Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

sodium methyl [(4-aminophenyl)sulphonyl] carbamate; sodium methyl (*EZ*)-sulfanilylcarbonimidate; asulam-sodium

EC Number: 218-953-8
CAS Number: 2302-17-2

CLH-O-0000001412-86-138/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

9 December 2016

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Substance Name: Asulam sodium

EC Number: **218-953-8**

CAS Number: **2302-17-2**

Index Number: **Not assigned**

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Version number: **1** **Date:** **December 2015**

CONTENTS

Part A.

Part B.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	**Asulam sodium**
EC number:	**218-953-8**
CAS number:	**2302-17-2**
Annex VI Index number:	**Not assigned**
Degree of purity:	***≥ 88.6%***
Impurities:	**There are a number of process impurities in the active substance. These have been taken into account but are not considered to contribute to the classification. Full information is provided in the technical document**

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

Current entry in Annex VI, CLP Regulation	Not listed
Current proposal for consideration by RAC	Skin Sens 1: H317 – May cause an allergic skin reaction Aquatic Acute 1; H400 – Very toxic to aquatic life Acute M factor = 1 Aquatic Chronic 1; H410 – Very toxic to aquatic life with long lasting effects. Chronic M factor = 1

Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Skin Sens 1: H317 – May cause an allergic skin reaction Aquatic Acute 1; H400 – Very toxic to aquatic life Acute M factor = 1 Aquatic Chronic 1; H410 – Very toxic to aquatic life with long lasting effects. Chronic M factor = 1

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.14.	Oxidising solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.15.	Organic peroxides	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
	Acute toxicity - dermal	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
	Acute toxicity - inhalation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
3.4.	Respiratory sensitisation	Not classified	Not applicable	Not classified	Data lacking
3.4.	Skin sensitisation	**Skin Sens 1; H317 - May cause an allergic skin reaction**	Not applicable	Not classified	-
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not classified	**inconclusive**
3.6.	Carcinogenicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Not classified	Not applicable	Not classified	**Fertility - inconclusive Development – conclusive but not sufficient for classification**
3.8.	Specific target organ toxicity –single exposure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	**H400; Very toxic to aquatic life** **H410; Very toxic to aquatic life with long lasting effects**	**Acute M = 1** **Chronic M = 1**	Not classified	-
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Pictogram(s): GHS07, GHS09

Signal word: Warning

Hazard statements: H317; May cause an allergic skin reaction

H410; Very toxic to aquatic life with long lasting effects

Precautionary statements: Not included in Annex VI of CLP

Proposed notes assigned to an entry: None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Asulam-sodium is a new pesticide active substance in the scope of Regulation 1107/2009. The classification and labelling has not previously been considered in the harmonised process.

At the time of submission the substance is not registered under REACH.

2.2 Short summary of the scientific justification for the CLH proposal

Asulam sodium does not meet the criteria for classification for acute toxicity as the ATE values are above those relevant for classification. There was no significant toxicity to specific organs or tissues following single exposure and there was no evidence of narcotic effects or respiratory tract irritation. Therefore, classification with STOT-SE is not appropriate. Asulam sodium was found to cause minimal skin irritation and reversible eye irritation, but the criteria for classification were not met. In a guinea pig maximisation test, 12/20 (60%) test animals exhibited a dermal response following topical challenge with the test item and therefore the criteria for classification as a skin sensitiser are met. There is insufficient data for sub-categorisation and therefore, asulam sodium should be classified as **Skin Sens 1; H317 – May cause an allergic skin reaction.**

In all of the available studies to investigate the repeated dose toxicity of asulam sodium, the lowest dose tested was in excess of the guidance value for classification for STOT RE (adjusted as necessary for study duration). In all cases, there were no adverse effects observed at these doses. Consequently, asulam sodium does not meet the criteria for classification for STOT RE.

There are weaknesses in the available genotoxicity dataset. *In vitro* there are good negative bacterial mutation studies. There are also two mouse lymphoma assays, one of which was positive at concentrations which exceed the maximum recommended concentration, whilst the other study was negative when tested up to the maximum concentration in accordance with current *in vitro* genotoxicity guideline requirements. In the only chromosome aberration study there was an increase in aberrations at the top concentration in the absence of S9, which was comparable to the vehicle (DMSO) control. However, due to high background of aberrations reported by DMSO in this study (likely attributed to the purity/grade of solvent used), the result was considered difficult to interpret. In the only available *in vivo* study (the mouse bone marrow micronucleus study), a marginal increase in PCEs with micronuclei was observed at a dose of 2000 mg/kg bw but not at a higher dose of 4000 mg/kg bw. The absence of a response at 4000 mg/kg bw/d cannot be accounted for by toxicity as clinical signs were reported at all dose levels and there was no impact on body weight. The study appears to be negative but, given the unusual study design, it is the dossier submitter's opinion that no clear conclusions can be drawn from this study. Overall, although there is no strong or reliable positive evidence that asulam sodium is mutagenic, the quality of the data package is such that no clear conclusion can be drawn.

In the carcinogenicity studies, an increased incidence (20%) of phaeochromocytomas was noted in male CD (Sprague Dawley origin) rats at the highest dose tested. Whilst the incidence of this neoplasm exceeded the laboratory's historical control data (2 – 16%), there is information in the publically-available literature on the incidence of this tumour type in Sprague Dawley rats which shows phaeochromocytomas can spontaneously occur at an incidence of up to 33%. Furthermore, this tumour is referenced in the CLP guidance as having a high spontaneous incidence rate in Sprague Dawley rats. The tumour was also not dose responsive, limited to a single sex and species, with no evidence of a multi-site response and no direct evidence from the toxicology package of studies to

support chemical induction of phaeochromocytomas in accordance with published literature [37]. In the mouse study, the incidence of hepatocellular adenomas and carcinomas were increased in both sexes at the lowest dose tested. However, there was no dose response, no accompanying histopathology, no toxicity at mid or high dose levels to account for the decrease in tumours and the neoplasm was restricted to one species and one site. Whilst the study had some methodological limitations, these tumours are not considered sufficient for classification purposes. Overall, no classification for carcinogenicity is proposed.

In a limited two-generation rat study, a reduction in litter size in the F_1 and F_2 generation was noted in the mid and high dose groups. The underlying reason for this effect was unclear as there was no difference in the number of fetuses dead at birth compared with controls and there were no effects on reproductive organs in this study or in the repeated dose studies. There was no effect on the fertility index so it was considered whether this could be a developmental effect, but in the developmental studies there were no adverse effects on the later stages of reproduction; including post-implantation loss, resorptions or a decrease in viable foetuses. This therefore suggests that the reduced litter loss was a chance finding in the two-generation study and was not related to treatment. However, the quality of the study is insufficient to allow for a conclusion on classification to be drawn.

In the rat developmental study, there was a slight increased incidence of absent 13^{th} rib in the top dose group (in excess of the limit dose) in the absence of maternal toxicity. There were no developmental findings at any dose in the rabbit. Overall, the presence of the finding in the rat, in isolation from other malformations of the ossification system, is considered insufficient for classification.

Asulam sodium is stable to hydrolysis, and photolysis is not expected to be a major route of degradation. From the available abiotic and biotic degradation information, asulam sodium is considered 'not rapidly degradable' for the purposes of classification. There is a low bioaccumulation potential with measured whole fish BCF values below the trigger of 500. Acute aquatic toxicity data are available on asulam sodium for fish, invertebrates, algae and aquatic plants. Algae and aquatic plants are the most acutely sensitive groups. The lowest reliable acute/short-term endpoint for classification purposes is the EC_{50} for *Lemna gibba* of 0.16 mg asulam sodium/L. This is in the range >0.1 to ≤1.0 and therefore asulam sodium should be classified as **Aquatic Acute 1; H400 with an Acute M-factor of 1.** Chronic studies are available in fish, invertebrates, algae and aquatic plants. Algae and aquatic plants are again the most sensitive organisms with lowest NOEC values from 0.011 to 0.54 mg asulam sodium/L. This is just within the range >0.01 to ≤0.1 and therefore, since the substance is also considered 'not rapidly degradable', it should be classified as **Aquatic Chronic category 1; H410 with a Chronic M-factor of 1.**

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Not currently listed

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

The following classification and labelling is currently notified in the C&L Inventory

Skin Sens 1; H317 – May cause an allergic skin reaction

Aquatic Acute 1; H400 – Very toxic to aquatic life

Wng

GHS07, GHS09

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Asulam-sodium is a pesticide active substance in the scope of Reg 1107/2009. It is subject to harmonised classification and labelling in accordance with Article 36(2) of CLP.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

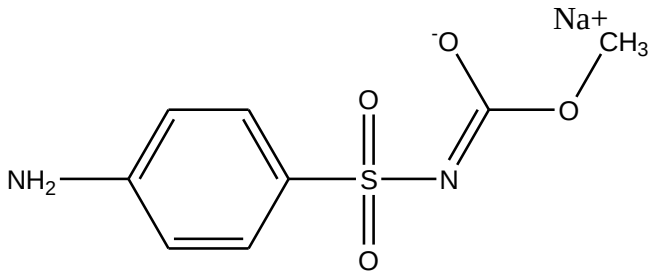
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	218-953-8
EC name:	sodium methyl [(4-aminophenyl)sulphonyl]carbamate
CAS number (EC inventory):	2302-17-2
CAS number:	2302-17-2
CAS name:	Carbamic acid, N-[(4-aminophenyl)sulfonyl]-, methyl ester, sodium salt (1:1)
IUPAC name:	sodium methyl (EZ)-sulfanilylcarbonimidate
CLP Annex VI Index number:	N/A
Molecular formula:	$C_8H_9N_2O_4S$ Na
Molecular weight range:	252.2

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Asulam sodium	≥ 88.6%		

Current Annex VI entry: N/A

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Confidential - see the IUCLID for full details and the confidential Annex to this report			

There are a number of process impurities in the substance. These have been taken into consideration and are not considered to impact on the classification proposed in this dossier. Further information on the impurities is considered to be confidential but full details are provided in the IUCLID.

Current Annex VI entry: Two of the impurities have a harmonised classification. However, at the concentration they are present in the substance and given the data on the substance itself, they are not considered to individually impact on the proposed classification. See the IUCLID for full details and the confidential Annex to this report.

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks

Current Annex VI entry: N/A

1.2.1 Composition of test material

The test material used for the majority of studies was asulam sodium and is considered to be representative of the material outlined above. A number of studies were conducted with Asulam (EC 222-077-1, CAS 33337-71-1). Where this is the case it is specified in the report.

1.3 Physico-chemical properties

All references are taken from Draft Assessment Report for Asulam-sodium - Annex B (Volume 3) B.2 Physical and chemical properties

All studies were conducted to appropriate quality standards and are considered relevant and reliable.

Table 8: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	White solid	Francon, 1999a[1]	Observation
Melting/freezing point	230°C	Francon, 1999a[1]	EPA/OPPTS series 830 (capillary method) Purity 99.1%
Boiling point	Substance decomposes at 230°C	Francon, 1999a[1]	-
Relative density	1.525	Francon, 1999a[1]	EPA/OPPTS series 830 (Pycnometer) Purity 99.1%
Vapour pressure	5×10^{-7} Pa at 45°C (test not conducted at lower temperature as the Vp is estimated to be low i.e., $< 5 \times 10^{-5}$)	Francon, 2000[2]	EEC method A 4 (gas saturation) Purity 99.1%
Surface tension	71.5 mN/m	Francon, 1999a[1]	EPA/OPPTS series 830 (ring method) Purity 89.6%
Water solubility	5.5 g/l at pH 4 962 g/l at pH 8 1048 g/l at pH 9 All at 20ºC	Francon, 1999b[3]	EEC method A 6 (flask method) Purity 99.1%
Partition coefficient n-octanol/water	Log P_{ow} = 0.11 at pH 4 and 25°C Log P_{ow} = 0.15 at pH 7 and 25°C Log P_{ow} = 0.77 at pH 9 and 25°C	Francon, 1999c[4]	EEC method A 8 (shake flask) Purity 99.1%

Flash point	Not applicable substance is a solid		
Flammability	Not considered highly flammable. In contact with the ignition source the test substance expanded and became discoloured, but did not ignite. Experience from handling and use indicates that the substance is not pyrophoric and does not emit flammable gases on contact with water.	van Helvoirt, 1993b[5]	EEC method A 10 (tested as a soluble granule formulation containing 81.2% active)
Explosive properties	Not explosive In a screening study, the heat of decomposition was 205 J/g and exothermal effects observed in the range 225-265ºC and 290-390ºC	Smeykal, 2001[6]	EEC method A 14 (tested as a soluble granule formulation containing 82.2% active)
Self-ignition temperature	No self-ignition up to a temperature of 140°C No self-ignition up to a temperature of 400°C	van Helvoirt, 1993a[7] Maarsingh, 2008[8]	UN-Bowes-Cameron-Cage-Test (tested as a soluble granule formulation containing 81.2% active) EEC method A 16 (tested as a soluble granule formulation containing 74% active)
Oxidising properties	Not oxidising	Tran Thanh Phong, 1999[9]	EEC method A 17 Purity 89.6%
Granulometry	No data	-	-

Stability in organic solvents and identity of relevant degradation products	Information on the solubility in organic solvents at 25 °C is available n-heptane: 0.00007 g/L xylene: 0.098 g/L dichloromethane: 0.033 g/L Methanol: 117 g/L n-octanol : 0.03 g/L acetone: 1.1 g/L ethyl acetate: 0.5 g/L acetonitrile: 2.3 g/L	Francon, 1999b[3]	Similar to EEC A6 Purity 99.1%
Dissociation constant	pKa1 = 1.29 at 20°C pKa2 = 4.68 at 20°C	Francon, 1999d[10]	OECD Test Guideline 112 Purity 99.1%
Viscosity	Not applicable	-	-

2 MANUFACTURE AND USES

2.1 Manufacture

The substances is manufactured outside of the EU and imported into the EU for use as a plant protection product.

2.2 Identified uses

Asulam sodium is an herbicide, which is effective against annual and perennial weeds, both monocotyledons and dicotyledons, and on some perennial pteridophytes. It is particularly effective against certain perennial grasses and broad-leaved weeds being readily translocated to the root systems of susceptible species.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 9: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Refer to table 8			

3.1 Physico-chemical Hazards

3.1.1 Summary and discussion of

In a standard study conducted in accordance with EECA10, asulam sodium did not ignite on contact with the ignition source, but expanded and turned black (van Helvoirt, 1993b). Experience from handling and use indicates that the substance is not pyrophoric and does not emit flammable gases on contact with water.

In a screening study conducted according to EEC A14, the exothermic decomposition energy was found to be 205J/g with exothermal effects in the range 225-265ºC and 290-390ºC. As such, the main study was not performed.

In a standard study conducted in accordance with EEC A17, the test material ignited but was rapidly extinguished in the preliminary test. As such, the main study was not performed.

3.1.2 Comparison with criteria

In the A10 study, asulam sodium failed to ignite and therefore does not meet the criteria for classification as a flammable solid (i.e., the burning time is not < 45 seconds). Further, experience from handling and use indicates that the substance is not pyrophoric and does not emit flammable gases on contact with water.

In the A14 screening study the exothermic decomposition energy was < 500 J/g and the onset of exothermic decomposition energy was below 500 ºC. Therefore, asulam sodium does not meet the criteria for classification as explosive.

In the A17 study, the test material did not burn in the preliminary study and therefore does not meet the criteria for classification.

3.1.3 Conclusions on classification and labelling

Not classified - Conclusive but not sufficient for classification

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

No classification is proposed by the Dossier Submitter (DS) for physical hazards based on the following observations:

- asulam-sodium does not meet criteria for flammable solids based on results of testing according to the EEC A10 method (van Helvoirt, 1993b).
- asulam-sodium does not exhibit explosive properties based on results of testing according to the EEC A14 method (Smeykal, 2001).
- asulam-sodium does not exhibit oxidizing properties based on results of testing according to the EEC A17 method (Tran Thanh Phong, 1999).

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

Asulam-sodium does not meet the criteria for classification for physico-chemical properties. RAC agrees with the proposal of the DS to **not classify asulam-sodium for physical hazards**

4 HUMAN HEALTH HAZARD ASSESSMENT

All references are taken from Draft Assessment Report for Asulam-sodium - Annex B (Volume 3) B.6A Toxicology and metabolislm

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

The absorption, metabolism, distribution and elimination of asulam, following single and repeated oral administration, were investigated in two toxicokinetic studies performed in the rat.

4.1.1 Non-human information

Asulam was rapidly and extensively absorbed following oral administration in the rat (T_{max} approximately 30 minutes). Oral absorption was determined to be in excess of 85-90%, based on excretion in urine and faeces. Asulam is widely distributed and rapidly cleared following oral administration, with no evidence of bioaccumulation in fat. There is some evidence for preferential binding to blood cells ([11],[12]).

The majority of the asulam detected in the excreta (82.0-92.3%) was found in the urine, following oral administration in the rat; a minor fraction was detected in the faeces. Asulam was excreted largely unchanged, with 69.0-80.3% of the administered dose detected as unchanged asulam in the urine. Following intravenous administration 3.3-4.6% of the dose was detected in the faeces, indicating limited biliary excretion. The major metabolite of asulam was identified as acetyl-asulam, which was present in both urine and faeces [12]. Other metabolites identified in the urine were sulphanilamide and acetyl-sulphanilamide. Metabolites were determined to be produced *via* sequences of N-deacetylation and / or N-acetlyation steps.

4.1.2 Human information

No data available.

4.1.3 Summary and discussion on toxicokinetics

Asulam is rapidly and extensively absorbed, distributed and eliminated following oral administration. There is no evidence of bioaccumulation. Asulam is predominantly excreted unchanged in the urine.

4.2 Acute toxicity

Acute toxicity studies have been conducted by the oral, inhalation and dermal routes.

