam/ha) that would occur at 5 m from the edge of the crop (Table B.9.9.7).

Table B.9.9.7 *Summary of probabilistic risk assessment for vegetative vigour for asulam (excluding > ER_{50} values)*

Distance from edge of the crop (m)	% drift	g asulam/ha	HC_{05} (g asulam/ha)	Acceptable risk?
1	2.77	66.48	13.82	No
5	0.57	13.68		Yes
10	0.29	6.96		Yes

Probabilistic seedling emergence risk assessment for non-target plants

As for vegetative vigour, a statistical analysis was carried out using the probabilistic HC_{05} approach (ETX 2.0).

The vegetative vigour input data are as shown in Table B.9.8.8 and the resulting species sensitive distribution is shown in Figure B.9.9.3.

Table B.9.9.8 *Seedling emergence ER_{50} values for asulam sodium on non-target plants*

Plant species	ER_{50} (seedling emergence) – g asulam sodium/ha
Cabbage	430
Maize	2900
Cucumber	>7400
Lettuce	250
Oat	560
Onion	1500
Ryegrass	710
Radish	430
Soya bean	3300
Tomato	5000

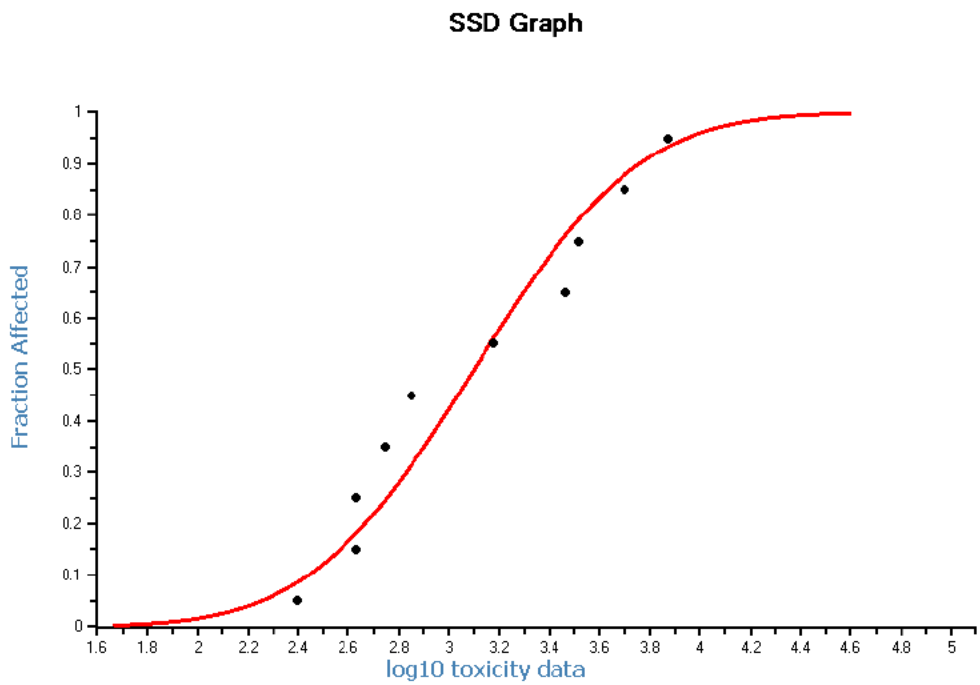


Figure B.9.9.3 *Species sensitivity distribution for asulam sodium on non-target plants (all ER_{50} values)*

The resultant median HC_{05} for seedling emergence using ER_{50} data = 167.7 g asulam sodium/ha (CL = 40.1 – 376.9 mL/ha), equivalent to 153.1 g asulam/ha.

Goodness of fit *– Three tests for normality were conducted (Anderson-Darling Kolmogorov-Smirnov and Cramer von Mises tests). All of the tests for normality are acceptable for all probability levels.*

The risk to off-field non-target plants is assessed using the following ratio:

$$\text{TER} = \frac{\text{HC}_{05}\ (\text{g asulam/ha})}{\text{PER by spray drift (g asulam/ha)}}$$

Using this approach, the risk to non-target plants is considered to be acceptable if the HC_{05} is greater than the PER (see Table B.9.9.9).

Table B.9.9.9 *Summary of probabilistic risk assessment for seedling emergence for asulam (all ER_{50} values).*

Distance from edge of the crop (m)	*% drift*	*g asulam/ha*	*HC_{05} (g asulam /ha)*	*Acceptable risk?*
1	*2.77*	*66.48*	*153.1*	*Yes*
5	*0.57*	*13.68*		*Yes*

The HC_{05} value for seedling emergence data is above the exposure level (g asulam/ha) that would occur at the default distance (1 m) from the edge of the crop.

RMS's evaluation of Notifier's probabilistic risk assessment

The Notifier's probabilistic risk assessment has been conducted broadly in line with the current advice included in SANCO/10329 guidance. This guidance requires the effects data (EC50 endpoints) to adequately fit a log-normal distribution (or other defined type of distribution) and as this only applies when vegetative vigour 'greater than' values are removed from the defined SSD distribution, only the more sensitive 'refined' vegetative vigour median HC_5 value = 13.82 g asulam /ha is relevant to the risk assessment. Also, a visual comparison of Figure B.9.9.1 (which includes all vegetative vigour EC50 values) and Figure B.9.9.2 (which excludes the > values) indicates there is a far better fit of the data to the predicted species sensitivity curve (assuming normal distribution) in Figure B.9.9.2.

The vegetative vigour median HC_5 value of 13.82 g asulam /ha is much more sensitive than the seedling emergence median HC_5 value of 153.1 g asulam /ha and is therefore the appropriate effects endpoint for use in the probabilistic risk assessment.

Based on SANCO/10329 guidance, the risk to terrestrial plants 'is assumed to be acceptable' when the HC_5 is below the highest predicted exposure level. Without spray drift risk mitigation, spray drift exposure is estimated to be 66.48 g asulam /ha – which is greater than the vegetative vigour HC5 of 13.82 g asulam /ha indicating a potential risk. However, when a 5 metre 'no-spray buffer zone is included, spray drift exposure is reduced to 13.68 g asulam /ha – which is less than the HC5 value – indicating an acceptable risk.

RMS's conclusions regarding risk to non-target plants

Risk mitigation measures are required to be included in order to provide an acceptable risk to non-target plants. However, as demonstrated by a higher tier probabilistic risk assessment, provided a 5 metre spray drift buffer zone is included (or other appropriate risk mitigation measures), the proposed use of asulam in spinach and flower bulb crops will pose an acceptable risk to non-target plants.

B.9.10 Effects on biological methods for sewage treatment (IIA 8.7)

B.9.10.1 Effects of active substance

i) Report: B.M. Luscombe, A.C.C. Craven (1981) ASULAM - Metabolism in and effect on the sewage treatment process. United Phosphorus Limited, Unpublished report No.: R000735; CA 8.15/01

This study has previously been evaluated at EU level.

Guidelines: Not specified. Deviations: Not specified

GLP: No

Executive Summary:

Model activated sludge systems were set up to determine if asulam in wastewater would be transformed, or would disrupt the treatment process. Asulam had no significant effect on activated sludge microflora and was not transformed in activated sludge at concentrations used in the study (up to 119.8 ppm).

Materials and Methods

Test Material:

Asulam-ring^{14}C, radiochemical purity: specific activity of 10.64 mCi/mM, Batch No.: not relevant.

Test Design:

The test material was diluted with non-labelled asulam to give a specific activity of 0.0993 mCi/mMol. Duplicate vessels containing activated sludge were treated to give nominal concentrations of either 0.1 ppm or 10 ppm asulam, while two control vessels were left untreated. The model activated sludge systems were incubated at 25°C with aeration for 23 hours. At the end of each of the three 23 hour cycle the sludge systems were analysed to determine the distribution of radiolabelled material, counts of aerobic bacteria were also made. After each sampling, asulam-ring^{14}C concentrations in activated sludge were increased: from 0.1 to 1 ppm and from 10 to 50 ppm after the first cycle, and from 1 to 10 ppm and from 50 to 100 ppm after the second cycle. After the third cycle the two vessels which had been brought to 10 ppm were sampled for a final time, while the two, which had been brought to 100ppm, were recharged to give a concentration of 11.8 ppm. Untreated controls were run alongside the asulam treated systems. The vessels, together with the controls, were incubated with aeration for 7 days before final sampling (on day 10).