Table 10: Summary table of relevant acute toxicity studies

Acute Oral			
Method	**LD_{50}**	**Observations and remarks**	**Reference**
Rat (CD) 5/sex 5000 mg/kg bw (25% solution in distilled water) Asulam sodium (88% purity) OECD 401, GLP	>5000 mg/kg bw	**Mortality:** None. **Clinical signs:** Hypoactivity, lethargy, ataxia and piloerection in all animals on day of administration. All animals were considered normal on day 2. **Necropsy:** No treatment-related abnormalities.	[13], 1987 (DAR B.6.2.1)
Rat (SD) 5/sex 5000 mg/kg bw (50% solution in distilled water) Asulam sodium (88% purity) USEPA 81-1, 82-1, GLP	>5000 mg/kg bw	**Mortality:** None. **Clinical signs:** Signs of toxicity were limited to brown perineal staining on day of administration. All animals were considered normal on day 2 (f) or day 3 (m). **Necropsy:** No treatment-related abnormalities.	[14], 1988 (DAR B.6.2.1)
Acute Inhalation			
Method	**LC_{50}**	**Observations and remarks**	**Reference**
Rat (SD) 5/sex 5.46 mg/L (4 h, whole body, dust exposure) Asulam sodium (88% purity) EPA 81-3, GLP	>5.46 mg/l	**Mortality:** None. **Clinical signs:** Periocular wetness immediately following exposure. No other overt signs of toxicity. **Necropsy:** No treatment-related abnormalities.	[15], 1988 (DAR B.6.2.3)
Acute Dermal			
Method	**LD_{50}**	**Observations and remarks**	**Reference**
Rat (CD) 5/sex 2000 mg/kg bw Asulam sodium (88% purity) OECD 402, GLP	>2000 mg/kg bw	**Mortality:** None. **Clinical signs:** No overt signs of toxicity. **Necropsy:** No treatment-related abnormalities.	[16], 1987 (DAR B.6.2.2)
Rabbit (NZ white) 5/sex/dose 2000, 4000 mg/kg bw Asulam sodium (88% purity) USEPA 81-1, 81-2, GLP	>4000 mg/kg bw	**4000 mg/kg bw:** **Mortality:** None. **Clinical signs:** Distension, emaciation and diarrhoea. **Necropsy:** Red/pink discoloration of the lungs in three males. **2000 mg/kg bw:** **Mortality:** 1(m) and 1(f). **Clinical signs:** Distension, emaciation and diarrhoea. **Necropsy:** Red/pink discoloration of the lungs in all animals.	[14], 1988 (DAR B.6.2.2)

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Two studies of acute oral toxicity of asulam sodium are available, both conducted in the rat. In both instances a single dose of 5000 mg/kg bw was tested. No deaths occurred in either study ([13],[14]). Clinical observations were limited to signs of general toxicity, including hypoactivity, piloerection, ataxia, lethargy and brown perineal staining. In both studies all animals had recovered by day 3 of the observation period. The oral LD_{50} of asulam sodium was >5000 mg/kg bw in both studies.

4.2.1.2 Acute toxicity: inhalation

A single study of acute inhalation toxicity of asulam sodium is available, conducted in the rat [15]. Five animals of each sex were exposed (whole body) to an atmosphere of asulam sodium generated as a dust at a concentration of 5.46 mg/L, for a period of four hours. It was not possible to make clinical observations during the exposure period because of the dusty atmosphere. There were no deaths. The inhalation LC_{50} (4 hours) of asulam sodium was >5.46 mg/L in the rat.

4.2.1.3 Acute toxicity: dermal

Two studies of acute dermal toxicity of asulam sodium are available.

In one study, the acute dermal toxicity of asulam sodium was examined in the rat at a single dose of 2000 mg/kg bw [16]. There were no mortalities, no overt signs of toxicity, and no treatment-related abnormalities were noted at necropsy.

A second study, conducted in the rabbit, examined the acute dermal toxicity of asulam sodium at doses of 2000 and 4000 mg/kg bw [14]. There were no deaths at 4000 mg/kg bw, but deaths of one male and one female were recorded at 2000 mg/kg bw. Clinical signs included distension, emaciation and diarrhoea at both dose levels. Gross necropsy revealed pink/red discolouration of the lungs in all animals at 2000 mg/kg bw and 3/5 males at 4000 mg/kg bw.

The dermal LD_{50} of asulam sodium was >2000 mg/kg bw in the rat and >4000 mg/kg bw in the rabbit.

4.2.1.4 Acute toxicity: other routes

No data available.

4.2.2 Human information

No data available.

4.2.3 Summary and discussion of acute toxicity

Refer to Section 4.2.1.

4.2.4 Comparison with criteria

In two studies of acute oral toxicity conducted in the rat, no deaths occurred at the only tested dose of 5000 mg/kg bw asulam sodium. The LD_{50} of asulam sodium is therefore in excess of the threshold for classification for acute oral toxicity (2000 mg/kg bw).

The acute inhalation toxicity of asulam sodium was investigated in a study in the rat. No deaths occurred at the tested concentration of asulam sodium (5.46 mg/L). The LC_{50} (4 hour) therefore exceeds the threshold for classification of dusts and mists for acute inhalation toxicity (5.0 mg/L).

Two studies of acute dermal toxicity of asulam sodium are available. The dermal LD_{50} of asulam sodium was >2000 mg/kg bw in the rat and >4000mg/kg bw in the rabbit, both of which were in excess of the threshold for classification for acute dermal toxicity (2000 mg/kg bw).

4.2.5 Conclusions on classification and labelling

No Classification – conclusive but not sufficient for classification

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

No classification is proposed by the DS for acute toxicity by the oral, inhalation or dermal route based on the following data:

Acute toxicity: oral route

Two GLP compliant studies addressing the acute oral toxicity of asulam-sodium were available (Report No. R001006, 1987; Report No R00163, 1988). Both were conducted in male and female rats, in accordance with test guidelines OECD TG 401 and USEPA 81-1, 82-1. Asulam-sodium was administered orally as 25 and 50% solutions in distilled water. No deaths were observed at the single dose tested, 5000 mg/kg bw. Signs of toxicity (reduced activity, lethargy, ataxia and piloerection) were observed in all animals on the day of administration only; all animals appeared normal on day 2 or 3 after treatment. Bodyweights were unaffected by treatment. Gross necropsy did not reveal any treatment-related findings.

No classification for acute oral toxicity was proposed since the LD_{50} was found to be > 5000 mg/kg bw for both males and females rats in both studies.

Acute toxicity: dermal route

Two GLP compliant studies of acute dermal toxicity of asulam-sodium were available.
In one study, which was conducted according to OECD TG 402, the acute dermal toxicity of asulam-sodium was examined in rats at a single dose of 2000 mg/kg bw (Report No. R001007, 1987). There were no mortalities, no overt signs of toxicity, and no treatment-related abnormalities were noted at necropsy.

A second study, conducted according to test guideline USEPA 81-1, 82-1, in rabbits, examined the acute dermal toxicity of asulam-sodium at doses of 2000 and 4000 mg/kg bw (Report No R00163, 1988). The test material (moistened with distilled water) was applied for 24 hours under occlusive conditions to the shorn dorsal skin of New Zealand White rabbits (5/sex) at dose levels of 2000 or 4000 mg/kg bw. Animals were observed for 14 days. There were no deaths at 4000 mg/kg bw, but deaths of one male and one female were recorded at 2000 mg/kg bw. Gross necropsy of the decedents revealed liquid-filled gastrointestinal tract and/or abdominal cavity; similar findings were also noted in one surviving male given 2000 mg/kg bw. Red/pink discoloration of the lungs was noted in all animals at 2000 mg/kg bw and in three animals at 4000 mg/kg bw. The deaths and necropsy findings in this study are not considered to be treatment-related, as similar findings were not seen at the top dose level.

No classification for acute dermal toxicity is proposed as the LD_{50} was found to be > 2000 mg/kg bw in the rat and > 4000 mg/kg bw in the rabbit.

Acute toxicity: Inhalation

Asulam-sodium was tested for acute inhalation toxicity in Sprague-Dawley rats (5 male and 5 female), in a GLP compliant study conducted according to the EPA OPP 81-3 guideline. Rats were exposed (whole body) to an atmosphere of asulam-sodium dust at a concentration of 5.46 mg/L, for 4 hours (Report No R001167, 1988). No deaths were observed. Signs of toxicity were limited to periocular wetness immediately following the exposure period. Slight weight loss (females) or reduced weight gain (males) was measured during the first week. However, all animals gained weight over the study period. Gross necropsy did not reveal any treatment-related findings.

No classification for acute inhalation was proposed as the LC_{50} was > 5.46 mg/L for both male and female rats.

Comments received during public consultation

One Member State Competent Authority (MSCA) agreed that based on the presented data, classification of asulam-sodium for acute toxicity is not warranted.

Assessment and comparison with the classification criteria

Oral

Taking into account that the oral LD_{50} value in male and female rats is above the threshold value for classification (2000 mg/kg bw), RAC agrees that asulam-sodium should **not be classified for acute oral toxicity** according to the CLP criteria.

Dermal

Taking into account that the dermal LD_{50} value in male and female rats and rabbits is above the threshold value for classification (2000 mg/kg bw), RAC agrees that asulam-sodium should **not be classified for acute dermal toxicity** according to the CLP criteria.

Inhalation

Taking into account that the inhalation LC_{50} value in male and female rats is above the threshold value for classification (5 mg/L air/4h), RAC considers that asulam-sodium should **not be classified for acute inhalation toxicity** according to the CLP criteria.

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

Acute toxicity studies of asulam sodium produced few signs of toxicity. In one of the available acute oral studies, signs of lethargy, reduced activity, ataxia and piloerection were observed at a dose of 5000 mg/kg bw and were considered to be indicative of general toxicity.

In a study of acute toxicity *via* the dermal route in the rabbit, red/pink discoloration of the lungs was observed in all animals at 2000 mg/kg bw and 3/5 males at 4000 mg/kg bw. This effect was not observed in any of the other available studies (including an acute inhalation study) and is not considered to clearly indicate a functional disturbance or morphological change which is of toxicological relevance to humans.

4.3.2 Comparison with criteria

Classification for specific organ toxicity-single exposure (STOT-SE) Category 1 or 2 is applicable to substances that have produced non-lethal toxicity in humans, or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant non-lethal toxicity in humans following a single exposure.

Classification for STOT-SE 3 is reserved for transient target organ effects and is limited to substances that have narcotic effects or cause respiratory tract irritation.

No significant toxicity of specific organs or tissues was observed after single exposure to asulam sodium via the oral, inhalation or dermal route. There was no evidence of narcotic effects or respiratory tract irritation.

4.3.3 Conclusions on classification and labelling

No classification – conclusive but not sufficient for classification

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

The DS did not propose classification of asulam-sodium for STOT SE based on the following observations.

Acute toxicity studies of asulam-sodium produced few signs of toxicity. In one of the available acute oral studies (Report No R001006, 1987), signs of lethargy, reduced activity, ataxia and piloerection were observed at a dose of 5000 mg/kg bw and were considered to be indicative of general toxicity.

In a study of acute toxicity via the dermal route in the rabbit (Report R00163, 1988), red/pink discoloration of the lungs was observed in all animals at 2000 mg/kg bw and 3/5 males at 4000 mg/kg bw. This effect was not observed in any of the other available studies (including an acute inhalation study) and was not considered by the DS to clearly indicate a functional disturbance or morphological change which is of toxicological relevance to humans.

Comments received during public consultation

One MSCA supported no classification of asulam-sodium for STOT SE.

Assessment and comparison with the classification criteria

There were no specific, non-lethal target organ toxicity arising during or after single oral, dermal and inhalation exposure to asulam-sodium. The observed effects were indicative of nonspecific, general acute toxicity, therefore RAC agrees with the DS that there is no clear evidence of specific effects on a target organ or tissue that were independent of mortalities, and no definitive signs of respiratory tract irritation or narcotic effects. Therefore RAC is of the opinion that **classification for specific target organ toxicity (single exposure) is not warranted**.

4.4 Irritation

4.4.1 Skin irritation

The potential for asulam sodium to cause dermal irritation was investigated in a guideline *in vivo* dermal irritation study, performed in the rabbit.

Table 11 Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Rabbit (NZ White) 3/sex 500 mg (4h) Asulam sodium (88% purity) OECD 404, GLP	Individual scores for each animal, calculated as mean of scores at 24, 48 and 72 hours were: Erythema: 0, 0, 0, 0, 1.0, 0 Oedema: 0, 0, 0, 0, 0, 0 All dermal reactions were reversed by 72 hours post application.	No classification	[17], 1987 (DAR B.6.2.4)

4.4.1.1 Non-human information

The potential for asulam sodium to cause skin irritation was investigated in a standard *in vivo* skin irritation study in the New Zealand white rabbit [17]. Signs of dermal irritation

were limited to a single animal (grade 1 erythema observed up to 48 hours post application). No other signs of dermal irritation were recorded. Average scores for each animal (calculated as the mean of scores at 24, 48 and 72 hours) for erythema were 0, 0, 0, 0, 1.0, 0; scores for oedema were 0, 0, 0, 0, 0, 0. All effects had reversed by 72 hours.

4.4.1.2 Human information

No data available.

4.4.1.3 Summary and discussion of skin irritation

Refer to Section 4.4.1.1.

4.4.1.4 Comparison with criteria

Asulam sodium was found to cause minimal skin irritation under the conditions of the available study [17]. Average scores for each animal (calculated as the mean of scores at 24, 48 and 72 hours) for erythema were 0, 0, 0, 0, 1.0, 0. Scores for oedema were 0, 0, 0, 0, 0, 0. In the case where studies of skin irritation are conducted using 6 animals, the criteria for classification for skin irritation - category 2 is a mean score of ≥2.3 for erythema/eschar or for oedema, in at least 4 of the 6 animals. A mean score of ≥2.3 for erythema/eschar or for oedema was not observed in any of the tested animals, therefore asulam sodium does not meet the criteria for classification for skin irritation.

4.4.1.5 Conclusions on classification and labelling

No classification – conclusive but not sufficient for classification

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for skin corrosion/irritation.

The skin irritation potential of asulam-sodium was assessed in a standard GLP-compliant skin irritation study (OECD TG 404) in three female and male New Zealand White (NZW) rabbits. Signs of dermal irritation were limited to a single animal (erythema grade 2 at 24 hours and grade 1 at up to 48 hours post application). No other signs of dermal irritation were recorded. Average scores for each animal (calculated as the mean of scores at 24, 48 and 72 hours) for erythema were 0, 0, 0, 0, 1.0, 0; scores for oedema were 0 for all animals. All effects had reversed by 72 hours (Report R001004, 1987).

Comments received during public consultation

One MSCA supported no classification of asulam-sodium for skin corrosion/irritation.

Assessment and comparison with the classification criteria

In the available study, the CLH criteria for skin irritation (a mean score of ≥ 2.3 for erythema/eschar or for oedema) were not met in any of the tested animals. RAC therefore considers that asulam-sodium **does not warrant classification for skin corrosion/irritation**.

4.4.2 Eye irritation

The potential for asulam sodium to cause eye irritation was investigated in a guideline *in vivo* eye irritation study, performed in the rabbit [18].

Table 12: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Rabbit (NZ) (6 washed, 6 unwashed) 100 mg Asulam sodium (88% purity) OECD 405, GLP	Individual scores for each animal, calculated as mean of scores at 24, 48 and 72 hours for the unwashed group were: Cornea: 0, 0, 0, 0, 0, 0 Iris: 0, 0.3, 0, 0, 0, 1.0 Conjunctival redness: 2, 1.7, 2, 1.0, 1.0, 1.7 Conjunctival chemosis: 0, 0.3, 0, 0, 0, 0.3 All ocular reactions were reversed by day 15.	No classification	[18], 1987 (DAR B.6.2.5)

4.4.2.1 Non-human information

The eye irritation potential of asulam sodium was tested in a standard *in vivo* eye irritation study in the rabbit. One hundred milligrams of the test item was instilled into one eye of 12 rabbits, 6 of which were washed 30 seconds following exposure. In the unwashed group (which is the relevant group for the purposes of classification) signs of eye irritation were observed in all animals. Iritis (grade 1) was observed in two animals at 1 hour, and a further two animals at 24 hours. Conjunctival redness (grade 1 or 2) was observed in all animals persisting beyond day 8 in four animals. Chemosis of the conjunctiva (grade 1) was recorded in four animals at 1 hour and two animals at 24 hours. No signs of corneal opacity were observed. All ocular reactions had resolved by day 15.

4.4.2.2 Human information

No data available.

4.4.2.3 Summary and discussion of eye irritation

Refer to Section 4.4.2.1

4.4.2.4 Comparison with criteria

Asulam sodium caused reversible eye irritation in unwashed eyes in an *in vivo* study in the rabbit. The criteria for classification in Category 1 (irreversible effects) were not met in any of the tested animals. Mean scores for specific ocular effects exceeding the criteria for classification in Category 2 (corneal opacity and/or iritis ≥1, conjunctival redness and/or oedema ≥2) were limited to conjunctival redness with a mean score of 2, in 2 of the 6 animals tested. All ocular effects were resolved by day 15 of the study. According to the guidance on the application of the CLP criteria, in the case where a study is conducted in 6 animals, effects exceeding the threshold for classification must be observed in at least 4 of 6 animals in order to classify the substance in Category 2. The observation of conjunctival redness with a mean score of 2, in 2 of the 6 animals, is not sufficient for classification.

4.4.2.5 Conclusions on classification and labelling

No classification– conclusive but not sufficient for classification

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS did not propose classification for eye effects based on the results of a reliable study.

The eye irritation potential of asulam-sodium was assessed in a standard GLP-compliant eye irritation study (OECD TG 405) in NZW rabbits.

0.1 g of the test item was instilled into one eye of 12 rabbits, 6 of which were washed 30 seconds following exposure. In the unwashed group (which is the relevant group for the purposes of classification), signs of eye irritation were observed in all animals. Iritis (grade 1) was observed in two animals at 1 hour, and a further two animals at 24 hours. Conjunctival redness (grade 1 or 2) was observed in all animals, persisting beyond day 8 in four animals. Chemosis of the conjunctiva (grade 1) was recorded in four animals at 1 hour and two animals at 24 hours. No signs of corneal opacity were observed. Individual scores for each animal, calculated as mean of scores at 24, 48 and 72 hours for the unwashed group were:

- cornea: 0, 0, 0, 0, 0, 0
- iris: 0, 0.3, 0, 0, 0, 1.0
- conjunctival redness: 2, 1.7, 2, 1.0, 1.0, 1.7
- conjunctival chemosis: 0, 0.3, 0, 0, 0, 0.3

All ocular reactions had been resolved by day 15 (Report R001002, 1987).

Comments received during public consultation

One MSCA supported the DS's proposal not to classify asulam-sodium for serious eye damage/eye irritation.

Assessment and comparison with the classification criteria

Asulam-sodium caused reversible eye irritation in unwashed eyes in an *in vivo* study in the rabbit.

The criteria for classification in Category 1 (irreversible effects within a 21-day observation period) were not met in any of the tested animals.

Mean scores for specific ocular effects exceeding the criteria for classification in Category 2 were limited to conjunctival redness with a mean score of 2, in 2 of the 6 animals tested. According to the guidance on the application of the CLP criteria, where a study is conducted in 6 animals, effects exceeding the threshold for classification must be observed in at least 4 out of 6 animals in order to classify the substance in Category 2. The observation of conjunctival redness with a mean score of 2 in only 2 of the 6 animals, is not sufficient for classification.

Therefore RAC agrees with the DS that **classification for eye damage/irritation is not warranted**.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

Specific studies investigating respiratory tract irritation are not available. No signs of respiratory tract irritation were observed in the acute inhalation toxicity study performed in the rat (refer to Section 4.2). There is no indication from the available data that classification for respiratory tract irritation is required.

4.4.3.2 Human information

No data available.

4.4.3.3 Summary and discussion of respiratory tract irritation

Refer to Section 4.4.3.1

4.4.3.4 Comparison with criteria

No signs of respiratory tract irritation (as set out in Annex 1: 3.8.2.2.1 of the Guidance on the Application of the CLP Criteria) were observed in the acute inhalation study (refer to Section 4.2).

4.4.3.5 Conclusions on classification and labelling

Not classified- data lacking

4.5 Corrosivity

4.5.1 Non-human information

In the available skin irritation study performed with asulam sodium, no observations of full thickness or irreversible skin damage were observed (refer to section 4.4.1, [**Error! ookmark not defined.**]). In the eye irritation study, asulam sodium did not produce corrosive effects (refer to Section 4.4.2, [17]).

4.5.2 Human information

No data available.

4.5.3 Summary and discussion of corrosivity

Refer to Section 4.5.1.

4.5.4 Comparison with criteria

Asulam sodium did not produce corrosive effects in studies of skin or eye irritation (refer to Sections 4.4.1 and 4.4.2, respectively). The pH of asulam sodium is not ≤ 2 or ≥ 11.5. Asulam sodium does not meet the necessary criteria for classification for corrosivity.

4.5.5 Conclusions on classification and labelling

No classification- conclusive but not sufficient for classification

4.6 Sensitisation

4.6.1 Skin sensitisation

The potential for asulam sodium to induce skin sensitisation was examined in a guideline guinea pig maximisation test.

Table 13: Summary table of relevant skin sensitisation studies

Species/Method	Doses	No. sensitised/total no.	Result	Reference
Guinea pig (Dunkin-Hartley) 10/sex/group Asulam sodium (88% purity) OECD 406 Maximisation test, GLP	Induction: Intradermal: 5% in distilled water Topical: 50% in distilled water Challenge: 10% or 50% in distilled water	Test: 12/20 at 24 hours, 9/20 at 48 hours Negative control (distilled water) : 0/20 No positive control used	Positive	[19], 1987 (DAR B.6.2.6)

4.6.1.1 Non-human information

The potential for asulam sodium to induce skin sensitisation was investigated in a guideline maximisation test in the guinea pig. Intradermal induction was performed at a concentration of 5% asulam sodium in distilled water. Challenge was performed at concentrations of 10

or 50% in distilled water. Dermal reactions were graded at 24 and 48 hours following challenge. Dermal reaction to the test item (grade 1 or 2 erythema) was observed in 12/20 and 9/20 of the test animals at 24 and 48 hours respectively in the 50% challenge group. No dermal reactions were observed in the control group.

4.6.1.2 Human information

No data available.

4.6.1.3 Summary and discussion of skin sensitisation

Refer to Section 4.6.1.1.

4.6.1.4 Comparison with criteria

In a guinea pig maximisation test, 12/20 (60%) test animals exhibited a dermal response following topical challenge with the test item (50% in distilled water). The intradermal induction dose in this study was 5% in distilled water. From the available data, asulam sodium can be concluded to be a low potency skin sensitiser and meets the criteria for classification for skin sensitisation, although there is insufficient data for sub-categorisation. Asulam sodium should be classified in Category 1 for skin sensitisation; H317.

4.6.1.5 Conclusions on classification and labelling

Skin sensitisation category 1; H317 May cause an allergic skin reaction.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The potential of asulam-sodium to cause skin sensitisation was investigated in a GLP-compliant Magnusson and Kligman Guinea Pig Maximisation test (GPMT; Report R001032, 1987), conducted according to OECD TG 406. Concentrations used for induction and challenge exposures were based on the results of a preliminary study. Intradermal induction was performed at a concentration of 5% asulam-sodium in distilled water. Challenge was performed at concentrations of 10% or 50% in distilled water. Dermal reactions were graded at 24 and 48 hours following challenge.

In the group challenged with 50% in distilled water a dermal reaction to the test item (grade 1 or 2 erythema) was observed in 12/20 (60%) and 9/20 (45%) of the test animals at 24 and 48 hours respectively.

In the test group challenged with 10% water solution of asulam-sodium, barely perceptible erythema was seen in 4/20 (20%) animals and grade 1 erythema was seen in 1/20 (5%) of the test animals at 24 hours, while at 48 hours barely perceptible erythema was seen in 4/20 test animals

No dermal reactions were observed in the control group.

The lack of a positive control group or reference to a separate positive control study (reliability check) was not considered by the DS to raise concerns, in view of the clear positive result in the 50% challenge group of this study.

According to the DS, it can be concluded that asulam-sodium is a low potency skin sensitiser and meets the criteria for classification for skin sensitisation, although there was insufficient data for sub-categorisation. The DS therefore proposed that it should be classified as Skin Sens. 1; H317.

Comments received during public consultation

Three MSCAs supported the DS proposal to classify of asulam-sodium as Skin Sens. 1; H317.

Assessment and comparison with the classification criteria

RAC agrees that asulam-sodium meets the classification criteria for Skin Sens. 1; H317, because 60% of animals were found sensitised after intradermal induction at a concentration of 5% asulam-sodium in distilled water in the GPMT.

For classification as Skin Sens 1A, the substance should sensitise at least 30% of the guinea pigs at intradermal induction concentrations ≤ 0.1% or should sensitise at least 60% of guinea pigs at intradermal induction concentrations in the range > 0.1% to ≤ 1%, which is a concentration 5 times lower than used in the actual test considered here. However, there are no data to exclude this possibility.

In the current Guidance on the Application of CLP Criteria (point 3.4.2.2.2) it is noted that classification into sub-categories is only recommended allowed if data are sufficient.

Since for Asulam-sodium such data for lower concentrations are absent, category 1A cannot be excluded, therefore classification as **Skin Sens. 1 (H317) without sub-categorisation is warranted**.

Specific concentration limit

The setting of an SCL is based on the potency of he substance, according to the Guidance on the Application of CLP Criteria (Version 4.1 – June 2015); itapplies for the most potent skin sensitisers classified in 1A.

Since the incidence of sensitised guinea pigs in the GPMT is ≥ 30% and the concentration used for intradermal induction > 1.0%, asulam-sodium is according to table 3.4.2-g in the CLP Guidance, a moderate potency skin sensitiser. Therefore, the generic concentration limit of 1% should be applied for asulam-sodium (according to table 3.4.2-i of the CLP Guidance).

4.6.2 Respiratory sensitisation

4.6.2.1 Non-human information

No data are available.

4.6.2.2 Human information

No data are available.

4.6.2.3 Summary and discussion of respiratory sensitisation

No data are available.

4.6.2.4 Comparison with criteria

No data are available.

4.6.2.5 Conclusions on classification and labelling

No classification- data lacking

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The potential of asulam-sodium to cause respiratory sensitisation was not investigated directly. However, no signs of respiratory tract irritation were observed in the acute inhalation toxicity study performed in the rat (Report R001002, 1987). As there was no indication from the available data that classification for respiratory sensitisation is warranted, it was not proposed by the DS.

Comments received during public consultation

One MSCA commented on absence of data on respiratory sensitisation.

Assessment and comparison with the classification criteria

In the opinion of RAC the available data from the acute inhalation toxicity study indicate that asulam-sodium does not cause respiratory sensitisation, hence RAC agrees with the DS that asulam-sodium **should not be classified**.

4.7 Repeated dose toxicity

Repeated dose toxicity studies *via* the oral route have been conducted in the rat and mouse (90 days and 8 weeks respectively) and in the dog (6 and 12 months). Chronic toxicity and carcinogenicity studies were performed in the rat and the mouse; non-neoplastic findings in these studies are discussed in this section, and neoplastic findings are discussed in Section 4.10. A 21-day repeated dose study *via* the dermal route in the rabbit is also available.

Table 14: Summary table of relevant repeated dose toxicity studies

Method	Dose Levels	Observations and Remarks[†‡]
90-day oral (dietary) Rat (Wistar) 10/sex/group Asulam sodium (89.6% purity) OECD 408, GLP Reference: [20], 2000 (DAR B.6.3.1) *Guidance value for classification:* *≤100 mg/kg bw/d*	0, 2000, 6000, 20000 ppm (♂/♀:0/0, 128.5/157.9, 387.0/479.4, 1327.3/1651.5 mg/kg bw/d –))	All the doses tested were above the guidance value for classification of 100 mg/kg bw/d. No adverse effects were observed at the dose level of 129 or 158 mg/kg bw/d for males and females respectively. **Effects at Doses ≥ guidance value for classification:** **20000 ppm - 1327.3 mg/kg bw/d (♂), 1651.5 mg/kg bw/d (♀):** **Observations:** ↓ BW gain: 12 % (♂) **Clinical Chemistry:** ↓ Total plasma protein : 9% (♂) ↑ Albumin/globulin ratio: 1.27 (♂), 1.43 (♀). *Control – 1.05 (♂), 1.19(♀)* ↓ RBC : 9% (♂), 8% (♀) ↓ HGB : 7% (♂), 8% (♀) ↑ PTT : 17% (♂) **Organ weights:** ↑ Spleen weight: 15 % abs, 16 % rel (♂) ↑ Thyroid weight: 18 % abs, 22 % rel (♀) **Histopathology:** Thyroid hypertrophy: 10/10 (♂), 8/10 (♀). *Control - 2/10 (♂), 1/10 (♀)* ↑ Severity of splenic haematopoiesis (♂) ↑ Severity of splenic haemosiderin deposition (♂ & ♀) Kidney mineralisation : 7/10 (♂), 9/10 (♀). *Control – 1/10 (♂), 5/10 (♀)* Focal urothelial hyperplasia: 6/10 (♂), 8/10 (♀). *Control - 2/10 (♂), 5/10 (♀)* Hydronephrosis: 7/10 (♀). *Control - 2/10* **6000 ppm - 387.0 mg/kg bw/d (♂), 479.4 mg/kg bw/d (♀):** **Observations:** No clinical signs of toxicity **Clinical Chemistry:** ↑ Albumin/globulin ratio: 1.19 (♂), 1.37 (♀). *Control – 1.05 (♂), 1.19(♀)* **Organ weights:** No significant changes in organ weight **Histopathology:** ↑ Severity of splenic haematopoiesis (♂) ↑ Severity of splenic haemosiderin deposition (♂ & ♀) Kidney mineralisation: 2/10 (♂), 7/10 (♀). *Control - 1/10 (♂), 5/10 (♀)* Focal urothelial hyperplasia: 5/10 (♂). *Control - 2/10* Hydronephrosis: 6/10 (♀). *Control - 2/10* NOAEL: 129 mg/kg bw/day (♂) and 158 mg/kg bw/day (♀).

Method	Dose Levels	Observations and Remarks[†‡]
Chronic toxicity / carcinogenicity (dietary) Rat (CD) 50/sex/dose Asulam sodium (Purity not reported) No guideline stated, but similar to OECD 453. Pre-GLP Reference: [21], 1981 (DAR B.6.5.1) *A guidance value for classification of ≤12 mg/kg bw/d can be calculated by application of Haber's rule*	0, 1000, 5000, 25000 ppm (♂/♀: 0/0, 36/47, 180/243, 953/1280 mg/kg bw/d)	All the doses tested were above the adjusted guidance value for classification of 12 mg/kg bw/d in a two-year rat study. No adverse effects were observed at the dose level of 36 and 47 mg/kg bw/d for males and females respectively. **<u>Effects at Doses ≥ guidance value for classification:</u>** **<u>25000 ppm - 953 mg/kg bw/d (♂), 1280 mg/kg bw/d (♀):</u>** **Observations:** ↓ BWG : 12% (♂), 15% (♀) **Clinical Chemistry:** Treatment related changes to red blood cell parameters; changes consistent with mild macrocytic anaemia in both sexes, predominantly in year 1. **Organ weights:** Enlarged thyroid (♂ & ♀) **Histopathology:** Thyroid hyperplasia in 11/50 (♂) and 3/50 (♀). *Control – zero incidence in both sexes* **<u>5000 ppm - 180 mg/kg bw/d (♂), 243 mg/kg bw/d (♀):</u>** **Observations:** ↓ BWG : 10% (♀) **Clinical Chemistry:** Treatment related changes to red blood cell parameters; changes consistent with mild macrocytic anaemia in both sexes, predominantly in year 1. **Histopathology:** Thyroid hyperplasia in 4/50 (♂). *Control – zero incidence.* NOAEL for non-neoplastic effects: 1000 ppm (36 and 47 mg/kg bw/d for males and females respectively)
8-week oral (dietary) range finding study Mouse (CD-1) 10/sex/group Asulam sodium (88% purity) Non guideline, GLP Reference: [22], 1988 (DAR B.6.3.2)	0, 3000, 10000, 30000, 50000 ppm (♂/♀: 0/0, 512/675, 1673/2263, 5103/6835, 9022/10828 mg/kg bw/d)	All the doses tested were above the adjusted guidance value for classification of 160 mg/kg bw/d in a 8 week mouse study. No adverse effects were observed at the dose level of 1673 and 6835 mg/kg bw/d for males and females respectively. NOAEL: 1673 mg/kg bw/day (♂) and 6835 mg/kg bw/day (♀).

Chronic toxicity / carcinogenicity (dietary) Mouse (CD-1) 75/sex/dose Asulam sodium (88% purity) EPA 83-2, GLP Reference: [23], 1992 (DAR B.6.5.2) *A guidance value for classification of ≤12 mg/kg bw/d can be calculated by application of Haber's rule*	0, 500, 5000, 50000 ppm (♂/♀: 0/0, 74/95, 730/938, 8040/10353 mg/kg bw/d)	All the doses tested were above the adjusted guidance value for classification of 12 mg/kg bw/d in a two-year mouse study. No adverse effects were observed at the dose level of 74 and 95 mg/kg bw/day for males and females respectively. **<u>Effects at Doses ≥ guidance value for classification:</u>** **<u>50000ppm - 8040 mg/kg bw/d (♂), 10353 mg/kg bw/d (♀):</u>** **Clinical Chemistry:** Treatment related changes to red blood cell parameters **Organ weights:** ↑ Spleen weight: 85% abs, 92 % rel (♂) and 114 % abs, 117 % rel (♀) *(female values calculated at 12 months)* **Histopathology:** Histopathological effects consistent with effects on RBC. **<u>5000ppm - 730 mg/kg bw/d (♂), 938 mg/kg bw/d (♀):</u>** **Clinical Chemistry:** Treatment related changes to red blood cell parameters **Organ weights:** ↑ Spleen weight: 69% abs, 78 % rel in (♂) and 18% rel (♀) *(♀values calculated at 12 months)* **Histopathology:** Histopathological effects consistent with effects on RBC. NOAEL for non-neoplastic effects: 500 ppm (74 and 95 mg/kg bw/d for males and females respectively)
6-month oral (gavage) Dog (beagle) 6/sex/dose Asulam sodium(98% purity) Non guideline, Non-GLP Reference: [24], 1980 (DAR B.6.3.3)	0, 60, 300, 1500 mg/kg bw/d	**<u>1500 mg/kg bw/d:</u>** **Observations:** 1 ♂ and 1 ♀ death. Vomiting (♂ & ♀). ↓ BW : 11% (♂) ↓ BWG : 23% (♂), 11% (♀) **Clinical Chemistry:** ↓ RBC : 9% (♂) ↓ HGB : 10% (♂) **Organ weights:** ↑ Kidney weight: 21 % rel (♂) ↓ Lung weight: 23 % abs, 13 % rel (♂) ↑ Thyroid weight: 130 % abs, 165 % rel (♂) and 144 % abs, 158 % rel (♀) ↓ Testes weight: 33% abs, 15 % rel **Histopathology:** No treatment related abnormalities. **<u>300 mg/kg bw/day:</u>** **Observations:** Vomiting (♀) **Organ weights:** ↑ Thyroid weight: 55 % abs, 54 % rel (♀)

		Histopathology: No treatment related abnormalities. NOAEL: 60 mg/kg bw/d
52-week oral (gavage) Dog (beagle) 5/sex/dose Asulam sodium (82.2% purity) OECD 409, GLP Reference: [25], 2004 (DAR B.6.3.3)	0, 100, 300 or 600 mg/kg bw/d	**<u>600 mg/kg bw/d:</u>** **Observations:** ↑ Salivation and vomiting **Organ weights:** ↑ Adrenal weight: 27% (♀) ↑ Thyroid weight: 67 % abs, 64 % rel (♂) and 72 % abs, 70 % rel (♀) **Histopathology:** Thyroid hypertrophy in all animals. *Control – zero incidence in both sexes* **<u>300 mg/kg bw/d:</u>** **Observations:** Vomiting (♀) **Organ weights:** ↑ Thyroid weight: 48% abs, 50 % rel (♂) and 33 % abs, 43 % rel (♀) **Histopathology:** Thyroid hypertrophy: 2/5 (♂) and 3/5(♀). *Control – zero incidence in both sexes* NOAEL: 100 mg/kg bw/d

†Reductions and increases in parameters are expressed by the use of ↓ and ↑ (respectively)
‡Values are expressed as percentage of controls and calculated from mean values at the end of the study period unless otherwise stated.