Results and Discussion:

At all asulam concentrations, over 94% of the applied radioactivity remained in the effluent and less than 0.1% was evolved as carbon dioxide, the rest of the radioactivity became absorbed onto the sludge solids.

Thin-layer chromatography of the effluents showed that the radioactivity remained mainly as unchanged asulam, although at 0.09 ppm up to 36% was left as unidentified material at the origin. Therefore, there was no evidence to show that asulam was transformed significantly in activated sludge at the concentrations employed.

The mean counts of aerobic bacteria are given in Table B.9.10.1.

Table B.9.10.1 Effects of asulam on activated sludge microflora

	Day 1			Day 2			Day 3			Day 10	
Initial concentration of asulam (ppm)	0	0.09	9.1	0	0.9	45.8	0	9.1	100.9	0	119.8
Mean counts of aerobic bacteria (x 10^6/mL)	1.8	1.9	2.2	1.5	1.9 [a]	1.6	1.6	1.4	1.9 [b]	0.96	0.61 [b]

[a] significantly higher than the control at the 5% level of probability.
[b] significantly higher than the control at the 5% level of probability.

Counts of aerobic bacteria and weights of sludge solids indicated that asulam at concentrations up to 119.8 ppm was not disrupting the activated sludge microflora.

Conclusions:

Asulam had no significant effect on activated sludge microflora and was not transformed in activated sludge at concentrations used in the study (up to 119.8 ppm).

RMS Comments:

This study has previously been evaluated at EU level. The conclusions presented are consistent with the EFSA Conclusion report (2010) and no concerns were raised regarding the study at EU level. Although not GLP compliant, the study was conducted before the need for this requirement and is considered to be scientifically valid and suitable for use in the regulatory risk assessment.

ii) Report: C. Mead (2001) Asulam sodium salt: Assessment of the inhibitory effect on the respiration of activated sewage sludge. United Phosphorus Limited, Unpublished report No.: C015300; CA 8.15/02.

This study has previously been evaluated at EU level.

Guidelines:

OECD 209 (1984), USEPA (= EPA) OPPTS 850.6800 (1996)
Deviations: In some instances, the initial and final dissolved oxygen concentrations were below those recommended in the test guidelines. This was considered to have no impact on the outcomes of the study.

GLP: Yes

Executive Summary:

A study was performed to assess the effect of asulam on the respiration of activated sewage sludge. The effects of the test material on the respiration of activated sewage sludge gave a 3-hour EC_{50} of greater than 1000 mg asulam/L. The No Observed Effect Concentration (NOEC) after 3 hours exposure was 1000 mg/L.

Materials and Methods

Test Material:

Asulam, purity: 822 g/kg, batch number: OP210109

Test Design:

Following a preliminary range-findings study, activated sewage sludge was exposed to an aqueous solution of asulam at a concentration of 1000 mg/L (three replicate flasks) for a period of 3 hours at 21°C with the addition of synthetic sewage as a respiratory substrate.

The rate of respiration was determined after 30 minutes and 3 hours contact time and compared to data for the control and a reference material, 3,5-dichlorophenol.

Observations were made on the test preparations throughout the study period, and the pH of the control, reference material and test material preparations was measured at the end of the exposure period prior to measurement of the oxygen consumption rate.

Results and Discussion:

At the end of the test the pH values ranged from 7.7 to 7.9.

Observations made on the test preparations throughout the study showed that at the test concentration of 1000 mg/L no undissolved test material was visible (see Table B.9.10.2).

Table B.9.10.2 Effects of asulam sodium on activated sludge microflora

Treatment group	O_2 consumption rates (mg O_2/L/min)		% inhibition	
	after 30 minutes	after 3 hours	after 30 minutes	after 3 hours
Control				
Replicate 1	0.45	0.46	-	-
Replicate 2	0.48	0.51	-	-
Asulam (1000 mg/L)				
Replicate 1	0.47	0.46	[1]	5
Replicate 2	0.47	0.46	[1]	5
Replicate 3	0.48	0.46	[3]	5
3.5-Dichlorophenol				
3.2 mg/L	0.43	0.37	8	24
10 mg/L	0.18	0.12	61	75
32 mg/L	0.10	0.06	78	88

[ ]: increase in respiration rate as compared to controls.

Conclusions:

The effects of the test material on the respiration of activated sewage sludge gave a 3-hour EC_{50} of greater than 1000 mg asulam/L. The No Observed Effect Concentration (NOEC) after 3 hours exposure was 1000 mg/L.

RMS Comments:

This study has previously been evaluated at EU level. The conclusions presented are consistent with the EFSA Conclusion report (2010) and no concerns were raised regarding the study at EU level. Although not GLP compliant, the study was conducted before the need for this requirement and is considered to be scientifically valid and suitable for use in the regulatory risk assessment.

B.9.10.2 Risk to biological methods of sewage treatment

Normal agricultural/horticultural use is not considered likely to result in significant exposure of sewage treatment works from the proposed crop uses of asulam. However, as further confirmation, two studies were conducted to determine if asulam in wastewater would be likely to disrupt the treatment process /microbial respiration (counts of aerobic bacteria not affected at concentrations up to 119.8 ppm asulam /L in one study, microbial respiration not affected at up to 1000 mg asulam /L in the other study).

Although the first study (Luscombe and Craven, 1981a) was not GLP compliant, it was conducted before the need for this requirement and is considered of an acceptable (although non-standard) methodology. The second study (Mead, 2001f) was GLP compliant and conducted to a standard methodology. Both studies are suitable for use in the risk assessment.

No significant effects were found in either of these studies at active substance concentrations that are much higher than that likely to occur in practice. It is therefore

concluded that the proposed uses of asulam pose a low and acceptable risk to biological methods of sewage treatment.

B.9.11 Overall summary of risk to non-target organisms

The proposed uses of asulam sodium in spinach and flower bulb crops pose an acceptable risk to birds, terrestrial crop dwelling 'other non-target arthropods', earthworms, soil macro-organisms, soil micro-organisms, and biological sewage treatment processes.

Bee acute oral and dermal toxicity studies indicate asulam to be of low toxicity to adult bees. Also, the standard bee 'hazard quotient' risk assessment for the proposed crop uses in spinach and flower bulb crops indicates a low risk. Results of a single published paper identified from a literature review suggest that the foraging of bees in treated crops may result in low level of residues of asulam and its metabolite sulphanilamide in hive honey. However, there are no data confirming the presence of such residues in the nectar of exposed plants and no risk assessment has been conducted in relation to this.

The conducted TER risk assessment for wild mammals indicates an acceptable risk, with the exception of the risk to small herbivorous mammals from late post-crop emergence use in flower bulbs (BBCH ≥ 40) – for which an acceptable long-term risk has not been demonstrated (refined long-term TER = 4.5). However, in this case the breaching of the wild mammal long-term TER trigger value of 5 is marginal and the RMS considers that the conducted refined risk assessment is conservative – particularly in assuming a 'PT' value of 1.0 (see Section B.9.1.5.7iii for more details). Therefore, taking this into account, the RMS considers that the long-term risk to wild mammals (including small herbivores) is acceptable. However, Member State's /EFSA may wish to consider this matter further.

Asulam sodium and its formulated product 'Asulox' ('Asulam 400 g/L SL') are of low to moderate toxicity to fish and aquatic invertebrates but of high toxicity to algae and higher aquatic plants. The log Pow values of asulam and its metabolites are well below the bioaccumulation trigger value of log 3.0, indicating a lack of potential for bioaccumulation in fish and other aquatic organisms.

The conducted aquatic life risk assessment indicates that the proposed uses in spinach and flower bulb crops require the inclusion of risk mitigation measures in order to provide an acceptable risk to aquatic life. The conducted 'FOCUS Step 4' risk assessment indicates an acceptable risk to aquatic life with the inclusion of a 5 metre 'no spray' spray drift buffer zone plus a vegetative field strip of sufficient width to reduce run-off by 80%. However, individual Member States may choose to use other risk mitigation measures.