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

<u>Rat</u>

The repeated-dose toxicity of asulam sodium in rats has been investigated in a 90-day [20] and a combined chronic toxicity / carcinogenicity study [21]. All the tested doses were above the guidance values for classification, adjusted as necessary for study duration. No adverse effects were observed in either study at the lowest doses tested (512 / 675 mg/kg bw/day in the 90-day study, 36 / 47 mg/kg bw/day in the chronic study, males and females respectively). Adverse effects at doses higher than these included reductions in body weight and body weight gain, changes in red blood cell parameters and clinical biochemistry, and histopathological changes in the spleen, thyroid and kidney.

<u>Mouse</u>

The repeated-dose toxicity of asulam sodium in mice has been investigated in an 8-week dietary range-finding study [22] and a combined chronic toxicity / carcinogenicity study [23]. All the tested doses were above the guidance values for classification, adjusted as necessary for study duration. In the range-finding study, the observed effects were limited to minor changes in bodyweight and food consumption at the highest-tested dose of approximately 10000 mg/kg bw/day. In the chronic study, adverse effects at doses ≥730 mg/kg/d were observed on red blood cell parameters, spleen, liver and kidney.

Dog

A six-month [24] and a one-year [25] repeated-dose toxicity study in dogs are available. All the tested doses exceeded the guidance values for classification, adjusted for study duration. No adverse effects were reported at the lowest dose in each study (60 mg/kg bw/day and 100 mg/kg bw/day, respectively). At doses ≥ 300 mg/kg bw/day, there were indications of general toxicity and adverse haematological, kidney and thyroid effects.

4.7.1.2 Repeated dose toxicity: inhalation

No data available.

4.7.1.3 Repeated dose toxicity: dermal

A 21-day dermal toxicity study was conducted in the rabbit.

Table 15: Summary table of relevant repeated dose toxicity studies

Method	Dose Levels	Observations and Remarks[†‡]
21-day dermal Rabbit (NZW) 10/sex/dose Asulam sodium (88% purity) US FIFRA 82-2, GLP Reference: [26], 1989 (DAR B.6.3.4)	0, 1000 mg/kg bw/d	No adverse effects were observed at the dose level of 1000 mg/kg bw/d. NOAEL: 1000 mg/kg bw/d (maximum recommended tested dose in accordance with current regulatory guidelines for sub-acute studies)

In a 21-day dermal toxicity study conducted in the rabbit, a single dose of 1000 mg/kg bw/day was tested. No adverse effects were observed.

4.7.1.4 Repeated dose toxicity: other routes

No data available.

4.7.1.5 Human information

No data available.

4.7.1.6 Other relevant information

None

4.7.1.7 Summary and discussion of repeated dose toxicity

Repeat dose toxicity of asulam *via* the oral route was investigated in short term studies performed in the rat, mouse and dog. Repeat dose data is also available from combined chronic toxicity / carcinogenicity studies in the rat and mouse. A 21-day repeat dose toxicity study *via* the dermal route was conducted in the rabbit.

Specific effects which were frequently observed in repeat dose studies *via* the oral route were predominantly haematological changes and effects on red blood cell parameters, increased thyroid weight and altered thyroid histopathology. Biochemistry and histopathology findings indicative of damage to red blood cells were also observed across multiple studies. Other specific effects observed in repeat dose studies *via* the oral route with less consistency included effects on the spleen, thymus, adrenals, kidneys, lung, testes, and bile ducts.

No adverse effects were observed in a repeat dose study *via* the dermal route in the rabbit.

All the dose levels in the available repeat dose toxicity studies are in excess of levels which are relevant for classification for STOT RE. In all available studies, no adverse effects were observed at the lowest tested dose.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

Refer to Section 4.8.2.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

STOT RE is assigned on the basis of a substance demonstrating evidence of significant or severe toxicity, generally at or below the oral guidance value of 100 mg/kg bw/day (for a classification in Category 2) obtained in a 90-day rat study. The equivalent guidance values for a one-year and a two-year study are ≤25 mg/kg bw/day and ≤12.5 mg/kg bw/day, respectively. The dermal guidance value for a classification in category 2 is ≤200 mg/kg bw/day obtained in a 90-day rat or rabbit study.

Studies to investigate the repeated-dose toxicity of asulam sodium were conducted in the rat, mouse and dog *via* the oral route, and in the rabbit *via* the dermal route. In all of the available studies, the lowest dose tested was in excess of the guidance value for classification for STOT RE (adjusted as necessary for study duration). In all cases, there were no adverse effects observed at these doses; consequently, asulam sodium does not meet the criteria for classification for STOT RE.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

No classification- conclusive but not sufficient for classification

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS considered the available animal data to be conclusive, andindicated no classification for STOT RE.

Oral

Repeated dose toxicity studies via the oral route have been conducted in the rat and mouse (90 days and 8 weeks respectively) and in the dog (6 and 12 months).

Rat

The repeated-dose toxicity of asulam-sodium in rats has been investigated in a 90-day (Report R007958, 2000) and a combined chronic toxicity / carcinogenicity study (Report R001275J, 1981). All the tested doses were above the guidance values for classification, adjusted as necessary for study duration. No adverse effects were observed in either study at the lowest doses tested (129 / 158 mg/kg bw/d in the 90-day study, 36 / 47 mg/kg bw/d in the chronic study, males and females respectively). Adverse effects at doses higher than these included reductions in body weight and body weight gain, changes in red blood cell parameters and clinical biochemistry, and histopathological changes in the spleen, thyroid and kidney.

Mouse

The repeated-dose toxicity of asulam-sodium in mice has been investigated in an 8-week dietary range-finding study (Report R001721, 1989) and a combined chronic toxicity / carcinogenicity study (Report R003662, 1992). All the tested doses were above the guidance values for classification, adjusted as necessary for study duration. In the range-finding study, the observed effects were limited to minor changes in bodyweight and food consumption at the highest-tested dose of approximately 10000 mg/kg bw/d. In the chronic study, adverse effects at doses ≥ 730 mg/kg/d were observed on red blood cell parameters, spleen, liver and kidney.

Dog

A six-month (Report R001265, 1980) and a one-year (Report C032927, 2004) repeated-dose toxicity study in dogs were available. All the tested doses exceeded the guidance values for classification, adjusted for study duration. No adverse effects were reported at the lowest dose in each study (60 mg/kg bw/d and 100 mg/kg bw/d, respectively). At doses ≥ 300 mg/kg bw/d, there were indications of general toxicity and adverse haematological, kidney and thyroid effects.

Inhalation

No data available.

Dermal

In a 21-day dermal toxicity study conducted in the rabbit, a single dose of 1000 mg/kg bw/d was tested. No adverse effects were observed.

Comments received during public consultation

One MSCA commented that there was not sufficient evidence for classification of asulam-sodium for STOT RE.

Another MSCA was of the opinion that taking into account some effects on the red blood cells, which possibly indicated anaemia in tested animals, repeated dose toxic effects cannot be excluded.

Teh DS responded that these findings only occurred at dose levels in excess of the guidance values for classification. Therefore, whilst an effect following repeated dosing had been noted, the criteria for classification with STOT-RE were not met.

Assessment and comparison with the classification criteria

Repeat dose toxicity of asulam-sodium via the oral route was investigated in short term studies performed in the rat, mouse and dog. Repeat dose toxicity data was also available from combined chronic toxicity / carcinogenicity studies in the rat and mouse. A 21-day repeat dose toxicity study via the dermal route was conducted in the rabbit.

Summary table of relevant repeated dose toxicity studies

Method	Dose Levels	Observations and Remarks[†‡]
90-day oral (dietary) Rat (Wistar) 10/sex/group Asulam-sodium (89.6% purity) OECD TG 408, GLP Reference: Report R007958, 2000 (DAR B.6.3.1) *Guidance value for classification: ≤ 100 mg/kg bw/d*	0, 2000, 6000, 20000 ppm (♂/♀:0/0, 128.5/157.9, 387.0/479.4, 1327.3/1651.5 mg/kg bw/d)	All the doses tested were above the guidance value for classification of 100 mg/kg bw/d. No adverse effects were observed at the dose level of 129 or 158 mg/kg bw/d for males and females respectively. **<u>Effects at Doses ≥ guidance value for classification:</u>** **<u>20000 ppm - 1327.3 mg/kg bw/d (♂), 1651.5 mg/kg bw/d (♀):</u>** **Observations:** ↓ BW gain: 12% (♂) **Clinical Chemistry:** ↓ Total plasma protein: 9% (♂) ↑ Albumin/globulin ratio: 1.27 (♂), 1.43 (♀). *Control – 1.05 (♂), 1.19(♀)* ↓ RBC: 9% (♂), 8% (♀) ↓ HGB: 7% (♂), 8% (♀) ↑ PTT: 17% (♂) **Organ weights:** ↑ Spleen weight: 15% abs, 16% rel (♂) ↑ Thyroid weight: 18% abs, 22% rel (♀) **Histopathology:** Thyroid hypertrophy: 10/10 (♂), 8/10 (♀). *Control - 2/10 (♂), 1/10 (♀)* ↑ Severity of splenic haematopoiesis (♂) ↑ Severity of splenic haemosiderin deposition (♂ & ♀) Kidney mineralisation: 7/10 (♂), 9/10 (♀). *Control – 1/10 (♂), 5/10 (♀)*

		Focal urothelial hyperplasia: 6/10 (♂), 8/10 (♀). *Control - 2/10 (♂), 5/10 (♀)* Hydronephrosis: 7/10 (♀). *Control - 2/10* **<u>6000 ppm - 387.0 mg/kg bw/d (♂), 479.4 mg/kg bw/d (♀):</u>** **Observations:** No clinical signs of toxicity **Clinical Chemistry:** ↑ Albumin/globulin ratio: 1.19 (♂), 1.37 (♀). *Control – 1.05 (♂), 1.19(♀)* **Organ weights:** No significant changes in organ weight **Histopathology:** ↑ Severity of splenic haematopoiesis (♂) ↑ Severity of splenic haemosiderin deposition (♂ & ♀) Kidney mineralisation: 2/10 (♂), 7/10 (♀). *Control - 1/10 (♂), 5/10 (♀)* Focal urothelial hyperplasia: 5/10 (♂). *Control - 2/10* Hydronephrosis: 6/10 (♀). *Control - 2/10* NOAEL: 129 mg/kg bw/d (♂) and 158 mg/kg bw/d (♀).
Chronic toxicity / carcinogenicity (dietary) Rat (CD) 50/sex/dose Asulam-sodium (Purity not reported) No guideline stated, but similar to OECD TG 453. Pre-GLP Reference: Report R001275J, 1981 (DAR B.6.5.1) *A guidance value for classification of ≤ 12 mg/kg bw/d can be calculated by application of Haber's rule*	0, 1000, 5000, 25000 ppm (♂/♀: 0/0, 36/47, 180/243, 953/1280 mg/kg bw/d)	All the doses tested were above the adjusted guidance value for classification of 12 mg/kg bw/d in a two-year rat study. No adverse effects were observed at the dose level of 36 and 47 mg/kg bw/d for males and females respectively. **<u>Effects at Doses ≥ guidance value for classification:</u>** **<u>25000 ppm - 953 mg/kg bw/d (♂), 1280 mg/kg bw/d (♀):</u>** **Observations:** ↓ BWG: 12% (♂), 15% (♀) **Clinical Chemistry:** Treatment related changes to red blood cell parameters; changes consistent with mild macrocytic anaemia in both sexes, predominantly in year 1. **Organ weights:** Enlarged thyroid (♂ & ♀) **Histopathology:** Thyroid hyperplasia in 11/50 (♂) and 3/50 (♀). *Control – zero incidence in both sexes* **<u>5000 ppm - 180 mg/kg bw/d (♂), 243 mg/kg bw/d (♀):</u>** **Observations:** ↓ BWG: 10% (♀) **Clinical Chemistry:** Treatment related changes to red blood cell parameters; changes consistent with mild macrocytic anaemia in both sexes, predominantly in year 1. **Histopathology:** Thyroid hyperplasia in 4/50 (♂). *Control – zero incidence.* NOAEL for non-neoplastic effects: 1000 ppm (36 and 47 mg/kg bw/d for males and females respectively)

8-week oral (dietary) range finding study Mouse (CD-1) 10/sex/group Asulam-sodium (88% purity) Non guideline, GLP Reference: Report R001721, 1989 (DAR B.6.3.2)	0, 3000, 10000, 30000, 50000 ppm (♂/♀: 0/0, 512/675, 1673/2263, 5103/6835, 9022/10828 mg/kg bw/d)	All the doses tested were above the adjusted guidance value for classification of 160 mg/kg bw/d in a 8 week mouse study. No adverse effects were observed at the dose level of 1673 and 6835 mg/kg bw/d for males and females respectively. NOAEL: 1673 mg/kg bw/d (♂) and 6835 mg/kg bw/d (♀).
Chronic toxicity / carcinogenicity (dietary) Mouse (CD-1) 75/sex/dose Asulam-sodium (88% purity) EPA 83-2, GLP Reference: Report R001721, 1992 (DAR B.6.5.2) *A guidance value for classification of ≤12 mg/kg bw/d can be calculated by application of Haber's rule*	0, 500, 5000, 50000 ppm (♂/♀: 0/0, 74/95, 730/938, 8040/10353 mg/kg bw/d)	All the doses tested were above the adjusted guidance value for classification of 12 mg/kg bw/d in a two-year mouse study. No adverse effects were observed at the dose level of 74 and 95 mg/kg bw/d for males and females respectively. **<u>Effects at Doses ≥ guidance value for classification:</u>** **<u>50000 ppm - 8040 mg/kg bw/d (♂), 10353 mg/kg bw/d (♀):</u>** **Clinical Chemistry:** Treatment related changes to red blood cell parameters **Organ weights:** ↑ Spleen weight: 85% abs, 92% rel (♂) and 114% abs, 117% rel (♀) *(female values calculated at 12 months)* **Histopathology:** Histopathological effects consistent with effects on RBC. **<u>5000 ppm - 730 mg/kg bw/d (♂), 938 mg/kg bw/d (♀):</u>** **Clinical Chemistry:** Treatment related changes to red blood cell parameters **Organ weights:** ↑ Spleen weight: 69% abs, 78% rel in (♂) and 18% rel (♀) *(♀values calculated at 12 months)* **Histopathology:** Histopathological effects consistent with effects on RBC. NOAEL for non-neoplastic effects: 500 ppm (74 and 95 mg/kg bw/d for males and females respectively)
6-month oral (gavage) Dog (beagle) 6/sex/dose Asulam-sodium(98% purity) Non guideline, Non-GLP Reference: Report R001265, 1980 (DAR B.6.3.3)	0, 60, 300, 1500 mg/kg bw/d	**<u>1500 mg/kg bw/d:</u>** **Observations:** 1 ♂ and 1 ♀ death. Vomiting (♂ & ♀). ↓ BW: 11% (♂) ↓ BWG: 23% (♂), 11% (♀) **Clinical Chemistry:** ↓ RBC: 9% (♂) ↓ HGB: 10% (♂) **Organ weights:** ↑ Kidney weight: 21% rel (♂) ↓ Lung weight: 23% abs, 13% rel (♂) ↑ Thyroid weight: 130% abs, 165% rel (♂) and 144% abs, 158% rel (♀) ↓ Testes weight: 33% abs, 15% rel

		Histopathology: No treatment related abnormalities. **<u>300 mg/kg bw/day:</u>** **Observations:** Vomiting (♀) **Organ weights:** ↑ Thyroid weight: 55% abs, 54% rel (♀) **Histopathology:** No treatment related abnormalities. NOAEL: 60 mg/kg bw/d
52-week oral (gavage) Dog (beagle) 5/sex/dose Asulam-sodium (82.2% purity) OECD TG 409, GLP Reference: Report C032927, 2004 (DAR B.6.3.3)	0, 100, 300 or 600 mg/kg bw/d	**<u>600 mg/kg bw/d:</u>** **Observations:** ↑ Salivation and vomiting **Organ weights:** ↑ Adrenal weight: 27% (♀) ↑ Thyroid weight: 67% abs, 64% rel (♂) and 72% abs, 70% rel (♀) **Histopathology:** Thyroid hypertrophy in all animals. *Control – zero incidence in both sexes* **<u>300 mg/kg bw/d:</u>** **Observations:** Vomiting (♀) **Organ weights:** ↑ Thyroid weight: 48% abs, 50% rel (♂) and 33% abs, 43% rel (♀) **Histopathology:** Thyroid hypertrophy: 2/5 (♂) and 3/5(♀). *Control – zero incidence in both sexes* NOAEL: 100 mg/kg bw/d

† Reductions and increases in parameters are expressed by the use of ↓ and ↑ (respectively)
‡ Values are expressed as percentage of controls and calculated from mean values at the end of the study period unless otherwise stated

Specific effects which were frequently observed in repeated dose studies via the oral route were predominantly haematological changes and effects on red blood cell parameters, increased thyroid weight and altered thyroid histopathology. Biochemistry and histopathology findings indicative of damage to red blood cells were also observed across multiple studies. Other specific effects observed in repeat dose studies via the oral route with less consistency included effects on the spleen, thymus, adrenals, kidneys, lung, testes, and bile ducts.

No adverse effects were observed in a repeat dose study via the dermal route in the rabbit.

All the dose levels in the available repeated dose toxicity studies where adverse effects were observed are in excess of levels which are relevant for classification for STOT RE. In all available studies, no adverse effects were observed at the lowest tested dose.

STOT RE is assigned on the basis of a substance demonstrating evidence of significant or severe toxicity, generally at or below the oral guidance value of 100 mg/kg bw/d (for a classification in Category 2) obtained in a 90-day rat study. The equivalent guidance values for a one-year and a two-year study are ≤ 25 mg/kg bw/d and ≤ 12.5 mg/kg bw/d,

respectively. The dermal guidance value for a classification in category 2 is ≤ 200 mg/kg bw/d obtained in a 90-day rat or rabbit study.

Studies to investigate the repeated-dose toxicity of asulam-sodium were conducted in the rat, mouse and dog via the oral route, and in the rabbit via the dermal route. In all of the available studies, the lowest dose tested was higher than the guidance value for classification for STOT RE (adjusted as necessary for study duration). In all cases, there were no adverse effects observed at these doses; consequently, asulam-sodium does not meet the criteria for classification for STOT RE.

RAC agrees with the DS that **no classification for specific target organ toxicity – repeated exposure (STOT RE) is warranted**.