The proposed spray treatment of 'Asulox' in spinach and flower bulb crops poses a high risk to non-target plants without the inclusion of risk mitigation measures. The results of a higher tier probabilistic risk assessment indicates that risk mitigation measures in the form of a 5 metre no-spray spray drift buffer zone will reduce exposure sufficiently to provide an acceptable risk to non-target plants. However, individual Member States may wish to include use of other alternative risk mitigation measures.

B.9.12. Literature review:

Background

A literature review has been carried out for asulam and its metabolites - for which details are summarised below.

The review has been conducted by the Notifier in accordance with requirements under Article 8(5) of Regulation (EC) No. 1107/2009, based on EFSA guidance as published in EFSA Journal 2011; 9(2):2092.

The objective of the review is to determine if any scientific peer-reviewed open literature, published in the last 10 years before the submission date of the dossier, is suitable for consideration in the risk assessment for asulam sodium.

The RMS has evaluated the quality of the conducted literature review in relation to its ability to identify relevant and reliable published information suitable for consideration in the ecotoxicology risk assessment.

i) **Evaluation of comprehensiveness of information databases used in the search**

A number of bibliographic databases were used in the conducted search, covering both human health and environmental aspects of the regulatory evaluation. The Notifier's justification for the use of these databases is included in the table below.

With respect to identifying ecotoxicology related published literature, the RMS considers that the comprehensiveness and range of different databases used was quite extensive and that there are therefore no significant deficiencies with respect to this aspect of the conducted literature review.

Provider	Database	Justification
STN International*	**ANABSTR – Analytical abstracts**	The Analytical Abstracts database covers worldwide literature on analytical chemistry. Amongst others subject coverage includes Agriculture, Environment, and Food. Sources include journals, books, conference proceedings, reports, and standards. Bibliographic information, indexing terms, abstracts, chemical names, and CAS Registry Numbers are all searchable.
STN International*	**BIOSIS**	BIOSIS Previews® is the largest and most comprehensive life science database in the world. Amongst others, subject coverage includes agriculture, biochemistry, biophysics, botany, environmental biology, physiology, toxicology. Sources include periodicals, journals, conference proceedings, reviews, reports, patents, and short communications. Nearly 6,000 life source journals, 1,500 international meetings as well as review articles, books, and monographs are reviewed for inclusion. Bibliographic information, indexing terms, abstracts, and CAS Registry Numbers are all searchable.
STN International*	**CAPLUS** Chemical Abstracts Plus	The Chemical Abstracts (CA) database covers all areas of biochemistry, chemistry and chemical engineering, and related sciences. Sources include over 8,000 journals, patents from 38 national patent offices and two international patent organizations, technical reports, books, conference proceedings, and dissertations. Electronic only journals and Web preprints are also covered. Bibliographic terms, indexing terms, roles, CAS Registry Numbers, International Patent Classification, and abstracts are searchable.
STN International	**EMBASE** Exerpta Medica	The Excerpta Medica database covers worldwide literature in the biomedical and pharmaceutical fields, including biological science, biochemistry, human medicine, forensic science, pediatrics, pharmacy, pharmacology and drug therapy, pharmacoeconomics, psychiatry, public health, biomedical engineering and instrumentation, and environmental science. Sources for EMBASE include more than 4,000 journals from approximately 70 countries, monographs, conference proceedings, dissertations, and reports.

Provider	Database	Justification
STN International	**MEDLINE**	The MEDLINE database is produced by the U.S. National Library of Medicine and contains more than 22.6 million records. The database contains information on all areas of medicine. Sources include journals and chapters in books or symposia.
STN International	**RTECS**	Registry of Toxic Effects of Chemical Substances contains factual toxicity data for commercially important substances from research and government reports. Coverage includes irritation data, federal standards and regulations, mutagenicity, tumoregenic effects, acute toxicity and multiple dose toxicity data, carcinogenicity reviews. Sources include journal articles, government reports, and unpublished EPA test submissions (TSCATS).
STN International	**SCISEARCH** Science Citation Index	Science Citation Index, one of the largest multidisciplinary scientific databases, is an international index to the literature covering virtually every subject area within the broad fields of science, technology, and biomedicine. Records include references from over 5,600 scientific, technical, and medical journals are contained in the database.
STN International*	**TOXCENTER**	Toxicology Center covers the pharmacological, biochemical, physiological, and toxicological effects of drugs and other chemicals. TOXCENTER is composed of the following subfiles: BIOSIS (1969 to date), CAplus (1907 to date), IPA (1970 to date), and MEDLINE (1953 to date). Sources include abstracts, books and book chapters, bulletins, conference proceedings, journal articles, letters, meetings, monographs, notes, papers, patents, presentations, research and project summaries, reviews, technical reports, theses, translations, unpublished material, web reprints. Records contain bibliographic data, abstracts, indexing terms, chemical names, and CAS Registry Numbers.
US National Library of Medicine National institutes of Health**	**PUBMED**	PubMed is a database comprising more than 21 million citations for biomedical literature from MEDLINE, life science journals, and online books. Citations may include links to full-text content from PubMed Central and publisher web sites.
Elsevier BV***	**ScienceDirect**	ScienceDirect is a data base of scientific literature published by Elsevier B.V. This database contains more than 10 million journal articles and book chapters from more than 2500 journals and 11,000 books.
John Wiley and Sons Inc****	**Wiley Online Library**	Wiley Online Library features 1500 journals, over 13,000 Online Books, and hundreds of multi-volume reference works, databases and other resources

* For STN databases reference is made to STN database summary sheets
http://www.stn-international.de/database_list.html?&no_cache=1&cHash=

** For details on Pubmed
http://www.ncbi.nlm.nih.gov/pubmed/

*** For more details on ScienceDirect
http://www.sciencedirect.com/

**** For more details on Wiley online library
http://onlinelibrary.wiley.com/

ii) Evaluation of database search strategy:

A 'single concept' search was used for both asulam sodium and its metabolites.

a) Search strategy for asulam sodium (includes human health and environment):

Within the STN, Pubmed, 'Wiley online library' and Science Direct databases, the search terms 'Asulam', 'Asulam sodium' and trade name 'Asulox' were used. It was considered (by the Notifier) that as asulam and asulam sodium are the common names for this active substance, any relevant article from the search period would make

reference to this term somewhere within the text of the article and that it was not therefore necessary to also search under their chemical (IUPAC) name or CAS /Registry number (as suggested in the EFSA guidance).

b) Search strategy for metabolites (includes human health and environmental):

The following metabolites were included in the conducted searches (common /code name first, followed by chemical name in square brackets): sulphanilamide [4-Aminobenzenesulphonamide], sulphanilic acid [Aminobenzenesulphonic acid], acetyl asulam (Methyl ((4-acetamidophenyl) sulphonyl)carbamate), AP formamide (N-(4-Aminophenyl)formamide), MCAPAP carbamic acid [4-(4-methoxycarbonylaminophenyl) aminophenyl) carbamic acid], malonyl asulam [4-(N-methoxycarbonylsulphamoyl) phenylcarbamoyl acetic acid] and asulam glc [Asulam glucosides].

For the Pubmed, Wiley online library and Science Direct databases, initial searches were conducted using the metabolite's common (or 'code'). For sulphanilamide and sulphanilic acid, this initial search resulted in a large number of articles being identified in the Wiley online library and Science Direct databases (> 5000 articles) and because of this it was necessary to refine the search using the common /code name plus the following search terms: Tox OR hazard OR adverse OR health OR NOAEL OR NOEL OR LOAEL OR LOEL OR BMD OR "vivo" OR "vitro" or 'storage stability' OR storage OR stability OR metabolic OR metabolism OR degradation OR breakdown OR 'residues' OR residue OR 'processing' OR hydrolysis OR rotation OR plant OR crop OR feed OR animal OR livestock OR hen OR cattle OR ruminant OR goat OR cow OR pig OR 'risk assessment' OR consume OR exposure or 'soil' OR 'water' OR 'air' OR environment OR fate OR endocrine disrupt OR bioaccumulation OR biomagnification OR bioconcentration OR poison OR effect. For the remaining potential metabolites, since there were none or only few articles available when searching with the common/code name, further searches were conducted using the chemical name and the CAS number (if available).