4.9 Germ cell mutagenicity (Mutagenicity)

To investigate the mutagenicity of asulam sodium, six *in vitro* studies with bacteria, mouse and human cells have been presented and one *in vivo* micronucleus study in mice has been evaluated.

Table 16: Summary table of relevant *in vitro* mutagenicity studies

Method	Organism/strain	Concentrations tested	Result
Bacterial reverse mutation assay Asulam sodium (purity 90.2%) Asulam- purity 82.3%Doses based on Asulam purity +/-S9 OECD 471 (plate incorporation), GLP Reference: [27], 2008 (DAR B.6.4.1 A)	*S. Typhimurium* TA98, TA100, TA1535, TA1537 *E. Coli* WP2 *uvr*A	0, 62, 185, 556, 1667 and 5000 µg/plate	Cytotoxicity was reported at ≥1667 µg/plate Vehicle and positive controls valid Asulam sodium was negative +/-S9.
Bacterial reverse mutation assay Asulam sodium (purity 44.2% aqueous concentrate) +/- S9 (5 or 10%) OECD 471 (plate incorporation), GLP Reference: [28], 2013 (DAR B.6.4.1 B)	*S. Typhimurium*- TA98, TA100, TA102, TA1535, TA1537	Preliminary study- 0.25-5000 µg/plate Main study- 0, 6.89, 20.58, 61.73, 185.19, 555.56 and 1666.67 µg/plate	Cytotoxicity was reported at 1667 µg/plate Vehicle and positive controls valid Asulam sodium was negative +/-S9.
Mouse Lymphoma assay	L5178Y mouse lymphoma cells	-S9 4000-5250 µg/mL	-S9

<table>
<tr><th>Method</th><th>Organism/strain</th><th>Concentrations tested</th><th>Result</th></tr>
<tr><td>Asulam-sodium (purity 99%)
+/ S9
Cytotoxicity was reported as relative survival
No guideline stated, but similar to OECD 476 (1984). Conducted pre-GLP

Reference:
[29], 1982 (DAR B.6.4.1C)</td><td></td><td><u>+ S9</u>
4400-5200 µg/mL</td><td>Cytotoxicity observed at 5250 µg/mL (equivalent to 21 mM)
No increase in mutation frequency with test substance
<table>
<tr><th>Conc. (µg/mL)</th><th>Mean relative survival (%)</th><th>Mean mutation colonies/ plate</th><th>Mutation frequency (/10^6 survivors)</th></tr>
<tr><td>0</td><td>100</td><td>90</td><td>46</td></tr>
<tr><td>4500</td><td>131</td><td>78</td><td>41</td></tr>
<tr><td>4750</td><td>153</td><td>97</td><td>39</td></tr>
<tr><td>5000</td><td>149</td><td>85</td><td>37</td></tr>
<tr><td>5250</td><td>66</td><td>94</td><td>50</td></tr>
<tr><td>EMS</td><td>55</td><td>910</td><td>667</td></tr>
</table>
<u>With S9</u>

Cytotoxicity observed at 5200 µg/mL in one duplicate.
Statistically significant increase in mutation frequency at 5200 µg/mL (131 vs. 45 in negative controls),
<table>
<tr><th>Conc. (µg/mL)</th><th>Mean relative survival (%)</th><th>Mean mutation colonies/ plate</th><th>Mutation frequency (/10^6 survivors)</th></tr>
<tr><td>0</td><td>100</td><td>65</td><td>45</td></tr>
<tr><td>4400</td><td>120</td><td>78</td><td>38</td></tr>
<tr><td>4600</td><td>106</td><td>57</td><td>31</td></tr>
<tr><td>4800</td><td>78</td><td>100</td><td>56</td></tr>
<tr><td>5000</td><td>82</td><td>119</td><td>68</td></tr>
<tr><td>5200</td><td>44</td><td>296</td><td>131**</td></tr>
<tr><td>20-MC</td><td>74</td><td>298</td><td>180</td></tr>
</table>
** $p \leq 0.001$
Although asulam-sodium showed increases in mutant frequency in the presence of S9, these increases were observed at a dose equivalent to 21 mM. The current regulatory guideline maximum recommended concentration for *in vitro* mammalian cell mutagenicity assay is 10 mM. Therefore the biological relevance of these increases are questionable,</td></tr>
<tr><td>Mouse Lymphoma assay
Asulam sodium (purity 90.2%)
Asulam- purity 82.3%
Doses based on Asulam</td><td>L5178Y mouse lymphoma cells</td><td><u>+/- S9</u>
6.0-2300 µg/mL</td><td>
<table>
<tr><th rowspan="2">Dose (µg/mL)</th><th colspan="2">-S9 (24h)</th><th colspan="2">+S9 (4h)</th></tr>
<tr><th>MF#</th><th>RTG@</th><th>MF#</th><th>RTG@</th></tr>
<tr><td>0</td><td>112</td><td>100</td><td>121</td><td>100</td></tr>
<tr><td>12</td><td>94</td><td>118</td><td>-</td><td>-</td></tr>
<tr><td>23</td><td>93</td><td>113</td><td>96</td><td>96</td></tr>
</table>
</td></tr>
</table>

<table>
<tr><th>Method</th><th>Organism/strain</th><th>Concentrations tested</th><th>Result</th></tr>
<tr><td>+/- S9
Exposure times: 4h +S9, 24h –S9
Cytotoxicity measured as RTG
OECD 476 (the MLA tk assay is now included in a separate guideline, OECD 490 (2015). The assay design also complies with this test guideline.
GLP

Reference:
[30], 2009 (DAR B.6.4.1 D)</td><td></td><td></td><td>
<table>
<tr><td>48</td><td>95</td><td>126</td><td>82</td><td>98</td></tr>
<tr><td>94</td><td>120</td><td>102</td><td>75</td><td>77</td></tr>
<tr><td>188</td><td>134</td><td>130</td><td>98</td><td>85</td></tr>
<tr><td>391</td><td>108</td><td>128</td><td>141</td><td>72</td></tr>
<tr><td>782</td><td>88</td><td>125</td><td>86</td><td>104</td></tr>
<tr><td>1127</td><td>-</td><td>-</td><td>73</td><td>90</td></tr>
<tr><td>1611</td><td>103</td><td>138</td><td>85</td><td>82</td></tr>
<tr><td>2300</td><td>111</td><td>138</td><td>94</td><td>80</td></tr>
<tr><td>+ve control</td><td>1014</td><td>31</td><td>499</td><td>56</td></tr>
</table>
[#] Mutation frequency
[@] Relative total growth
Asulam was negative for mammalian cell mutagenicity in the presence and absence of S9.</td></tr>
<tr><td>Chromosome aberration test

Asulam (purity not stated)
+/-S9
Exposure times: 1 h +S9, 49 h
No guideline, pre-GLP

Reference: [31], 1984 (DAR B.6.4.1 F)</td><td>Human lymphocytes</td><td><u>- S9</u>
125- 1000 µg/mL
<u>+ S9</u>
1000-2500 µg/mL</td><td>Study does not comply with modern guidelines: only 100 metaphase cells scored/dose level, exposure times are not as recommended, lack of a repeat assay and historical control data was not submitted as part of the report.
<u>- S9</u>
Cytotoxicity observed at 1000 µg/mL. Negative and solvent control values were within the laboratory’s historical control range according to the study author (data not provided).
<table>
<tr><th>Conc. (µg/mL)</th><th>Cells scored</th><th>No. of aberrations/ cell</th><th>% cells with aberrations</th></tr>
<tr><td>0</td><td>100</td><td>0.03</td><td>3.0 (0.0)</td></tr>
<tr><td>125</td><td>100</td><td>0.03</td><td>3.0 (0.0)</td></tr>
<tr><td>250</td><td>100</td><td>0.03</td><td>3.0 (0.0)</td></tr>
<tr><td>500</td><td>100</td><td>0.03</td><td>3.0 (0.0)</td></tr>
<tr><td>1000</td><td>34</td><td>0.06</td><td>5.8 (0.0)</td></tr>
<tr><td>+ve control: MMC 0.2µg/ml</td><td>23</td><td>0.78</td><td>39.1 (26.1)</td></tr>
</table>
Values in parenthesis refer to % cells with >1 aberration
<u>+ S9</u>
<table>
<tr><th>Conc. (µg/mL)</th><th>Cells scored</th><th>No. of aberrations/ cell</th><th>% cells with aberrations</th></tr>
<tr><td>0</td><td>100</td><td>0.03</td><td>3.0 (0.0)</td></tr>
<tr><td></td><td></td><td></td><td></td></tr>
<tr><td>1000</td><td>100</td><td>0.01</td><td>1.0 (0.0)</td></tr>
<tr><td>1500</td><td>100</td><td>0.02</td><td>2.0 (0.0)</td></tr>
<tr><td>2000</td><td>100</td><td>0.00</td><td>0.0 (0.0)</td></tr>
<tr><td>2500</td><td>100</td><td>0.00</td><td>0.0 (0.0)</td></tr>
</table></td></tr>
</table>

Method	Organism/strain	Concentrations tested	Result
			+ve control: CPA 50 µg/ml | 50 | 0.16 | 12.0 (4.0) Values in parenthesis refer to % cells with >1 aberration
Unscheduled DNA Synthesis (UDS) assay Asulam-sodium (purity not stated +/-S9 Exposure time- 2 h No guideline, pre-GLP Reference: [32], 1982 (DAR B.6.4.1 E)	HeLa S3 cells	+/-S9 0-250 µg/mL	Hydroxyurea was included in cell medium to reduce normal DNA replication No increase in UDS was observed in cells exposed to Asulam.

Table 17: Summary table of relevant *in vivo* mutagenicity studies

Method	Organism /strain	Concentrations tested	Result
Micronucleus test Asulam sodium (purity 89.3%) Vehicle- water 2000 PCEs evaluated for each animal OECD 474, GLP Reference: [33], 2004 (DAR B.6.4.2)	Male NMRI mice (5/group)	0, 1000, 2000 and 4000 mg/kg bw/d on two consecutive days, with sacrifice 24 h post final administration	Clinical observations- apathy, digging, grooming movements, loss of weight, spasm, ptosis and difficulty breathing at all doses. **24 h**- PCEs with micronuclei were not statistically significantly or dose-dependently increased compared to controls. Negative control Animal number | No of NCE/ 2000 PCEs (%PCE) | MNNCE/ 2000 PCE | MNPCE/ 2000 PCE 6 | 2988(40%) | 0 | 4 7 | 2350 (46%) | 1.7 | 3 12 | 1152 (63%) | 3.5 | 4 14 | 1997 (50%) | 1.0 | 4 24 | 1693 (54%) | 3.5 | 4 Mean ±SD | 2036 ±690 (50%) | 1.9 ±1.6 | 3.8 ±0.4 1000 mg/kg bw/d Animal number | No of NCE/ 2000 PCEs (%PCE) | MNNCE/ 2000 PCE | MNPCE/ 2000 PCE 9 | 2064 (49%) | 1.0 | 3 13 | 2357 (46%) | 0.8 | 6 17 | 3794 (35%) | 2.1 | 2

<table>
<tr><th>Method</th><th>Organism /strain</th><th>Concentrations tested</th><th>Result</th></tr>
<tr><td></td><td></td><td></td><td>
<table>
<tr><td>27</td><td>1735 (54%)</td><td>0</td><td>1</td></tr>
<tr><td>29</td><td>1791 (53%)</td><td>4.5</td><td>6</td></tr>
<tr><td>Mean ±SD</td><td>2348 ±845 (46%)</td><td>1.7 ±1.7</td><td>3.6 ±2.3</td></tr>
</table>

2000 mg/kg bw/d
<table>
<tr><th>Animal number</th><th>No of NCE/ 2000 PCEs (%PCE)</th><th>MNNCE/ 2000 PCE</th><th>MNPCE/ 2000 PCE</th></tr>
<tr><td>2</td><td>1732 (53%)</td><td>1.2</td><td>3</td></tr>
<tr><td>11</td><td>1321 (60%)</td><td>3.0</td><td>8</td></tr>
<tr><td>20</td><td>1976 (50%)</td><td>0</td><td>7</td></tr>
<tr><td>26</td><td>922 (68%)</td><td>2.2</td><td>9</td></tr>
<tr><td>28</td><td>1513 (57%)</td><td>1.3</td><td>3</td></tr>
<tr><td>Mean ±SD</td><td>1493 ±402 (57%)</td><td>1.5 ±1.1</td><td>6.0 ±2.8</td></tr>
</table>

4000 mg/kg bw/d
<table>
<tr><th>Animal number</th><th>No of NCE/ 2000 PCEs (%PCE)</th><th>MNNCE/ 2000 PCE</th><th>MNPCE/ 2000 PCE</th></tr>
<tr><td>1</td><td>3817 (34%)</td><td>1.0</td><td>2</td></tr>
<tr><td>4</td><td>2145 (48%)</td><td>0.9</td><td>1</td></tr>
<tr><td>5</td><td>1616 (55%)</td><td>1.2</td><td>6</td></tr>
<tr><td>15</td><td>1199 (63%)</td><td>1.7</td><td>1</td></tr>
<tr><td>25</td><td>6112 (25%)</td><td>3.3</td><td>4</td></tr>
<tr><td>Mean</td><td>2978 ±2015 (40%)</td><td>1.6 ±1.0</td><td>2.8 ±2.2</td></tr>
</table>

Positive control-
<table>
<tr><th>Animal number</th><th>No of NCE/ 2000 PCEs (%PCE)</th><th>MNNCE/ 2000 PCE</th><th>MNPCE/ 2000 PCE</th></tr>
<tr><td>3</td><td>1085 (65%)</td><td>0</td><td>16</td></tr>
<tr><td>16</td><td>1614 (55%)</td><td>1.2</td><td>32</td></tr>
<tr><td>18</td><td>1269 (61%)</td><td>6.3</td><td>10</td></tr>
<tr><td>19</td><td>2418 (45%)</td><td>3.3</td><td>19</td></tr>
<tr><td>23</td><td>1433 (58%)</td><td>0</td><td>12</td></tr>
<tr><td>Mean</td><td>1564 ±516 (56%)</td><td>2.2 ±2.7</td><td>17.8 ±8.7</td></tr>
</table>

Historical control data range for vehicle - 2.0- 5.8
</td></tr>
</table>

4.9.1 Non-human information

4.9.1.1 *In vitro* data

There are a total of 4 bacterial reverse mutation assays available but only the two most recent studies conform to OECD guidelines and are GLP compliant. These two bacterial studies ([27],[28]) were negative for mutagenicity in the presence and absence of S9 and support the previous findings from the older supplementary studies.

Two mouse lymphoma studies have been reported for asulam sodium. The 1982 [29] study is limited as it was conducted in principle to an earlier version of OECD 476 (1984), and was not GLP; in addition, covered a narrow range of concentrations. Although cytotoxicity was not marked at the highest concentration in accordance with current recommendations, the maximum concentration was equivalent to 21 mM, a concentration which exceeds the current regulatory guideline maximum recommended concentration for the *in vitro* mammalian cell mutagenicity assay (10 mM). Whilst an increase in mutation frequency was observed at 5200 µg/mL (21 mM) in the presence of S9, this concentration exceeded the maximum recommended concentration for this assay type. Therefore the biological relevance of these increases is questionable. In a recent GLP and guideline compliant study [30], asulam sodium was negative with and without S9 in the mouse lymphoma assay. Although the highest concentration tested was much lower than that in the 1982 study, the maximum concentration tested was in accordance with current regulatory guidelines for this assay type, with the maximum concentration equivalent to 10 mM. Overall, the more recent study is deemed to be more robust and adequately addresses the *in vitro* mammalian gene mutation endpoint.

The only study available to address clastogenicity is a chromosomal aberration test in human lymphocytes [31]. There was an increase in aberrations (5.8%) at the top dose (1000 µg/mL) in the absence of metabolic activation but this was not statistically significantly different from controls (medium and solvent). There is also evidence within the report that shows the solvent alone (DMSO +S9) can induce aberrations up to 6% which exceeds the percentage reported at 1000 µg/mL (5.8%). In the presence of S9, there was no increase in the percentage of aberrations at any dose level. Overall, although the study authors concluded that asulam did not induce chromosomal aberrations, it is unclear why a common solvent such as DMSO has been reported to cause an increase of up to 6% in aberrations (likely attributed to the purity/grade of solvent used). Therefore, no clear conclusion can be drawn from this study.

A negative result was reported in the UDS assay [32] but the study is limited as it was not validated nor guideline compliant at the time of conduct.

4.9.1.2 *In vivo* data

One GLP and guideline-compliant *in vivo* study has been evaluated to determine the potential for asulam sodium to induce cytogenetic damage in mice [33]. The current OECD guideline recommendations for dose selection are either a limit dose (2000 mg/kg bw) in this study design or the maximum tolerated dose where data is available. The inclusion of 4000 mg/kg bw/day does appear excessive and inconsistent with OECD recommendations, however, at this dose the ratio of polychromatic to normochromatic erythrocytes was altered (when expressed as %PCE, bone marrow toxicity was evident, with %PCE dropping to 40%) and supports the test substance reaching the bone marrow.

An increase in micronuclei formation was only reported at the mid-dose which was marginally outside the historical control data for the vehicle (6.0 versus 5.8). This result was not statistically significant compared to the control and was not reported in the top dose group i.e. no dose response was evident.

In addition, the absence of a response at 4000 mg/kg bw/d cannot be accounted for by toxicity as clinical signs were reported at all dose levels of test substance and there was no impact on body weight.

Overall the study appears to be negative; however, given the unusual study design no clear conclusions can be drawn from this study.

No studies on germ cells have been submitted.

4.9.2 Human information

No data available.

4.9.3 Other relevant information

No data available.

4.9.4 Summary and discussion of mutagenicity

The mutagenicity of asulam sodium has been investigated in 8 studies.

Of the 4 bacterial reverse mutation assays available only the two most recent studies conform to OECD guidelines and are GLP compliant. These two bacterial studies (2008 and 2013) were negative for mutagenicity in the presence and absence of S9 and support the previous findings from the older supplementary studies.