For the STN databases, initial searches were conducted using the common/ code name, with additional searches also conducted using the CAS number and registry number (where available). For the metabolites acetyl asulam, AP formamide, MCAPAP carbamic acid, malanoyl asulam and asulam glc, given that these initial searches resulted in no or limited articles being available, further searches were also performed on their chemical names.

c) 'Limitations' included in active substance and metabolite searches:

The searches were limited to literature published since 2004. This is in line with Article 8(5) of Regulation (EC) No 1107/2009 which states 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. Also, since some of the databases used included patent information, as well as peer reviewed literature, the results were filtered to limit the output to non-patent documents.

d) RMS's evaluation of quality of search strategy:

With respect to ecotoxicology related requirements, the RMS is in agreement with the active substance and metabolite search strategies and included 'limitations'.

In addition to the active substance, non-target organisms may be exposed in soil to sulphanilamide and MBSC; in surface water to sulphanilamide, sulphanilic acid, AP

formamide, MCAPAP and MBSC; and in sediment to sulphanilamide (ref. Table B.9.02). The literature search includes all of these metabolites with the exception of the recently identified potential major water metabolite but minor soil metabolite MBSC (maximum of 6% AR in one soil type) – for which no searches were conducted. However, given the close similarity of the chemical structure of MBSC to that for asulam (MBSC differing only in the lack of a NH2 group), the RMS's view is that its toxicity is not likely to be greater than that for asulam and on this basis a specific literature search for this metabolite is not considered essential by the RMS.

It is concluded with respect to ecotoxicology aspects, that the conducted 'single concept' search strategy adequately meets the 'literature search' related requirements in this area, with no significant deficiencies.

iii) Evaluation of 'relevance' of publications

The Notifier's initial assessment conducted to determine 'relevance or 'non-relevance' is broadly in line with the 'Step 1' 'rapid assessment' procedure recommended in Section 5.3 of EFSA (2011) guidance, although this guidance also includes a third categorisation of 'unclear relevance' – which should be treated in the same way as 'relevant' publications (i.e. require a 'Step 2' full document assessment of relevance).

At 'Step 2', the Notifier appears to have assessed the full text of the document for <u>both</u> relevance and <u>reliability</u>, instead of conducting a 'Step 2' relevance assessment first, followed by a subsequent assessment for reliability – as recommended in the EFSA (2010) guidance. However, this procedural difference is not considered a critical factor effecting the validity of the relevance (or reliability) assessment.

<u>Step 1 and Step 2 relevance criteria and assessment procedure:</u>

With respect to the relevance criteria applied and the methodology used, the Notifier has stated the following in Section 4.2 of their report:

'Publications meeting the relevance criteria are those showing new/unknown effects or information potentially contradictory to the regulatory data package for the active substance, its relevant metabolites and/or the plant protection products on human health, animal health and/or the environment, which could impact the endpoints or the risk assessment parameters. An initial assessment of studies for relevance was carried out by reference to their titles and if necessary abstracts. Those studies that were felt to meet the relevance criteria following review of their abstracts were obtained and the full text of the document was assessed further to determine it the information contained in the study could impact on the endpoints and risk assessment parameters related to the active substance. Reviews of the relevance and reliability of the articles brought up in the literature search were carried out by experts in the particular fields.'

The Notifier's relevance criteria (Section 4.2 of their Literature Review Report') is broadly in line with that specified in Section 5.1 of EFSA's (2009) guidance document, which states: 'Studies relevant to the dossier are those that inform one or more data requirement(s), including hazard identification, hazard characterisation and exposure assessment, for the active substance under assessment, its relevant metabolites or plant protection products.'

The use of titles and /or abstract information at Step 1 and full text details at Step 2 is also in agreement with that advised in this guidance.

<u>Step 1 relevance evaluation for active substance and its metabolites (all data requirement areas)</u>:

After removing replicates, summary records for a total of 344 active substance and 10,060 metabolite related publications were obtained based on the conducted searches covering both human health and environmental related data requirements.

The RMS has briefly checked the 344 active substance search article titles and a sample of the 10,060 metabolite search article titles and agrees with the Notifier's 'Step 1' relevance conclusions that 11 active substance related search publications and 4 metabolite related search publications are potentially relevant (in relation to human health and /or environmental areas) and therefore require a further Step 2 full document relevance check.

It is the RMS's view that the metabolite search should have been refined further to reduce the very large number of irrelevant publications and as a result make the 'Step 1' relevance check less time consuming. However, this does not affect the validity of the Step 1' relevance check.

Step 2 relevance evaluation for the active substance (Ecotoxicology related areas):

The identified 11 'Step 1 relevant' active substance related publications are summarised below in Table B.9.12.1, together with the Notifier's 'Step 2' 'relevance' evaluation – which was conducted based on a 'full text review' of each publication.

Based on the brief summarised publication details provided by the Notifier (included in the 'Title', 'Reference' and 'Result' columns of Table B.9.12.1), the following publications do not appear to relate to ecotoxicology data requirements and are therefore considered by the RMS as 'not relevant' with respect to this area of the regulatory assessment: publication numbers 27, 104, 211, 236, 247 and 283. Also, given that publication number 40 relates to an assessment of effects of a mixed active containing formulation, it is not possible to distinguish the reported effects caused by asulam from that due to the other substances present, therefore this paper is also regarded as 'not relevant'.

The RMS agrees with the Notifier, that publication 110 is potentially relevant to effects on aquatic plants and therefore requires a ecotoxicology related 'reliability' assessment based on the full text content of this publication (for which details were provided by the Notifier in the original submission).

However, based on the limited details provided (which did not include the full text of the publication), it is unclear why publication numbers 85, 121 and 330 are regarded by the Notifier as 'not relevant'. Therefore, the full text of these publications was requested by the RMS (CRD's emailed request and Notifier's response of 9th and 21st September 2015 respectively), with a full text 'Step 2' relevance assessment subsequently conducted by the RMS as detailed below:

RMS's 'Step 2' full text relevance evaluation for active substance search publication number 85:

Study ref: Kaufmann A & Kaenzig A (2004) 'Contamination of honey by the herbicide asulam and its antibacterial active metabolite sulfanilamide'; Food Addit Contam 2004 Jun 21(6): pp564-571.

This paper discusses potential sources of asulam and its metabolite sulphanilamide found in honey from hives in Swizerland, with contamination via the foraging of asulam treated grassland pastures being identified as a potential source – although no

direct evidence in the form of the presence of asulam and sulphanilamide residues in foraged pollen and nectar from treated meadows was reported.

The indications in this published paper of the possibility of contamination of honey in bee hives due to the use of asulam on grassland pastures have implications with respect to the environmental safety of agricultural use of asulam to bees. Therefore, the paper is considered ecologically relevant by the RMS and as such also requires a 'reliability' assessment to determine whether it should be considered further in the regulatory risk assessment.

RMS's 'Step 2' full text relevance evaluation for active substance search publication number 121:

Study ref: Baran et al (2011) Effects of the presence of sulphonamides in the environment and their influence on human health; Journal of Hazardous Materials 196(0):pp1-15.

This paper summarises the results of other publications in relation to the presence of sulphonamides (including asulam and its metabolite sulphanilamide) in the environment and concludes that they are widespread. Potential environmental sources of sulphonamides are stated to include their use as an antimicrobial veterinary medicine, in commercial bee keeping (to protect honey bees against bacterial diseases e.g. American foalbrood) and in agriculture as a herbicide (as the sulphonamide asulam). However, no details of the referenced studies supporting the quoted environmental residue values (other than the publication reference) are included in the paper – the actual evidence being lacking. Therefore, it is considered that the publication does not adequately 'inform one or more data requirement/s' as required in EFSA's (2009) definition of 'relevant'. On this basis, the RMS's view is that the publication should be classified as 'not relevant' and as such does not require further consideration.

RMS's 'Step 2' full text relevance evaluation for active substance search publication number 330:

Study ref: Matsumoto et al (2009) Toxicity of Agricultural Chemicals in *Daphnia magna*; Oasaki City Medical Journal (2009) 55(2), pp89-97.

This paper reports the results of acute (2 days) and subchronic (8 day) tests on the toxicity to Daphnia magna of a total of 30 agricultural chemicals including asulam. For asulam, in acute and chronic toxicity studies respectively a 48 hour and 8 day LC50 > 10mg asulam /L was reported, with no significant reductions in numbers of offspring in the subchronic toxicity study. Such studies are 'relevant' to ecotoxicology regulatory requirements and therefore require a 'reliability' assessment to determine whether they should be considered further in the regulatory risk assessment (considered below).

Step 2 relevance evaluation for metabolites (Ecotoxicology related areas):

The 4 metabolite related publications determined as 'relevant' in the Step 1 'rapid assessment' are summarised in Table B.9.12.2, together with the Notifier's 'Step 2' 'relevance' evaluation for these publications (based on a 'full text' review).

Two of the four publications (numbers 988 and 9357 in the Notifier's metabolite search table) do not relate to ecotoxicology requirements and are therefore 'not relevant' in relation to the ecotoxicology evaluation. However, the other two

publications (numbers 3152 and 3944 Notifier's metabolite search table), which also occur in the Notifier's active substance search table (numbers 85 and 121) both appear to be 'relevant' in relation to potential effects of metabolites on bees and as such require a 'reliability' assessment.

Table B.9.12.1 Details of Notifier's 'Step 2' active substance related relevance evaluation for 11 publications identified as potentially relevant at 'Step 1':

Number	Author	Year	Title	Reference	Full text reviewed Y or N	Meet Relevance/Reliability Criteria	
						Y, N or N/A	Result of reliability/relevance assessment/Justification if not reliable
27.	GÃ¡miz-Gracia,Laura;GarcÃ±Ã a-CampaÃ±a,Ana M.;Soto-Chinchilla,Jorge J.;Huertas-PÃ®rez,JosÃ® F.;GonzÃ¡lez-Casado,A.	2005	Analysis of pesticides by chemiluminescence detection in the liquid phase	Gámiz-Gracia L, García-Campaña AM, Soto-Chinchilla JJ, Huertas-Pérez JF, González-Casado A. 2005. Analysis of pesticides by chemiluminescence detection in the liquid phase. TrAC Trends in Analytical Chemistry 24(11):927-42.	Y	N	Methodology plus application, water matrix only for asulam. No relevance to asulam consumer risk assessment.
40.	van Wijngaarden RP, Cuppen JG, Arts GH, Crum SJ, van den Hoorn MW, van den Brink PJ, Brock TC.	2004	Aquatic risk assessment of a realistic exposure to pesticides used in bulb crops: a microcosm study.	Environ Toxicol Chem. 2004 Jun;23(6):1479-98.	Y	N	Assesses effects of a pesticide mixture to an aquatic community in a laboratory microcosm and so is not relevant to a specific risk assessment for asulam. However, it is concluded that the first-tier risk assessment procedure for individual compounds was adequate for protecting sensitive populations exposed to realistic combinations of pesticides.
85.	Kaufmann A, Kaenzig A.	2004	Contamination of honey by the herbicide asulam and its antibacterial active metabolite sulfanilamide.	Food Addit Contam. 2004 Jun;21(6):564-71.	Y	N	Paper is relevant to local authorised use of asulam in Switzerland and the potential for transfer of residues to honey but is not relevant for EU evaluation of asulam on spinach or flower bulbs. Not relevant for consumer risk assessment. On this basis, also not relevant for honey bee risk assessment.
104.	Knapp, M. F.	2005	Diffuse pollution threats to groundwater: A UK water company perspective	Quarterly Journal of Engineering Geology and Hydrogeology (2005), 38(1), 39-51	Y	N	Relates only to control of bracken by aerial and ground-based application of asulam in upland catchments, mainly by estates managing shooting rights. It is therefore not relevant for the EU evaluation of use on spinach and flower-bulbs.
110.	Michel A, Johnson RD, Duke SO, Scheffler BE.	2004	Dose-response relationships between herbicides with different modes of action and growth of Lemna paucicostata: an improved ecotoxicological method.	Environ Toxicol Chem. 2004 Apr;23(4):1074-9.	Y	Y	Lemna paucicostata is not a standard species but it is considered that the esults should eb tansferable to other Lemna species. Provides a useful additional toxicity value for this sensitive group. Klimisch Score 2
121.	Baran,Wojciech;Adamek,Ewa;ZiemiaÃ„ska,Justyna;Sobczak,Andrzej	2011	Effects of the presence of sulfonamides in the environment and their influence on human health	Baran W, Adamek E, Ziemiańska J, Sobczak A. 2011. Effects of the presence of sulfonamides in the environment and their influence on human health. J Hazard Mater 196(0):1-15.	Y	N	Paper will become relevant to local authorised use which links the application of asulam to sulphanilamide residues in honey. Little assessment on human health, but a greater emphasis on environmental risk assessment. Not relevant for EU evaluation of asulam on spinach or flower bulbs. On this basis, also not relevant for honey bee risk assessment.

Table B.9.12.1 Details of Notifier's 'Step 2' active substance related relevance evaluation for 11 publications identified as potentially relevant at 'Step 1' (continued)

Number	Author	Year	Title	Reference	Full text reviewed Y or N	Meet Relevance/Reliability Criteria	
						Y, N or N/A	Result of reliability/relevance assessment/Justification if not reliable
211.	Santaladchaiyakit,Yanawath;Srijaranai, Supalax;Burakham, Rodjana	2012	Methodological aspects of sample preparation for the determination of carbamate residues: A review	Santaladchaiyakit Y, Srijaranai S, Burakham R. 2012. Methodological aspects of sample preparation for the determination of carbamate residues: A review. Journal of Separation Science 35(18):2373-89.	Y	N	Methodology plus application. No relevance to asulam consumer risk assessment
236.	Munz, Nicole; Leu, Christian; Wittmer, Irene	2012	Pesticide measurements in watercourses	Aqua & Gas (2012), 92(11), 32-41	Y	N	Paper is relevant to local authorised use of asulam in Switzerland and not relevant for EU evaluation of asulam on spinach or flower bulbs.
247.	Devipriya,Suja;Yesodharan,Suguna	2005	Photocatalytic degradation of pesticide contaminants in water	Devipriya S and Yesodharan S. 2005. Photocatalytic degradation of pesticide contaminants in water. Solar Energy Mater Solar Cells 86(3):309–48.	Y	N	Not relevant as this describes artificial systems only. The reference to asulam applies to another paper (Tanaka et al, Environ. Sci. Technol. 26 (1992) 2534-2536) which will be equally not relevant for the same reason.
283.	Polgar, Laszlo; Garcia-Reyes, Juan F.; Fodor, Peter; Gyepes, Attila; Dernovics, Mihaly; Abranko, Laszlo; Gilbert-Lopez, Bienvenida; Molina-Diaz, Antonio	2012	Retrospective screening of relevant pesticide metabolites in food using liquid chromatography high resolution mass spectrometry and accurate-mass databases of parent molecules and diagnostic fragment ions	Journal of Chromatography A (2012), 1249, 83-91	Y	N	Methodology plus application. No relevance to asulam consumer risk assessment
330.	Matsumoto, Ken-ichi; Hosokawa, Mamoru; Kuroda, Koichi; Endo, Ginji	2009	Toxicity of agricultural chemicals in Daphnia magna	Osaka City Medical Journal (2009), 55(2), 89-97	Y	N	Non-standard methodology and results consistent with studies already presented in dossier (paper indicates lower toxicity).

Table B.9.12.3 Details of Notifier's 'Step 2' metabolite related relevance evaluation for 4 publications identified as potentially relevant at 'Step 1':