Two mouse lymphoma studies have been reported for asulam sodium. The 1982 study is limited as it was not conducted to GLP nor available guidelines (contemporary or recent), and only covered a narrow range of concentrations. Although cytotoxicity was not marked at the highest concentration in accordance with current recommendations, the maximum concentrations was equivalent to 21 mM, a concentration which exceeds the current regulatory guideline maximum recommended concentration for the *in vitro* mammalian cell mutagenicity assay (10 mM). Whilst an, an increase in mutation frequency was observed at 5200 µg/mL (21 mM) in the presence of S9, this concentration exceeded the maximum recommended concentration for this assay type. Therefore the biological relevance of this increase is questionable. In a recent GLP and guideline compliant study, asulam sodium was negative with and without S9 in the mouse lymphoma assay. Although the highest concentration tested was much lower than that in the 1982 study, the maximum concentration tested was in accordance with current regulatory guidelines for this assay type, with the maximum concentration equivalent to 10 mM.. Overall, the more recent study is deemed to be more robust and adequately addresses the *in vitro* mammalian gene mutation endpoint.

The only study available to address clastogenicity is a chromosomal aberration test in human lymphocytes. There was an increase in aberrations (5.8%) at the top dose (1000 µg/mL) in the absence of metabolic activation but this was not statistically significantly different from controls (medium and solvent). There is also evidence within the report that shows the

solvent alone (DMSO +S9) can induce aberrations up to 6% which exceeds the percentage reported at 1000 µg/mL (5.8%). In the presence of S9, there was no increase in the percentage of aberrations at any dose level. Overall, although the study authors concluded that asulam did not induce chromosomal aberrations, it is unclear why a common solvent such as DMSO has been reported to cause an increase of up to 6% in aberrations (likely attributed to the purity/grade of solvent used). Overall therefore, no clear conclusion can be drawn from this study.

A negative result was reported in the UDS assay but the study is limited as it was not validated nor guideline compliant at the time of conduct.

In an *in vivo* micronucleus test with mice, micronuclei formation was only reported at the mid-dose (2000 mg/kg/day) which was marginally outside the historical control data for the vehicle (6.0 versus 5.8). This result was not statistically significant compared to the control and was not reported in the top dose group i.e. no dose response was evident.

In addition, the absence of a response at 4000 mg/kg bw/d cannot be accounted for by toxicity as clinical signs were reported at all dose levels of test substance and there was no impact on body weight.

Overall the study appears to be negative; however, given the unusual study design no clear conclusions can be drawn from this study.

Overall, there is no strong or reliable evidence that asulam sodium is mutagenic in the test systems used, but it is recognised that there are weaknesses in the available data set.

4.9.5 Comparison with criteria

For classification in Category 1A or 1B, the substance should be known to induce heritable changes or be regarded as if it will induce heritable changes in germ cells of humans, or produce positive results in *in vivo* somatic cell tests in combination with evidence that the substance has the potential to cause mutations in germ cells. There are no human data and the results of the in vivo mouse micronucleus study are considered to be inconclusive. Therefore it does not meet the criteria for classification as a Category1A or Category 1B mutagen.

For classification in Category 2 under CLP, the substance should show positive results in mammals and/or in some cases in *in vitro* experiments. As outlined in the sections above, there are weaknesses in the available dataset for asulam sodium. *In vitro* there are good negative bacterial mutation studies. There are also two mouse lymphoma assays, one of which is positive at concentrations which exceed the maximum recommended concentration, whilst the other study was negative when test up to the maximum concentration in accordance with current *in vitro* genotoxicity guideline requirements. In the only chromosome aberration study there was an increase in aberrations at the top concentration in the absence of S9, which was comparable to the vehicle (DMSO) control; however due to high background of aberrations reported by DMSO in this study (likely attributed to the purity/grade of solvent used), the result was considered difficult to interpret. In the only available *in vivo* study (the mouse bone marrow micronucleus study), a marginal increase in PCEs with micronuclei was observed at a dose of 2000 mg/kg bw but not at 4000 mg/kg bw. The absence of a response at 4000 mg/kg bw/d cannot be accounted for by toxicity as clinical signs were reported at all dose levels of test substance and there was no impact on body weight.

The study appears to be negative; however, given the unusual study design no clear conclusions can be drawn from this study.

Overall, although, there is no strong or reliable positive evidence that asulam sodium is mutagenic, the quality of the data package is such that no clear conclusion can be drawn. .

4.9.6 Conclusions on classification and labelling

Not classified- inconclusive, data not sufficient for classification

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The mutagenicity of asulam-sodium has been investigated in six *in vitro* studies with bacteria, mouse and human cells and one *in vivo* micronucleus study in mice. No studies on germ cells have been submitted.

Summary table of relevant *in vitro* mutagenicity studies

Method	Organism/strain	Concentrations tested	Result
1st study Bacterial reverse mutation assay Asulam-sodium (purity 90.2%) Asulam purity 82.3% Doses based on Asulam purity +/-S9 OECD TG 471 (plate incorporation), GLP Reference: Report V7905/05, 2008 (DAR B.6.4.1 A)	*S. Typhimurium* TA98, TA100, TA1535, TA1537, *E. Coli*, WP2 *uvr*A	0, 62, 185, 556, 1667 and 5000 µg/plate	Cytotoxicity was reported at ≥ 1667 µg/plate Vehicle and positive controls valid **Asulam-sodium was negative +/-S9.**
2nd study Bacterial reverse mutation assay Asulam-sodium (purity 44.2% aqueous concentrate) +/- S9 (5 or 10%) OECD TG 471 (plate incorporation), GLP Reference: Report 481-1-06-6691, 2013 (DAR B.6.4.1 B)	*S. Typhimurium* - TA98, TA100, TA102, TA1535, TA1537	Preliminary study - 0.25 - 5000 µg/plate Main study - 0, 6.89, 20.58, 61.73, 185.19, 555.56 and 1666.67 µg/plate	Cytotoxicity was reported at 1667 µg/plate Vehicle and positive controls valid **Asulam-sodium was negative +/-S9.**
3rd study Mouse Lymphoma assay	L5178Y mouse lymphoma cells	-S9;	-S9

<table>
<tr>
<td>Asulam-sodium (purity 99%)
+/ S9
Cytotoxicity was reported as relative survival
No guideline stated, but similar to OECD TG 476 (1984). Conducted pre-GLP

Reference: Report R001258, 1982 (DAR B.6.4.1C)</td>
<td></td>
<td>4000 - 5250 µg/mL

<u>+ S9;</u>
4400 - 5200 µg/mL</td>
<td>Cytotoxicity observed at 5250 µg/mL (equivalent to 21 mM)
No increase in mutation frequency with test substance
<table>
<tr><th>Conc. (µg/mL)</th><th>Mean relative survival (%)</th><th>Mean mutation colonies/ plate</th><th>Mutation frequency (/10^6 survivors)</th></tr>
<tr><td>0</td><td>100</td><td>90</td><td>46</td></tr>
<tr><td>4500</td><td>131</td><td>78</td><td>41</td></tr>
<tr><td>4750</td><td>153</td><td>97</td><td>39</td></tr>
<tr><td>5000</td><td>149</td><td>85</td><td>37</td></tr>
<tr><td>5250</td><td>66</td><td>94</td><td>50</td></tr>
<tr><td>EMS</td><td>55</td><td>910</td><td>667</td></tr>
</table>
<u>With S9</u>
Cytotoxicity observed at 5200 µg/mL in one duplicate.
Statistically significant increase in mutation frequency at 5200 µg/mL (131 vs. 45 in negative controls),
<table>
<tr><th>Conc. (µg/mL)</th><th>Mean relative survival (%)</th><th>Mean mutation colonies/ plate</th><th>Mutation frequency (/10^6 survivors)</th></tr>
<tr><td>0</td><td>100</td><td>65</td><td>45</td></tr>
<tr><td>4400</td><td>120</td><td>78</td><td>38</td></tr>
<tr><td>4600</td><td>106</td><td>57</td><td>31</td></tr>
<tr><td>4800</td><td>78</td><td>100</td><td>56</td></tr>
<tr><td>5000</td><td>82</td><td>119</td><td>68</td></tr>
<tr><td>5200</td><td>44</td><td>296</td><td>131**</td></tr>
<tr><td>20-MC</td><td>74</td><td>298</td><td>180</td></tr>
</table>
** p≤0.001
Although asulam-sodium showed increases in mutant frequency in the presence of S9, these increases were observed at a dose equivalent to 21 mM. The current regulatory guideline maximum recommended concentration for in vitro mammalian cell mutagenicity assay is 10 mM. Therefore the biological relevance of these increases are questionable.</td>
</tr>
<tr>
<td>4th study
Mouse Lymphoma assay
Asulam-sodium (purity 90.2%)
Asulam purity 82.3%
Doses based on Asulam
+/- S9
Exposure times: 4h +S9, 24h –S9
Cytotoxicity measured as RTG
OECD TG 476 (the MLA tk assay is now included in a separate guideline, OECD TG 490</td>
<td>L5178Y mouse lymphoma cells</td>
<td><u>+/- S9</u>
6.0-2300 µg/mL</td>
<td>
<table>
<tr><th rowspan="2">Dose (µg/mL)</th><th colspan="2">-S9 (24h)</th><th colspan="2">+S9 (4h)</th></tr>
<tr><th>MF#</th><th>RTG@</th><th>MF#</th><th>RTG@</th></tr>
<tr><td>0</td><td>112</td><td>100</td><td>121</td><td>100</td></tr>
<tr><td>12</td><td>94</td><td>118</td><td>-</td><td>-</td></tr>
<tr><td>23</td><td>93</td><td>113</td><td>96</td><td>96</td></tr>
<tr><td>48</td><td>95</td><td>126</td><td>82</td><td>98</td></tr>
<tr><td>94</td><td>120</td><td>102</td><td>75</td><td>77</td></tr>
<tr><td>188</td><td>134</td><td>130</td><td>98</td><td>85</td></tr>
<tr><td>391</td><td>108</td><td>128</td><td>141</td><td>72</td></tr>
<tr><td>782</td><td>88</td><td>125</td><td>86</td><td>104</td></tr>
<tr><td>1127</td><td>-</td><td>-</td><td>73</td><td>90</td></tr>
<tr><td>1611</td><td>103</td><td>138</td><td>85</td><td>82</td></tr>
<tr><td>2300</td><td>111</td><td>138</td><td>94</td><td>80</td></tr>
</table>
</td>
</tr>
</table>

<table>
<tr>
<td>(2015). The assay design also complies with this test guideline. GLP

Reference: Report V7903/02, 2009 (DAR B.6.4.1 D)</td>
<td></td>
<td></td>
<td>
<table>
<tr><td>positive control</td><td>1014</td><td>31</td><td>499</td><td>56</td></tr>
</table>
Mutation frequency
@ Relative total growth
Asulam was negative for mammalian cell mutagenicity in the presence and absence of S9.
</td>
</tr>
<tr>
<td>5th study
Chromosome aberration test

Asulam (purity not stated)
+/-S9
Exposure times: 1 h +S9, 49 h
No guideline, pre-GLP

Reference: Report R001262, 1984 (DAR B.6.4.1 F)</td>
<td>Human lymphocytes</td>
<td><u>- S9</u>
125- 1000 µg/mL
<u>+ S9</u>
1000-2500 µg/mL</td>
<td>Study does not comply with modern guidelines: only 100 metaphase cells scored/dose level, exposure times are not as recommended, lack of a repeat assay and historical control data was not submitted as part of the report.
<u>- S9</u>
Cytotoxicity observed at 1000 µg/mL. Negative and solvent control values were within the laboratory’s historical control range according to the study author (data not provided).
<table>
<tr><th>Conc. (µg/mL)</th><th>Cells scored</th><th>No. of aberrations/ cell</th><th>% cells with aberrations</th></tr>
<tr><td>0</td><td>100</td><td>0.03</td><td>3.0 (0.0)</td></tr>
<tr><td>125</td><td>100</td><td>0.03</td><td>3.0 (0.0)</td></tr>
<tr><td>250</td><td>100</td><td>0.03</td><td>3.0 (0.0)</td></tr>
<tr><td>500</td><td>100</td><td>0.03</td><td>3.0 (0.0)</td></tr>
<tr><td>1000</td><td>34</td><td>0.06</td><td>5.8 (0.0)</td></tr>
<tr><td>positive control: MMC 0.2µg/ml</td><td>23</td><td>0.78</td><td>39.1 (26.1)</td></tr>
</table>
Values in parenthesis refer to % cells with > 1 aberration
<u>+ S9</u>
<table>
<tr><th>Conc. (µg/mL)</th><th>Cells scored</th><th>No. of aberrations/ cell</th><th>% cells with aberrations</th></tr>
<tr><td>0</td><td>100</td><td>0.03</td><td>3.0 (0.0)</td></tr>
<tr><td></td><td></td><td></td><td></td></tr>
<tr><td>1000</td><td>100</td><td>0.01</td><td>1.0 (0.0)</td></tr>
<tr><td>1500</td><td>100</td><td>0.02</td><td>2.0 (0.0)</td></tr>
<tr><td>2000</td><td>100</td><td>0.00</td><td>0.0 (0.0)</td></tr>
<tr><td>2500</td><td>100</td><td>0.00</td><td>0.0 (0.0)</td></tr>
<tr><td>positive control: CPA 50 µg/ml</td><td>50</td><td>0.16</td><td>12.0 (4.0)</td></tr>
</table>
Values in parenthesis refer to % cells with > 1 aberration</td>
</tr>
<tr>
<td>6th study
Unscheduled DNA Synthesis (UDS) assay
Asulam-sodium (purity not stated)
+/-S9
Exposure time- 2 h
No guideline, pre-GLP

Reference: Report</td>
<td>HeLa S3 cells</td>
<td><u>+/-S9</u>
0-250 µg/mL</td>
<td>Hydroxyurea was included in cell medium to reduce normal DNA replication
No increase in UDS was observed in cells exposed to Asulam.</td>
</tr>
</table>

C030509, 1982 (DAR B.6.4.1 E)			

Evaluation of *in vitro* data

There are a total of 4 bacterial reverse mutation assays available but only the two most recent studies conform to OECD guidelines and are GLP compliant. These two bacterial studies (Report V7905/05, 2008; Report 481-1-06-6691, 2013) were negative for mutagenicity in the presence and absence of S9 and support the previous findings from the older supplementary studies.

Two mouse lymphoma studies have been reported for asulam-sodium. The 1982 study (Report R001258) is limited as it was conducted to an earlier version of OECD TG 476 (1984), and was not GLP-compliant; in addition, it covered a narrow range of concentrations. Although cytotoxicity was not marked at the highest concentration in accordance with current recommendations, the maximum concentration was equivalent to 21 mM, a concentration which exceeds the current regulatory guideline maximum recommended concentration for the *in vitro* mammalian cell mutagenicity assay (10 mM). Whilst an increase in mutation frequency was observed at 5200 µg/mL (21 mM) in the presence of S9, this concentration exceeded the maximum recommended concentration for this assay type. Therefore the biological relevance of these increases is questionable. In a recent GLP and guideline compliant study (Report V7903/02), asulam-sodium was negative with and without S9 in the mouse lymphoma assay. Although the highest concentration tested was much lower than that in the 1982 study, the maximum concentration tested was in accordance with current regulatory guidelines for this assay type, with the maximum concentration equivalent to 10 mM. Overall, the more recent study is deemed to be more robust and adequately addresses the *in vitro* mammalian gene mutation endpoint.

The only study available with which to address clastogenicity was an *in vitro* chromosomal aberration test in human lymphocytes (Report R001262, 1984). There was an increase in aberrations (5.8%) at the top dose (1000 µg/mL) in the absence of metabolic activation but this was not statistically significantly different from controls (medium and solvent). There was also evidence within the report that showed that the solvent alone (DMSO +S9) can induce aberrations up to 6% which exceeds the percentage reported at 1000 µg/mL (5.8%). In the presence of S9, there was no increase in the percentage of aberrations at any dose level. Overall, although the study authors concluded that asulam did not induce chromosomal aberrations, it is unclear why a common solvent such as DMSO has been reported to cause an increase of up to 6% in aberrations (likely attributed to the purity/grade of solvent used). Therefore, no clear conclusion can be drawn from this study.

A negative result was reported in the UDS assay (Report C030509, 1982) but it should be noted that at the time it was conducted no guidelines were available.

Summary table of relevant *in vivo* mutagenicity studies

<table>
<tr><th>Method</th><th>Organism /strain</th><th>Concentrations tested</th><th>Result</th></tr>
<tr><td>Micronucleus test

Asulam-sodium (purity 89.3%)
Vehicle- water
2000 PCEs evaluated for each animal
OECD TG 474, GLP</td><td>Male NMRI mice (5/group)</td><td>0, 1000, 2000 and 4000 mg/kg bw/d on two consecutive days, with sacrifice 24 h post final administration</td><td>Clinical observations: apathy, digging, grooming movements, loss of weight, spasm, ptosis and difficulty breathing at all doses.

Negative control
<table>
<tr><th>Animal number</th><th>No of NCE/ 2000 PCEs (%PCE)</th><th>MNNCE/ 2000 PCE</th><th>MNPCE/ 2000 PCE</th></tr>
<tr><td>6</td><td>2988 (40%)</td><td>0</td><td>4</td></tr>
<tr><td>7</td><td>2350 (46%)</td><td>1.7</td><td>3</td></tr>
</table>
</td></tr>
</table>

Reference: Report C032928, 2004 (DAR B.6.4.2)

12	1152 (63%)	3.5	4
14	1997 (50%)	1.0	4
24	1693 (54%)	3.5	
Mean ± SD	2036 ± 690 (50%)	1.9 ± 1.6	3.8 ±0.4

1000 mg/kg bw/d

Animal number	No of NCE/ 2000 PCEs (%PCE)	MNNCE/ 2000 PCE	MNPCE/ 2000 PCE
9	2064 (49%)	1.0	3
13	2357 (46%)	0.8	6
17	3794 (35%)	2.1	2
27	1735 (54%)	0	1
29	1791 (53%)	4.5	6
Mean ±SD	2348 ±845 (46%)	1.7 ±1.7	3.6 ±2.3

2000 mg/kg bw/d

Animal number	No of NCE/ 2000 PCEs (%PCE)	MNNCE/ 2000 PCE	MNPCE/ 2000 PCE
2	1732 (53%)	1.2	3
11	1321 (60%)	3.0	8
20	1976 (50%)	0	7
26	922 (68%)	2.2	9
28	1513 (57%)	1.3	3
Mean ±SD	1493 ±402 (57%)	1.5 ±1.1	6.0 ±2.8

4000 mg/kg bw/d

Animal number	No of NCE/ 2000 PCEs (%PCE)	MNNCE/ 2000 PCE	MNPCE/ 2000 PCE
1	3817 (34%)	1.0	2
4	2145 (48%)	0.9	1
5	1616 (55%)	1.2	6
15	1199 (63%)	1.7	1
25	6112 (25%)	3.3	4

Mean	2978 ±2015 (40%)	1.6 ±1.0	2.8 ±2.2

Positive control

Animal number	No of NCE/ 2000 PCEs (%PCE)	MNNCE/ 2000 PCE	MNPCE/ 2000 PCE
3	1085 (65%)	0	16
16	1614 (55%)	1.2	32
18	1269 (61%)	6.3	10
19	2418 (45%)	3.3	19
23	1433 (58%)	0	12
Mean	1564 ±516 (56%)	2.2 ±2.7	17.8 ±8.7

Historical control data range for vehicle - 2.0- 5.8

24 h- PCEs with micronuclei were not statistically significantly or dose-dependently increased compared to controls.