Number	Author	Year	Title	Reference	Full text reviewed Y or N	Meet Relevance/Reliability Criteria	
						Y, N or N/A	Result of reliability/relevance assessment/Justification if not reliable
121.	Baran,Wojciech;Adamek,Ewa;ziemiaÂ„ska,Justyna;Sobczak,Andrzej	2011	Effects of the presence of sulfonamides in the environment and their influence on human health	Baran W, Adamek E, Ziemiańska J, Sobczak A. 2011. Effects of the presence of sulfonamides in the environment and their influence on human health. J Hazard Mater 196(0):1-15.	Y	N	Paper will become relevant to local authorised use which links the application of asulam to sulphanilamide residues in honey. Little assessment on human health, but a greater emphasis on environmental risk assessment. Not relevant for EU evaluation of asulam on spinach or flower bulbs. On this basis, also not relevant for honey bee risk assessment.
988.	Chen, Jiabin; Zhou, Xuefei; Zhang, Yalei; Gao, Haiping	2012	Potential toxicity of sulfanilamide antibiotic: Binding of sulfamethazine to human serum albumin	Science of the Total Environment (2012), 432, 269-274	Y	N	Paper not relevant for toxicological endpoints. Paper discusses analytical methods for detecting sulphanilamide in human albumin serum
3152.	Kaufmann A, Kaenzig A.	2004	Contamination of honey by the herbicide asulam and its antibacterial active metabolite sulfanilamide.	Food Addit Contam. 2004 Jun;21(6):564-71.	Y	N	Paper is relevant to local authorised use of asulam in Switzerland and the potential for transfer of residues to honey but is not relevant for EU evaluation of asulam on spinach or flower bulbs. Not relevant for consumer risk assessment. On this basis, also not relevant for honey bee risk assessment.
9357.	BiaÂ,k-BieliÂ„ska,Anna;Stolte,Stefan;Matzke,Marianne;FabiaÂ„ska,Aleksandra;Maszkowska,Joanna;KoÂ,odziejska,Marta;Liberek,Beata;Stepnowski,Piotr;Kumirska,Jolanta	2012	Hydrolysis of sulphonamides in aqueous solutions	Bialk-Bielińska A, Stolte S, Matzke M, Fabiańska A, Maszkowska J, Kołodziejska M, Liberek B, Stepnowski P, Kumirska J. 2012. Hydrolysis of sulphonamides in aqueous solutions. J Hazard Mater 221–222(0):264-74.	Y	N	Not relevant as neither sulphanilic acid nor sulphanilamide is one of the compounds examined (and none that are examined are closely related to them structurally).

Note: Publication number 121 (in 'Notifiers active substance search list) is number 3944 in Notifier's metabolite search list.

iv) Evaluation of 'reliability' of literature search publications

Given their determined 'relevance', a full text 'reliability' assessment is required for active substance search related publication numbers 85, 110 and 330. Publications 85 and 110 were also identified in the metabolite search (under numbers 3152 and 3944) as 'relevant' and therefore these publications also require a 'reliability' assessment in relation to metabolite related ecotoxicology requirements. Full text details were only provided in the original submission for publication 110 (ref. Michel et al 2004). However, following a request from the RMS, full details were subsequently provided also for the other publications (CRD's emailed request and Notifier's response of 9th and 21st September 2015 respectively).

Reliability is defined in the EFSA 2011 guidance (Section 5.4.2) as 'the extent to which a study is free from bias and its findings reflect true facts'. As suggested in the

guidance, the RMS has classified the studies according to their likely reliability for use in the risk assessment. In making this assessment, the RMS has used the classification scheme of Klimisch et (2009), based on the following four categories:

Category 1 'Reliable without restriction'

Category 2 'Reliable with restrictions'

Category 3 'Not reliable'

Category 4 'Not assignable'

Both category 1 and 2 relate to studies that are scientifically acceptable and as such are suitable for use in the regulatory risk assessment.

In making the reliability classification, the RMS has taken into account that 'GLP compliance' and the conducting of the studies to standard test guidelines are not considered under EFSA (2011) guidance as a 'guarantee of reliability' and as such are not necessary requirements for 'Category 1 classification, study reliability being 'judged solely on the basis of the accuracy and reproducibility of the facts reported'.

Reliability evaluation for active substance search publication number 110:

Study ref: Michel A, Johnson RD, Duke SO, and Scheffler BE, Environ Toxicol Chem. (2004) Apr;23(4):1074-9 'Dose-response relationships between herbicides with different modes of action and growth of *Lemna paucicostata*: an improved ecotoxicological method.

Details for this study are included in Section B.9.2.4.1ii). Based on the information provided, although not GLP compliant and not conducted to the standard *Lemna* growth inhibition OECD 221 test guideline, the study appears to have been conducted to an acceptable standard, although the lack of chemical analysis to confirm test substance concentrations is considered a significant deficiency affecting the reliability of the study. Also, although growth in the untreated control is stated as 'exponential', specific details for frond doubling time in the control were not included and hence it is not possible to verify that the study passes the OECD221 test guideline 'validity' requirement of a doubling time of 2.5 days. However, given asulam's high solubility in water, solubility issues affecting the test concentrations are not considered likely. Also, the specified 'exponential growth' in the untreated control, suggests that growth rates were rapid and not inhibited by any nutrient deficiencies or environmental effects. Taking account of the available evidence, which indicates the study is not full reliable, the study has been classified by the RMS as 'Reliable with restrictions' and as such is included in the 'effects' evidence considered in the aquatic risk assessment (Table B.9.2.14).

Reliability evaluation for active substance search publication number 85:

Study ref: Kaufmann A & Kaenzig A (2004) 'Contamination of honey by the herbicide asulam and its antibacterial active metabolite sulfanilamide'; Food Addit Contam 2004 Jun 21(6): pp564-571.

Although an assessment of the reliability /validity of the analytical methods used to identify asulam and sulphanilamide residues in honey has not been undertaken, the paper is considered to be well written and of a good scientific standard. However, given that the paper only includes a summary of the residue data and also of the analytical methods used, it is considered appropriate to categorise it as 'Reliable with

restrictions' (Category 2). Given this categorisation, the publication is considered further in the regulatory risk assessment for bees (Sections B.9.4.3).

Reliability evaluation for active substance search publication number 330:

Study ref: Matsumoto et al (2009) Toxicity of Agricultural Chemicals in *Daphnia magna*; Oasaki City Medical Journal (2009) 55(2), pp89-97.

Although the reported *Daphnia magna* acute and chronic studies were stated to have been conducted to OECD guidelines, this does not appear to be the case - at least for the 'chronic toxicity' tests which were only of 8 days in duration compared with 21 days required in the standard OECD 211 (2012) test guideline. Also, there is insufficient information in the published paper to determine whether these tests passed the required OECD study validity criteria for acute and chronic test (in relation to levels of control mortality and for the reproductive tests also in relation to numbers of control off-spring produced). Additional, no chemical analysis was conducted in either acute or chronic studies to verify the test concentrations over the duration of the study (which a standard requirement).

Given the above deficiencies, the RMS considers the results in the reported paper are 'Not reliable' (Category 3) for regulatory use and as such has not considered this paper further.

B.9.13 References relied on

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, 8.1.1/01	[redacted]	2000	Asulam sodium salt: Acute oral toxicity (LD_{50}) to the bobwhite quail [redacted] GLP, Unpublished	Y	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.1.4/01	[redacted]	2003	Asulam sodium salt: A reproduction study with Japanese quail, amended final report [redacted] GLP, Unpublished	Y	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.1.4/02	[redacted]	2003	Asulam: A study to evaluate egg shell quality with the Japanese quail [redacted] GLP, Unpublished	Y	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.1.4/03	[redacted]	2004	Expert Opinion (EBAWX004): Impact of asulam on eggshell quality [redacted] Not GLP, Unpublished	n/a	Yes	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, 8.2.1.1/01	[redacted]	1988a	Acute toxicity of Asulam technical to rainbow trout (*Salmo gairdneri*) under static conditions [redacted] GLP, Unpublished	Y	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.2.1.2/01	[redacted]	2000	Asulam Sodium salt - Acute toxicity to Bluegill Sunfish (*Lepomis macrochirus*) under static-renewal conditions [redacted] GLP, Unpublished	Y	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.2.2.1/01	[redacted]	1997a	Asulam - Fish, juvenile growth test - 28 Days - under flow-through conditions [redacted] GLP, Unpublished	Y	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.2.3.1/01	[redacted]	1981	Asulam - Absorption, metabolism and elimination studies with fish [redacted] Not GLP, Unpublished	Y	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.3.1.1/01	Manning, C.S.	1988 b	Asulam technical acute toxicity to the water flea (*Daphnia magna*) under flow-through conditions Hunter/ESE Inc. Hunter/ESE Inc. Study No.: ESE 89325-0200-2130 United Phosphorus Limited Report No.: R001268 GLP, Unpublished	N	Yes	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, 8.3.2.1/01	McElligott, A.	1997 b	Asulam - Chronic toxicity (21-day) to Daphnids under static renewal conditions Rhone-Poulenc Agro Rhone-Poulenc Agro Study No.: SA 97050 United Phosphorus Limited Report No.: R005639 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.3.2.1/02	Herrmann, Voigt, Benz	1992	Toxicity of Asulox® 80SG to water-fleas (*Daphnia magna*) (20 days) Ökolimna Ökolimna Study No.: 11/90/286 United Phosphorus Limited Report No.: R001808 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.4/01a	Hoberg, J.R.	1992a	Asulam sodium - Toxicity to the freshwater green alga, *Selenastrum capricornutum* Springborn Laboratories, Inc. Springborn Laboratories, Inc. Study No.: 92-8-4391 United Phosphorus Limited Report No.: R003656 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.4/01b	Dorgerloh, M.	2004a	Non-GLP recalculation report: Influence of Asulam-sodium on the growth of green alga, *Pseudokirchnerellia subcapitata* (formerly named *Selenastrum capricornutum*) Bayer CropScience AG originally reported from Springborn Laboratories, Inc. Study No.: 92-8-4391 United Phosphorus Limited Report No.: C040380 Not GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.4/02a	Hoberg, J.R.	1992 b	Asulam sodium - Toxicity to the freshwater alga, *Anabaena flos-aquae* Springborn Laboratories, Inc. Springborn Laboratories, Inc. Study No.: 92-10-4457 United Phosphorus Limited Report No.: R003654 GLP, Unpublished	N	Yes	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, 8.4/02b	Dorgerloh, M.	2004 b	Non-GLP recalculation report: Influence of Asulam-sodium on the growth of the freshwater alga, *Anabaena flos-aquae* Bayer CropScience AG originally reported from Springborn Laboratories, Inc. Study No.: 92-10-4457 United Phosphorus Limited Report No.: C040383 Not GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.4/03	Hoberg, J.R.	1992c	Asulam sodium - toxicity to the freshwater diatom, *Navicula pelliculosa* Springborn Laboratories, Inc. Springborn Laboratories, Inc. Study No.: 92-12-4519 United Phosphorus Limited Report No.: R003655 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.4/04a	Hoberg, J.R.	1992 d	Asulam sodium - toxicity to the marine diatom, *Skeletonema costatum* Springborn Laboratories, Inc. Springborn Laboratories, Inc. 92-8-4395 United Phosphorus Limited Report No.: R003657 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.4/04b	Dorgerloh, M.	2004c	Non-GLP recalculation report: influence of asulam-sodium on the growth of the marine diatom, *Skeletonema costatum*. Bayer CropScience AG originally reported from Springborn Laboratories, Inc. Study No.: 92-8-4395 United Phosphorus Limited Report No.: Report No.: C040376 Not GLP, Unpublished	N	Yes	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, 8.4.1/01	Gosch, H., Sowig, P.	2003	Algal growth inhibition - *Pseudokirchneriella subcapitata* under static testing conditions AE C473799; substance, pure sulfanilamide, metabolite of asulam code: AE C473799 00 1B99 0001 Bayer CropScience GmbH, DEU; Ecotoxicology, Frankfurt United Phosphorus Limited Report No.: C027726 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.5.2/01	Heintze, A.	2002	Assessment of side effects of asulam sodium salt on the larvae of the midge, *Chironomus riparius* with the laboratory test method Arbeitsgemeinschaft GAB GmbH & IFU GmbH Arbeitsgemeinschaft GAB GmbH & IFU GmbH Study No.: 20011081/01-ASCr United Phosphorus Limited Report No.:C030755 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.6/01	Hoberg, J.R.	1992e	Asulam sodium - Toxicity to the duckweed (*Lemna gibba*) Springborn Laboratories, Inc. Springborn Laboratories, Inc. Study No.: 92-9-4397 United Phosphorus Limited Report No.: R003653 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.6.1/01	Juckeland, D.	2011	Effects of Sulfanilamide on *Lemna minor* in growth inhibition test under semi-static test conditions BioChem agrar, GmbH BioChem agrar, GmbH, Study No.: 11 10 48 021 W United Phosphorus Limited Report No.: 11 10 48 021 W GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.7.1/01 and 8.7.2/01	Schmitzer, S.	1998	Laboratory testing for toxicity (acute contact and oral LD_{50}) of asulam on honey bees (*Apis mellifera* L.) (Hymenoptera, Apidae) IBACON IBACON Study No.: 3560036 United Phosphorus Limited Report No.: C015416 GLP, Unpublished	N	Yes	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, 8.8.1.1/01	Moll, M., Bützler, R.	2001	Effects of EXP 04668 A on the parasitoid *Aphidius rhopalosiphi* in the laboratory - dose response test IBACON GmbH IBACON GmbH Study No.: 6465001, United Phosphorus Limited No.: C017898 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.8.1.1/02	Moll, M.	1999a	Effects of EXP04668A on the parasitoid *Aphidius rhopalosiphi* (Hymenoptera, Aphidiidae) in the laboratory IBACON GmbH IBACON GmbH Study No.: 6461001 United Phosphorus Limited Report No.: R006295 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.8.1.2/01	Goßmann, A.	2001	Effects of EXP 04668 A on the predatory mite *Typhlodromus pyri* in the laboratory - dose response test - IBACON GmbH IBACON GmbH Study No.: 6466063 United Phosphorus Limited Report No.: C017897 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.8.1.2/02	Luehrs, U.	1999a	Effects of EXP04668A on the predatory mite *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) in the laboratory. IBACON GmbH IBACON GmbH Study No.: 6462063 Rhone-Poulenc Report No.: R006339 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.8.1.3/01	Schmitzer, S.	2002	Effects of EXP04668A (AE F074383 00 SL33) on the wolf spider *Pardosa spec.* in the laboratory IBACON GmbH IBACON GmbH Study No.: 13502065 United Phosphorus Limited Report No.: C030817 GLP, Unpublished	N	Yes	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, 8.8.1.3/02	Mead-Briggs, M.	1991	An evaluation of the side-effects of Asulox® 80 SG on lycosid spiders University Southampton University Southampton Study No.: RP-91-1 United Phosphorus Limited Report No.: R001634 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.8.1.4/01	Waltersdorfer, A.	2002	Toxicity to the foliage dwelling predator *Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae) in the laboratory Asulam water soluble concentrate Bayer CropScience GmbH Bayer CropScience GmbH Study No.: CW02/017 United Phosphorus Limited Report No.: C028606 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.8.2.1/01	Moll, M.	1999 b	Effects of EXP04668A on the parasitoid *Aphidius rhopalosiphi* (Hymenoptera, Aphidiidae) - extended Laboratory study. IBACON GmbH IBACON GmbH Study No.: 6463002 United Phosphorus Limited Report No.: R007954 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.8.2.2/01	Luehrs, U.	1999 b	Effects of EXP04668A on the predatory mite *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) - extended laboratory study IBACON GmbH IBACON GmbH Study No.: 6464062 United Phosphorus Limited Report No.: R007956 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.8.2.3/01	Drexler, A.	2002	Effects of EXP04668A on the reproduction of rove beetles *Aleochara bilineata* - Extended laboratory study IBACON GmbH IBACON GmbH Study No.: 13501071 United Phosphorus Limited Report No.: C030818 GLP, Unpublished	N	Yes	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, 8.8.2.5/01	Feije, R.	2002	Asulam (EXP 04668 A = AE F074383): An extended laboratory single-dose test to evaluate the effects on survival and reproduction of the predaceous mite *Hypoaspis aculeifer Canestrini* (Acari: Laelapidae) in standard soil (LUFA 2.1) MITOX Trial Management BV MITOX Trial Management BV Study No.: AV011HAE United Phosphorus Limited Report No.: C030819 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.8.2.5/02	Vinall, S.	2011	Sulfanilamide – Laboratory determination of toxicity to the springtail *Folsomia candida* (Collembola, Isotomidae) in an artificial soil substrate Mambo-Tox Ltd Mambo-Tox Ltd Study No.: UP-11-11 United Phosphorus Limited Report No.: UP-11-11 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.9.1/01	Handley, J.W.; Wetton, P.M.	1992	The acute toxicity of asulam sodium to earthworms (*Eisenia foetida*) Safepharm Laboratories Limited Safepharm Laboratories Limited Study No.: 282/234 United Phosphorus Limited Report No.: R001746 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.9.1/02	Sowig, P.	2002	Acute toxicity to earthworms (*Eisenia fetida*) Sulfanilamid substance, pure Code: AE C473799 00 1B99 0001 Bayer CropScience GmbH United Phosphorus Limited Report No.: C023318 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.9.2/01	Luehrs, U.	2000	Effects of ASULAM sodium salt on reproduction and growth of earthworms *Eisenia fetida* (Savigny 1826) in artificial soil IBACON GmbH IBACON GmbH Study No.: 6960022 United Phosphorus Limited Report No.: C010993 GLP, Unpublished	N	Yes	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, 8.9.2/02	McCormac, A.	2011	Sulfanilamide – Determination of Chronic (sub-lethal) toxicity to the earthworm *Eisenia fetida* in artificial soil substrate Mambo-Tox Ltd Mambo-Tox Ltd Study No.: UP-11-10 United Phosphorus Limited Report No.: UP-11-10 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.10.1/01	Luscombe, B.M.	1981a	Asulam - Effect on soil micro-organisms: II. studies on soil processes - carbon cycle, mobilization of organic nitrogen, denitrification and enzyme activity May & Baker Ltd. May & Baker Ltd. Study No.: R.Ag.18 United Phosphorus Limited Report No.: R000731 Not GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.10.1/02	Luscombe, B.M.	1981 b	Asulam: Effects on soil micro-organisms - III. Studies on soil processes - nitrogen fixation May & Baker Ltd. May & Baker Ltd. Study No.: R.Ag 19 United Phosphorus Limited Report No.: R000733 Not GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.10.1/03	Kölzer, U.	2002	Assessment of the side effects of ASULAM sodium salt on the activity of the soil microflora Arbeitsgemeinschaft GAB GmbH & IFU GmbH Arbeitsgemeinschaft GAB GmbH & IFU GmbH Study No.: 20011081/01-ABMF United Phosphorus Limited Report No.: C021214 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.10.1/04	Schulz, L.	2011	Sulfanilamide – Effects on the activity of soil microflora (Nitrogen transformation test) BioChem agrar, GmbH BioChem agrar, GmbH, Study No.: 11 10 48 021 W United Phosphorus Limited Report No.: 11 10 48 048 N GLP, Unpublished	N	Yes	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, 8.10.2/01	Luscombe, B.M.	1981a	Asulam - Effect on soil micro-organisms: II. studies on soil processes - carbon cycle, mobilization of organic nitrogen, denitrification and enzyme activity May & Baker Ltd. May & Baker Ltd. Study No.: R.Ag.18 United Phosphorus Limited Report No.: R000731 Not GLP, Unpublished ⇒ CA 8.10.1/01	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.10.2/02	Kölzer, U.	2002	Assessment of the side effects of ASULAM sodium salt on the activity of the soil microflora Arbeitsgemeinschaft GAB GmbH & IFU GmbH Arbeitsgemeinschaft GAB GmbH & IFU GmbH Study No.: 20011081/01-ABMF United Phosphorus Limited Report No.: C021214 GLP, Unpublished ⇒ CA 8.10.1/03	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.12/01	Christensen, K.P.	1992	Asulam Sodium - Determination of effects on seed germination, seedling emergence and vegetative vigor of ten plant species Springborn Laboratories, Inc. Springborn Laboratories, Inc. Study No.: 92-9-4431 United Phosphorus Limited Report No.: R003652 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA, 8.15/01	Luscombe, B.M., Craven, A.C.C.	1981	ASULAM - Metabolism in and effect on the sewage treatment process May & Baker Ltd. May & Baker Ltd. Study No.: R.Ag 21 United Phosphorus Limited Report No.: R000735 Not GLP, Unpublished	N	Yes	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, 8.15/02	Mead, C.	2001	Asulam sodium salt: Assessment of the inhibitory effect on the respiration of activated sewage sludge Safepharm Laboratories Ltd. Safepharm Laboratories Ltd. Study No.: 1392/015 United Phosphorus Limited Report No.: C015300 GLP, Unpublished	N	Yes	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	