Evaluation of *in vivo* data

One GLP and guideline-compliant *in vivo* study has been evaluated to determine the potential for asulam-sodium to induce cytogenetic damage in mice (Report C032928, 2004). The current OECD guideline recommendations for dose selection are either a limit dose (2000 mg/kg bw/d) in this study design or the maximum tolerated dose where data is available. The inclusion of 4000 mg/kg bw/d does appear excessive and inconsistent with OECD recommendations, however, at this dose the ratio of polychromatic to normochromatic erythrocytes was altered (when expressed as %PCE, bone marrow toxicity was evident, with %PCE dropping to 40%) and supports the test substance reaching the bone marrow.

An increase in micronucleus formation was only reported at the mid-dose which was marginally outside the historical control data for the vehicle (6.0 versus 5.8). This result was not statistically significant compared to the control and was not reported in the top dose group i.e. no dose response relationship was evident.

In addition, the absence of a response at 4000 mg/kg bw/d cannot be accounted for by toxicity as clinical signs were reported at all dose levels of test substance and there was no impact on body weight.

Overall the study appears to be negative; however, given the unusual study design no clear conclusions can be drawn from this study. Consequently the Dossier Submitter assessed the available data as inconclusive and not sufficient for classification.

Comments received during public consultation

Two MSCAs agreed with the DS that available genotoxicity data are inconclusive, therefore no clear conclusion on classification can be drawn.

Assessment and comparison with the classification criteria

The two bacterial reverse mutation assays (2008 and 2013) which conform to OECD guidelines and were GLP compliant were negative for mutagenicity in the presence and absence of S9 and are consistent with the findings from the older supplementary bacterial studies.

Two mouse lymphoma studies have been reported for asulam-sodium. The 1982 study (Report R001258) is limited as it only covered a narrow range of concentrations. Although cytotoxicity was not marked at the highest concentration in accordance with current recommendations, the maximum concentrations was equivalent to 21 mM, a concentration which exceeds the current regulatory guideline maximum recommended concentration for the *in vitro* mammalian cell mutagenicity assay (10 mM). Whilst an increase in mutation frequency was observed at 5200 µg/mL (21 mM) in the presence of S9, this concentration exceeded the maximum recommended concentration for this assay type. Therefore the biological relevance of this increase is questionable. It was not conducted to GLP or to pre-OECD guidelines available at the time.

In a recent GLP and guideline compliant study (Report V7903/02), asulam-sodium was negative with and without S9 in the mouse lymphoma assay. Although the highest concentration tested in the 2009 study (2300 µg/mL) was much lower than that in the 1982 study (5200 µg/mL), the maximum concentration tested was in accordance with current regulatory guidelines for this assay type, with the maximum concentration equivalent to 10 mM. Overall, the more recent study is deemed to be more robust and adequately addresses the *in vitro* mammalian gene mutation endpoint.

The only study available to address clastogenicity is a chromosomal aberration test in human lymphocytes. There was an increase in aberrations (5.8%) at the top dose (1000 µg/mL) in the absence of metabolic activation but this was not statistically significantly different from controls (medium and solvent). There is also evidence within the report that shows the solvent alone (DMSO +S9) can induce aberrations up to 6% which exceeds the percentage reported at 1000 µg/mL (5.8%). In the presence of S9, there was no increase in the percentage of aberrations at any dose level. Overall, although the study authors concluded that asulam did not induce chromosomal aberrations, it is unclear why a common solvent such as DMSO has been reported to cause an increase of up to 6% in aberrations (likely attributed to the purity/grade of solvent used). Overall therefore, no clear conclusion can be drawn from this study.

In an *in vivo* micronucleus test with mice, micronuclei formation was only reported at the mid-dose (2000 mg/kg bw/d) which was marginally outside the historical control data for the vehicle (6.0 versus 5.8). This result was not statistically significant compared to the control and was not reported in the top dose group i.e. no dose response was evident. In addition, the absence of a response at 4000 mg/kg bw/d cannot be accounted for by toxicity as clinical signs were reported at all dose levels of test substance and there was no impact on body weight. Overall the study appears to be negative; however, given the unusual study design no clear conclusions can be drawn from this study.

Overall, there is no strong or reliable evidence that asulam-sodium is mutagenic in the test systems used, but it is recognised that there are weaknesses in the available data set.

Comparison with the criteria

For classification in Category 1A or 1B, the substance should be known to induce heritable changes or be regarded as if it will induce heritable changes in germ cells of humans, or produce positive results in in vivo somatic cell tests in combination with evidence that the substance has the potential to cause mutations in germ cells.

There are no human data and the results of the *in vivo* mouse micronucleus study are considered to be inconclusive. Therefore, it does not meet the criteria for classification as a Category 1A or Category 1B mutagen.

For classification in Category 2, the substance should show positive results in mammals and/or in some cases in *in vitro* experiments. As outlined in the sections above, there are weaknesses in the available dataset for asulam-sodium.

There are good negative bacterial mutation *in vitro* studies. There are also two mouse lymphoma assays, one of which is positive at concentrations which exceed the maximum recommended concentration, whilst the other study was negative when tested up to the maximum concentration in accordance with current *in vitro* genotoxicity guideline requirements.

In the only chromosome aberration study, there was an increase in aberrations at the top concentration in the absence of S9, however that increase in aberrations was comparable to that observed in the vehicle (DMSO) control group. Due to high background of aberrations reported in DMSO control group in this study (likely attributed to the purity/grade of solvent used), the result was considered difficult to interpret.

In the only available *in vivo* study (the mouse bone marrow micronucleus study), a marginal increase in PCEs with micronuclei was observed at a dose of 2000 mg/kg bw but not at 4000 mg/kg bw. The absence of a response at 4000 mg/kg bw/d cannot be accounted for by toxicity as clinical signs were reported at all dose levels of test substance and there was no impact on body weight. The study appears to be negative; however, given the unusual study design no clear conclusions can be drawn from this study.

Overall, although, there is no strong or reliable positive evidence that asulam-sodium is mutagenic and due to the poor quality of the data package, no clear conclusion can be drawn.

Taking the above analysis of data into account RAC agrees with the DS that the available data are inconclusive and that it is therefore not possible to classify asulam-sodium for germ cell mutagenicity according to the CLP criteria.

4.10 Carcinogenicity

Two carcinogenicity studies are available, one in rats and one in mice. Non-neoplastic findings are reported that are associated with general toxicity or effects associated with neoplasms. Other non-neoplastic findings are reported in the repeat dose Section 4.7.

Table 18: Summary table of relevant carcinogenicity studies

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
108 week carcinogenicity study Asulam sodium (purity not stated) CD rats (Sprague Dawley origin) 50/sex/group Satellite group (sacrificed at 78 weeks)- 15/sex/group Clinical signs, palpations, body weights, food consumption ophthalmoscopy, haematology, clinical chemistry, urine analysis, gross necropsy and histopathology were recorded. No guideline stated, but similar to OECD 453. Pre-GLP Reference: [21], 1981 (DAR 6.5.1)	0, 1000, 5000, 25000 ppm (♂/♀: 0/, 36/47, 180/243, 953/1280 mg/kg bw/d)	♂- Non-neoplastic findings **1000 ppm (36 mg/kg bw/d) -** No test item-related effects reported that would be associated with neoplasms or general toxicity. **5000ppm (180 mg/kg bw/d) -** No test item-related effects reported that would be associated with neoplasms or general toxicity. **25000 ppm (953 mg/kg bw/d)** - reduced weight gain (13%), increased incidence of adrenal medullary hyperplasia (17/50). ♀- Non-neoplastic findings **1000 ppm (47 mg/kg bw/d)-** No test item-related effects reported that would be associated with neoplasms or general toxicity. **5000 ppm (243 mg/kg bw/d) -** reduced weight gain in females between weeks 6-52 (13%). **25 000 ppm (1280 mg/kg bw/d) -** reduced weight gain between weeks 6-52 (18%) Males- Neoplastic findings Phaeochromocytomas- 6% (3/50), 10% (5/50), 8% (4/50) and 20% (10/50) at 0, 1000, 5000 and 25 000 ppm Laboratory historical control incidence- 2-16% (HCD taken from 6 studies conducted in1978 with the same strain of rat *i.e.* CD rats of Sprague Dawley origin) With the exception of two tumours (1 at 5000 ppm week 77 and 1 at 25 000 ppm week 76), the phaeochromocytomas occurred in aged rats (>80 weeks) in all groups. There was no decrease in latency observed across the treated groups compared to controls. ♀- Neoplastic findings No test item-related effects
Two-year carcinogenicity study in mice Asulam sodium (purity 88%) CD-1 mice 75/sex/group Satellite group (sacrificed at12 months)- 10/sex/group Clinical investigations only- 15/sex/group	0, 500, 5000 and 50 000 ppm (♂/♀: 0/0, 74/95, 730/938, 8040/10353 mg/kg bw/d)	♂- Non-neoplastic findings **500 ppm (74 mg/kg bw/d) -** No test item-related effects reported that would be associated with general toxicity or neoplasms. **5000 ppm (730 mg/kg bw/d)** - No test item-related effects reported that would be associated with general toxicity or neoplasms. **50 000 ppm (8040 mg/kg bw/d)** - decreased mean bodyweight throughout study period (range 3-10%), increased food consumption, accumulation of brown pigment in hepatic Kupffer cells. ♀- Non-neoplastic findings **500 ppm (95 mg/kg bw/d) -** No test item-related effects reported that would be associated with general toxicity or neoplasms. **5000 ppm (938 mg/kg bw/d) -** No test item-related effects reported that would be associated with general toxicity or neoplasms.

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Clinical signs, palpations, body weights, haematology, gross necropsy and histopathology were recorded. EPA 83-2 guideline and GLP Reference: [23], 1992 (DAR 6.5.2)		**50 000 ppm (10 535 mg/kg bw/d) -** Mean bodyweight reduced at week 80 (6%), increased food consumption, accumulation of brown pigment in hepatic Kupffer cells. ♂- Neoplastic findings Hepatocellular adenoma- 16, 32, 8 and 12% at 0, 500, 5000 and 50 000 ppm Hepatocellular carcinoma- 6, 20, 18, 4% at 0, 500, 5000 and 50 000 ppm *Historical Control Data in CD-1 male mice conducted from 1986-1996 in dietary, gavage and drinking water studies:* *Hepatocellular adenoma incidence range- 7-22%* *Hepatocellular carcinoma incidence range- 0-10.0%* ♀- Neoplastic findings Hepatocellular adenoma- 0, 8, 4 and 0% at 0, 500, 5000 and 50 000 ppm Hepatocellular carcinoma- 2, 8, 2 and 0% at 0, 500, 5000 and 50000 ppm *Historical Control Data in CD-1 female mice conducted from 1986-1996 in dietary, gavage and drinking water studies:* *Hepatocellular adenoma incidence range- 0-8.0%* *Hepatocellular carcinoma incidence range- 0-2.0%*

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

Two studies have been submitted, one in rats and one in mice. The rat study pre-dates GLP and no guidelines have been stated, but generally conforms to OECD 453. For the mouse study, GLP has been adhered to and it was conducted in accordance with EPA 83-2.

Rat

Male and female CD rats were exposed to asulam sodium for 108 weeks [21]. Mortality was high across all dose groups but was not linked to treatment with asulam; deaths occurred in 34/50, 34/50, 39/50 and 26/50 males at 0, 36, 180 and 953 mg/kg bw/day and in 35/50, 37/50, 33/50, 30/50 females at 0, 47, 243 and 1280 mg/kg bw/day. The incidence of mortality was insufficient for termination in accordance with OECD guidance (< 25% survivors in controls and low dose group), but 50% of animals were not present in each group at study termination. Whilst the mortality rate was not linked to asulam sodium toxicity or neoplasms, the low numbers of animals at termination compromises the integrity of this study.

The main non-neoplastic findings focussed on changes in haematological parameters at mid and high dose groups which were consistent with microcytic anaemia and minor reductions in body weight gain particularly in female rats (13% ♂, 18% ♀). In addition, both sexes reported increased incidence of enlarged thyroid (11/50 ♂, 11/50 ♀) with accompanying hyperplasia (11/50 ♂, 3/50 ♀) and epithelial whorls (5/50 ♂, 4/50 ♀) and increased incidence of bile duct hyperplasia at the highest dose (17/50 ♂, 13/50 ♀). Top dose males also reported increased incidence of adrenal medullary hyperplasia (17/50), increased incidence of splenic siderocytes (7/50) and increased incidence of pituitary hyperplasia (13/50).

Neoplastic findings were reported in male rats only and an increase in phaeochromocytomas (20%) was observed at the top dose which was slightly outside the historical control range (2-16%); this was accompanied by adrenal medullary hyperplasia. There was no dose response seen for this tumour type and no change in latency period across controls and treated groups (initiated week 77 onwards). Although the histopathology report did not differentiate between benign or malignant tumours, the authors state in the summary section that benign phaeochromocytomas were increased with no sign of malignancy.

Mouse

In the mouse study [**Error! Bookmark not defined.**], deaths occurred in males at 47/75, 2/75, 50/75 and 47/75 with 0, 74, 730 and 8040 mg asulam sodium/kg bw/d whilst in females the incidence was 45/75, 37/75, 46/75 and 53/75 with 0, 95, 938 and 10 353 mg asulam sodium/kg bw/d. The high mortality incidence was not treatment related in either sex and was insufficient for termination in accordance with OECD guidance (< 25% survivors in controls and low dose group). There was not, however, 50% present in each group at study termination although 50% were alive at 86 weeks for males and 89 weeks for females. Whilst the mortality rate was not linked to asulam sodium toxicity or neoplasms, the low numbers of animals available at termination does compromise the integrity of the study.

There were no test item-related effects at the lowest dose in either male or female mice. At mid-and high doses, haematological effects were reported which were consistent with mild microcytic anaemia. The only other non-neoplastic finding at the mid-dose was accumulation of brown pigment in the spleen in males. At the high dose, non-neoplastic findings included decreased bodyweight in males throughout the study period (max. 10%), decreased body weight in females at week 80 (6%), increased food consumption in both sexes, accumulation of brown pigment in the spleen and hepatic Kupffer cells in both sexes and brown pigment in the renal proximal tubule in females only.

Neoplastic events were limited to an increase in hepatocellular adenoma and carcinoma in both sexes at the lowest dose tested.

In females, the hepatocellular carcinomas were elevated and outside the HCD but no dose response was observed, there was no accompanying liver toxicity i.e. histopathological changes or variation in liver weight and the lack of tumours in higher dose groups cannot be explained by the toxicity of the test compound i.e. there was no increase in mortality or exceedance of the maximum tolerated dose. Equally, the increase in hepatocellular adenoma was within the collated historical control data incidence, no dose response was observed and no accompanying liver histopathology was reported.

In males, the incidence of hepatocellular adenomas and carcinomas was highest at the lowest tested dose and both were outside the historical control data. Neither tumour type showed a dose-response relationship, there was no accompanying liver toxicity, *i.e*. histopathological changes or variation in liver weight and the lack of tumours in higher dose groups cannot be explained by the toxicity of the test compound.

Overall, the adenoma and carcinomas are not sufficient evidence of a carcinogenic effect particularly in the absence of a dose-response relationship and no toxicity to asulam sodium at higher dose levels to account for the lack of tumour incidence. Although the study had some methodological limitations overall it is sufficient to conclude that asulam sodium was not carcinogenic in this study

4.10.1.2 Carcinogenicity: inhalation

No information available.

4.10.1.3 Carcinogenicity: dermal

No information available.

4.10.2 Human information

No information available.

4.10.3 Other relevant information

Rats

Whilst the phaeochromocytomas were outside the laboratory's historical control data, data published by the animal supplier (11 studies performed during 1977-85) report control incidences for benign tumours of 0-18.0% (mean 6.0%). In addition, published data from other studies with Sprague Dawley rats are supportive of the spontaneous nature of phaeochromocytomas in aging animals and the incidence is variable from 4-33% (Suzuki *et al* [34], Chandra *et al* [35] and McMartin *et al* [36]), the most relevant paper being the paper by McMartin which employed Charles River Sprague Dawley rats (Crl:CD) during 1984-1991. The mean incidence of benign phaeochromocytomas in males was 19% with a range of 10.2-30%.

The conditions leading to chemically induced phaeochromocytomas in animal studies include hypoxia, uncoupling of oxidative phosphorylation, disturbances in calcium homeostasis and disturbance of the hypothalamic endocrine axis ([37]). Taking into consideration all available data, there is no evidence that asulam directly generates the required conditions for phaeochromocytoma formation *i.e.* there is no pulmonary toxicity leading to low oxygen levels, calcium concentrations have not been reported to be affected by treatment, kidney function is not altered and there is no evidence that asulam uncouples mitochondrial respiration as no increase in brown adipose has been reported.

In long-term studies, chemically induced phaeochromocytomas can occur together with other tumours or toxic effects in other organs. Typically phaeochromocytomas concurrently cause nephrotoxic effects, neoplastic liver changes or endocrine disturbances, with tumours in different endocrine glands such as the thyroid, pancreas, preputial gland, zymbal gland or Harderian gland previously reported ([34], [37]). With the exception of thyroid effects at high doses in repeat dose studies, asulam-sodium does not impact on endocrine organs or produce tumours in endocrine tissues except the adrenal gland but the phaeochromocytomas occur in isolation, it is concluded that they are spontaneous in nature and are not chemically induced by asulam- sodium.