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*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
CP10.3.2.1 (and CA 5.2.1/01)	[redacted]	1987a	FR 1398/1: Acute oral toxicity study in the rat [redacted] GLP, Unpublished Study required to determine acute oral toxicity of the test article	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CP10.3.2 (and CA 5.6.1/01)	[redacted]	1981	Asulam: two generation reproduction study in the rat [redacted] Not GLP, Unpublished Study required to assess reproductive toxic potential of the test article	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CP, 10.5.1/01	Moll, M.; Buetzler, R.	2001	Effects of EXP 04668 A on the parasitoid *Aphidius rhopalosiphi* in the laboratory - dose response test – United Phosphorus Limited Report No.: C017898 IBACON GmbH Study No.: 6465001 IBACON GmbH GLP, Unpublished ⇒ 8.8.1.1/01	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CP, 10.5.1/02	Moll, M.	1999a	Effects of EXP04668A on the parasitoid *Aphidius rhopalosiphi* (Hymenoptera, Aphidiidae) in the laboratory United Phosphorus Limited Report No.: R006295 IBACON GmbH Study No.: 6461001 IBACON GmbH GLP, Unpublished ⇒ 8.8.1.1/02	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
CP, 10.5.1/03	Gossmann, A.	2001	Effects of EXP04668A on the predatory mite *Typhlodromus pyri* in the laboratory - dose response test United Phosphorus Limited Report No.: C017897 IBACON GmbH IBACON GmbH Study No.: 6466063 GLP, Unpublished ⇒ 8.8.1.2/01	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CP, 10.5.1/04	Luehrs, U.	1999a	Effects of EXP04668A on the predatory mite *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) in the laboratory. United Phosphorus Limited Report No.: R006339 IBACON GmbH Study No.: 6462063 IBACON GmbH GLP, Unpublished ⇒ 8.8.1.2/02	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CP, 10.5.1/05	Schmitzer, S.	2002	Effects of EXP04668A (AE F074383 00 SL33) on the wolf spider *Pardosa* spec. in the laboratory United Phosphorus Limited Report No.: C030817 IBACON GmbH Study No.: 13502065 IBACON GmbH GLP, Unpublished ⇒ 8.8.1.3/01	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CP, 10.5.1/06	Mead-Briggs, M., Thomas, M.	1991	An evaluation of the side-effects of Asulox 80 SG on lycosid spiders United Phosphorus Limited Report No.: R001634, University Southampton, England Study No.: RP-91-1 University Southampton, England GLP, Unpublished ⇒ 8.8.1.3/02	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CP, 10.5.1/07	Waltersdorfer, A.	2002	Toxicity to the foliage dwelling predator *Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae) in the laboratory, Asulam watersoluble concentrate 392 g/L	N	Y	Data protection is claimed in accordance with	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
			United Phosphorus Limited Report No.: C028606 Bayer CropScience GmbH Study No.: CW02/017 Bayer CropScience GmbH GLP, Unpublished ⇒ 8.8.1.4/01			Article 59 of Regulation (EC) No 1107/2009		
CP, 10.5.2/01	Moll, M.	1999b	Effects of EXP04668A on the parasitoid *Aphidius rhopalosiphi* (Hymenoptera, Aphidiidae) - Extended Laboratory study and amended final report. United Phosphorus Limited Report Nos.: R007954 and C031135 IBACON GmbH, Rossdorf, DEU; GLP, Unpublished ⇒ 8.8.2.1/01	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CP, 10.5.2/02	Luehrs, U.	1999b	Effects of EXP04668A on the predatory mite *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) - Extended laboratory study United Phosphorus Limited Report No.: R007956 IBACON GmbH Study No.: 6464062 IBACON GmbH GLP, Unpublished ⇒ 8.8.2.2/01	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CP, 10.5.2/03	Drexler, A.	2002	Effects of EXP04668A on the reproduction of rove beetles *Aleochara bilineata* Extended laboratory study United Phosphorus Limited Report No.: C030818, IBACON GmbH Study No.: 13501071 IBACON GmbH GLP, Unpublished ⇒ 8.8.2.3/01	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CP, 10.6.6/01	Feije, R.	2002	Asulam (EXP 04668 A = AE F074383): An extended laboratory single-dose test to evaluate the effects on survival and reproduction of the predaceous mite *Hypoaspis aculeifer* Canestrini (Acari: Laelapidae) in	N	Y	Data protection is claimed in accordance with Article 59 of	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
			standard soil (LUFA 2.1) and amendment United Phosphorus Limited Report No.: C030819 and C038575 MITOX Trial Management BV Study No.: AV011HAE MITOX Trial Management BV GLP, Unpublished ⇒ 8.8.2.5/01			Regulation (EC) No 1107/2009		