4.10.4 Summary and discussion of carcinogenicity

The only neoplasm in rats was phaeochromocytomas in males at the highest dose tested (953 mg/kg bw/d). Whilst the incidence of this neoplasm exceeded the laboratory's historical control data, there is information in the publically-available literature on the incidence of this tumour type in Sprague Dawley rats which shows phaeochromocytomas

can spontaneously occur at an incidence of up to 33%. Furthermore, this tumour is referenced in the CLP guidance as having a high spontaneous incidence rate in Sprague Dawley rats. The tumour was also not dose responsive, limited to a single sex and species, with no evidence of a multi-site response to asulam and no direct evidence from the toxicology package of studies to support chemical induction of phaeochromocytomas in accordance with published literature [37].

In the mouse study, the incidence of hepatocellular adenomas and carcinomas were increased in both sexes at the lowest dose tested. However, there was no dose response over the test range (100-fold), no accompanying histopathology, no toxicity at mid or high dose levels to account for the decrease in tumours and the neoplasm was restricted to one species and one site. Overall whilst the study had some methodological limitations, these tumours are not considered sufficient for classification purposes.

4.10.5 Comparison with criteria

In carcinogenicity studies an increased incidence of adrenal phaeochromocytomas in male rats in the highest dose group of 953 mg/kg/day was observed, but without a clear dose-response relationship and in the presence of general toxicity, *i.e*. a reduction in weight gain of 13%. Although at study termination there was high mortality at all dose levels, including controls, this was not due to the presence of tumours. In addition, mortality increased significantly towards the end of the study such that > 50% of each sex were alive at week 93 for males and week 101 for females (~90% or more of the study completed) and > 45% were alive at the top dose for both sexes at week 104. Furthermore, all tumours for decedents were recorded to give a full tumour profile for asulam.

A substance may be placed in Category 2 for carcinogenicity on the basis of limited evidence in experimental animals or human studies. 'Limited evidence' includes data that suggest a carcinogenic effect but are limited for making a definitive evaluation because, for example, the evidence of carcinogenicity is restricted to a single study; there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential. Other supporting information can be taken into account to increase or decrease the category of classification.

The phaeochromocytomas in rats could be interpreted as limited evidence of carcinogenicity, but based on other considerations highlighted in the CLP guidance it is concluded that they do not lead to classification of asulam sodium. The justifications for non-classification based on adrenal tumours are as follows:

1. The tumour type is consistent with high spontaneous tumour incidence highlighted in the CLP guidance (e.g. RIVM, [38]- male rat incidence is 0.12-45% in Sprague Dawley, 2.8-45% in F344, 10.6-69.2% in Wistar, 0-69% in general).

2. Although the incidence slightly exceeded the laboratory's HCD in the high-dose group, there are relevant examples in the literature which show that the incidence can be as high as 33% compared with 20% observed in the asulam rat study. There was not a statistically significant increase compared with the controls.

3. A dose-response relationship was not evident over the wide dose range tested (36/47 to 953/1280 mg/kg/day in males and females, respectively), although it is noted that there were high mortality rates across the groups which could impact on this.

4. Neoplasms were not reported in any other organs or tissues even at the high doses tested of up to 1280 mg/kg/d in rats and > 10000 mg/kg/day in mice.
5. The lesions did not appear to progress to malignancy.
6. The response was limited to a single sex and species, although it is appreciated that female rats and mice are generally less susceptible to this tumour type ([38] and [39]).
7. The development of phaeochromocytomas was exclusively associated with senescent animals, with the latency period not being decreased.
8. No evidence of a mechanism of action attributed to chemically induced phaeochromocytomas, as noted in the public domain.
9. There are no reported effects in the whole toxicity package that support the generation of phaeochromocytomas by chemical induction according to published literature ([34]) and the RIVM report [38].

The liver adenomas and carcinomas in mice could be interpreted as limited evidence of carcinogenicity but these were not dose responsive, lacked accompanying liver toxicity and the decrease in incidence at mid- and high doses cannot be explained by treatment-related toxicity. Although at study termination there was high mortality at all dose levels including controls, this was not due to the presence of tumours. In addition, mortality increased significantly towards the end of the study such that > 50% of animals was still alive at 86 weeks for males and 89 weeks for females (i.e. at a time by which any liver tumours had already started to develop). Furthermore, all tumours for decedents were recorded to give a full tumour profile for asulam. Overall, therefore, although there were methodological limitations in the study, the liver adenomas and carcinomas in the mouse are not considered sufficient for classification.

4.10.6 Conclusions on classification and labelling

Not classified- conclusive but not sufficient for classification

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification based on the data from two oral carcinogenicity studies, one in rats and one in mice.

The carcinogenicity studies with asulam-sodium given in the diet were performed on rats and mice. The rat study pre-dates GLP and no guidelines have been stated, but generally conforms to OECD TG 453. For the mouse study, GLP has been adhered to and it was conducted in accordance with EPA 83-2.

Summary table of carcinogenicity studies

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
108 week carcinogenicity study Asulam-sodium (purity not stated) CD rats (Sprague Dawley origin) 50/sex/group Satellite group (sacrificed at 78 weeks) 15/sex/group Clinical signs, palpations, body weights, food consumption ophthalmoscopy, haematology, clinical chemistry, urine analysis, gross necropsy and histopathology were recorded. No guideline stated, but similar to OECD TG 453. Pre-GLP Reference: Report R001275J, 1981 (DAR 6.5.1)	0, 1000, 5000, 25000 ppm ♂: 0, 36, 180, 953 mg/kg bw/d) ♀: 0, 47, 243, 1280 mg/kg bw/d)	♂ Non-neoplastic findings **1000 ppm (36 mg/kg bw/d) -** No test item-related effects reported that would be associated with neoplasms or general toxicity. **5000ppm (180 mg/kg bw/d) -** No test item-related effects reported that would be associated with neoplasms or general toxicity. **25000 ppm (953 mg/kg bw/d)** - reduced weight gain (13%), increased incidence of adrenal medullary hyperplasia (17/50). ♀ Non-neoplastic findings **1000 ppm (47 mg/kg bw/d) -** No test item-related effects reported that would be associated with neoplasms or general toxicity. **5000 ppm (243 mg/kg bw/d) -** reduced weight gain in females between weeks 6-52 (13%). **25 000 ppm (1280 mg/kg bw/d) -** reduced weight gain between weeks 6-52 (18%) Males - Neoplastic findings Phaeochromocytomas - 6% (3/50), 10% (5/50), 8% (4/50) and 20% (10/50) at 0, 1000, 5000 and 25 000 ppm, respectively Laboratory historical control incidences were in the range 2% - 16%, (from 6 studies conducted in1978 with the same strain of rat i.e. CD rats of Sprague Dawley origin) With the exception of two tumours (1 at 5000 ppm observed on week 77 and 1 at 25000 ppm on week 76), the phaeochromocytomas occurred in aged rats (> 80 weeks) in all groups. There was no decrease in latency observed across the treated groups compared to controls. ♀- Neoplastic findings No test item-related effects
Two-year carcinogenicity study in mice Asulam-sodium (purity 88%) CD-1 mice 75/sex/group Satellite group (sacrificed at12 months)- 10/sex/group Clinical investigations only- 15/sex/group Clinical signs, palpations, body weights, haematology, gross necropsy and histopathology were recorded. EPA 83-2 guideline and GLP Reference: Report R003662, 1992 (DAR 6.5.2)	0, 500, 5000 and 50 000 ppm ♂: 0, 74, 730, 8040 mg/kg bw/d) ♀: 0, 95, 938, 10353 mg/kg bw/d)	♂- Non-neoplastic findings **500 ppm (74 mg/kg bw/d) -** No test item-related effects reported that would be associated with general toxicity or neoplasms. **5000 ppm (730 mg/kg bw/d)** - No test item-related effects reported that would be associated with general toxicity or neoplasms. **50000 ppm (8040 mg/kg bw/d)** - decreased mean bodyweight throughout study period (range 3 - 10%), increased food consumption, accumulation of brown pigment in hepatic Kupffer cells. ♀- Non-neoplastic findings **500 ppm (95 mg/kg bw/d) -** No test item-related effects reported that would be associated with general toxicity or neoplasms. **5000 ppm (938 mg/kg bw/d) -** No test item-related effects reported that would be associated with general toxicity or neoplasms. **50000 ppm (10535 mg/kg bw/d) -** Mean bodyweight reduced at week 80 (6%), increased food consumption, accumulation of brown pigment in hepatic Kupffer cells. ♂- Neoplastic findings Hepatocellular adenoma - 16, 32, 8 and 12% at 0, 500, 5000 and 50000 ppm Hepatocellular carcinoma - 6, 20, 18, 4% at 0, 500, 5000 and 50000 ppm *Historical Control Data in CD-1 male mice conducted from 1986-1996 in dietary, gavage and drinking water studies:* *Hepatocellular adenoma incidence range was 7 - 22%* *Hepatocellular carcinoma incidence range was 0 - 10.0%*

		♀- Neoplastic findings Hepatocellular adenoma - 0, 8, 4 and 0% at 0, 500, 5000 and 50000 ppm Hepatocellular carcinoma - 2, 8, 2 and 0% at 0, 500, 5000 and 50000 ppm *Historical Control Data in CD-1 female mice conducted from 1986-1996 in dietary, gavage and drinking water studies:* *Hepatocellular adenoma incidence range – 0 - 8.0%* *Hepatocellular carcinoma incidence range – 0 - 2.0%*

Rat

Male and female CD rats were exposed to asulam-sodium for 108 weeks (Report R001275J, 1981). Mortality was high across all dose groups but was not considered linked to treatment with asulam due to lack of dose response relationship; deaths occurred in 34/50, 34/50, 39/50 and 26/50 males at 0, 36, 180 and 953 mg/kg bw/d, respectively, and in 35/50, 37/50, 33/50, 30/50 females at 0, 47, 243 and 1280 mg/kg bw/d, respectively. The incidence of mortality was insufficient for an earlier termination of the study in accordance with OECD guidance 116 (> 25% in controls and low dose group), but 50% of animals were not present in each group at study termination. Overall, the low numbers of animals at termination compromises the integrity of this study.

The main non-neoplastic findings focussed on changes in haematological parameters at mid and high dose groups which were consistent with microcytic anaemia and minor reductions in body weight gain, particularly in female rats (13% ♂, 18% ♀). In addition, both sexes reported increased incidences of enlarged thyroid (11/50 ♂, 11/50 ♀) with accompanying hyperplasia (11/50 ♂, 3/50 ♀) and epithelial whorls (5/50 ♂, 4/50 ♀) and increased incidences of bile duct hyperplasia at the highest dose (17/50 ♂, 13/50 ♀). Top dose males also displayed increased incidences of adrenal medullary hyperplasia (17/50), splenic siderocytes (7/50) and pituitary hyperplasia (13/50).

Neoplastic findings were reported in male rats only and an increase in phaeochromocytomas (20%) was observed at the top dose which was slightly outside the historical control range (2-16%); this was accompanied by adrenal medullary hyperplasia. No dose response relationship was seen for this tumour type and no change in latency period across controls and treated groups. Although the histopathology report did not differentiate between benign or malignant tumours, the authors state in the summary section that benign phaeochromocytomas were increased with no sign of malignancy. Whilst the phaeochromocytomas were outside the laboratory's HCD, data published by the animal supplier (11 studies performed during 1977-85) reported control incidences for benign tumours of 0-18.0% (mean 6.0%). In addition, published data from other studies with Sprague Dawley rats are supportive of the spontaneous nature of phaeochromocytomas in aging animals and the incidence is variable, ranging between 4 - 33% (Suzuki *et al.*, 1979; Chandra *et al.*, 1992; McMartin *et al.*, 1992), the most relevant paper being the paper by McMartin which employed Charles River Sprague Dawley rats (Crl:CD) during 1984-1991. The mean incidence of benign phaeochromocytomas in males was 19% with a range of 10.2 - 30%.

The conditions leading to chemically induced phaeochromocytomas in animal studies include hypoxia, uncoupling of oxidative phosphorylation, disturbances in calcium homeostasis and disturbance of the hypothalamic endocrine axis (Griem *et al.*, 2009). Taking into consideration all available data, there is no evidence that asulam-sodium directly generates the required

conditions for phaeochromocytoma formation i.e. there is no pulmonary toxicity leading to low oxygen levels, calcium concentrations have not been reported to be affected by treatment, kidney function is not altered and there is no evidence that asulam uncouples mitochondrial respiration as no increase in brown adipose tissue has been reported.

In long-term studies, chemically induced phaeochromocytomas can occur together with other tumours or toxic effects in other organs. Typically phaeochromocytomas cause nephrotoxic effects, neoplastic liver changes or endocrine disturbances, concurrently with tumours in different endocrine glands such as the thyroid, pancreas, preputial gland, zymbal gland or Harderian gland previously reported (Suzuki *et al.*, 1979, Griem *et al.*, 2009). With the exception of thyroid effects at high doses in repeat dose studies, asulam-sodium does not impact on endocrine organs or produce tumours in endocrine tissues except the adrenal gland. Since the phaeochromocytomas occured in isolation, it is concluded that they are spontaneous in nature and are not chemically induced by asulam-sodium.

Mouse

In the mouse study (Report R003662, 1992), deaths occurred in males at 47/75, 42/75, 50/75 and 47/75 with 0, 74, 730 and 8040 mg asulam-sodium/kg bw/d, respectively, whilst in females the incidence was 45/75, 37/75, 46/75 and 53/75 with 0, 95, 938 and 10 353 mg asulam-sodium/kg bw/d, respectively. The high mortality incidence was not treatment related in either sex and was insufficient for early termination of the study in accordance with OECD guidance 116 (i.e. mortality was < 25% in controls and low dose group). However, survival was less than 50% in each group at study termination although 50% were alive at 86 weeks for males and 89 weeks for females. Whilst the mortality rate was not linked to asulam-sodium toxicity, the low numbers of animals available at termination was not considered to compromise the integrity of the study.

There were no test item-related effects at the lowest dose in either male or female mice. At mid-and high doses, haematological effects were reported which were consistent with mild microcytic anaemia. The only other non-neoplastic finding at the mid-dose was accumulation of brown pigment in the spleen in males. At the high dose, non-neoplastic findings included decreased bodyweight in males throughout the study period (up to 10%), decreased body weight in females at week 80 (by 6%), increased food consumption in both sexes, accumulation of brown pigment in the spleen and hepatic Kupffer cells in both sexes and brown pigment in the renal proximal tubule in females only.

Neoplastic events were limited to an increase in hepatocellular adenoma and carcinoma in both sexes at the lowest dose tested.

In females, the incidences of hepatocellular carcinomas were elevated and outside the HCD but no dose response relationship was observed, there was no accompanying liver toxicity and therefore the histopathological changes or variation in liver weight and the lack of tumours in higher dose groups cannot be explained by the toxicity of the test compound, since there was no increase in mortality and the maximum tolerated dose did not appear to have been exceeded. Similarly, the increase in incidence of hepatocellular adenoma was within the HCD incidence, no dose response relationship was observed and no accompanying liver histopathology was reported.

In males, the incidence of hepatocellular adenomas and carcinomas was highest at the lowest tested dose and both were outside the HCD range. Neither tumour type showed a dose-response relationship, there was no accompanying liver toxicity, and therefore the

histopathological changes or variation in liver weight and the lack of tumours in higher dose groups cannot be explained by the toxicity of the test compound.

Overall, the incidence of adenoma and carcinomas in mice are not considered to be sufficient evidence of a carcinogenic effect particularly in the absence of a dose-response relationship and the absence of toxicity to asulam-sodium at higher dose levels to account for the lack of tumour incidence. Although the study had some methodological limitations, overall it was adequate for the conclusion to be drawn that asulam-sodium was not carcinogenic in this study.

Comments received during public consultation

Two MSCAs agreed with the justification for no classification of asulam-sodium for carcinogenicity, as proposed by the DS.

Assessment and comparison with the classification criteria

As pointed out by the DS, the phaeochromocytomas in rats could be interpreted as limited evidence of carcinogenicity, but based on considerations highlighted in the CLP guidance it is concluded that classification of asulam-sodium for carcinogenicity is not warrented. The justifications for non-classification based on adrenal tumours are as follows:

1. The tumour type is consistent with high spontaneous tumour incidence highlighted in the CLP guidance (e.g. RIVM, (2001) tumour incidence in male rats was 0.12-45% in Sprague Dawley, 2.8-45% in F344, 10.6-69.2% in Wistar, 0-69%).
2. Although the incidence slightly exceeded the laboratory's HCD in the high-dose group, there are relevant examples in the literature which show that the incidence can be as high as 33% compared with 20% observed in the asulam rat study. There was not a statistically significant increase compared with the controls.
3. A dose-response relationship was not evident over the wide dose range tested (36/47 to 953/1280 mg/kg bw/d in males and females, respectively).
4. Neoplasms were not reported in any other organs or tissues even at the high doses tested of up to 1280 mg/kg bw/d in rats and > 10000 mg/kg bw/d in mice.
5. The lesions did not appear to progress to malignancy.
6. The response was limited to a single sex and species, although it is apparent that female rats and mice are generally less susceptible to this tumour type (RIVM, 2001; Tischler *et al.*, 2004).
7. The development of phaeochromocytomas was exclusively associated with senescent animals, with the latency period not being decreased.
8. There are no reported effects in the toxicity studies that support the generation of phaeochromocytomas by chemical induction according to published literature (Suzuki *et al.*, 1979) and the RIVM report (2001).

The liver adenomas and carcinomas in mice could be interpreted as limited evidence of carcinogenicity but these were not dose responsive, lacked accompanying liver toxicity and

the decrease in incidence at mid- and high doses cannot be explained by treatment-related toxicity. There were methodological limitations in the study but overall, the evidence for liver adenomas and carcinomas in the mouse are not considered sufficient for classification.

Taking into account the above outlined justification, RAC, in agreement with the proposal of the DS, is of the opinion that asulam-sodium **does not warrant classification for carcinogenicity.**

4.11 Toxicity for reproduction

A two-generation reproduction study in rats was performed with asulam sodium to investigate fertility effects. Studies in rats and rabbits have been conducted to evaluate the developmental effect of the test substance.

4.11.1 Effects on fertility

Table 19: Summary table of relevant reproductive toxicity studies - Fertility

<table>
<tr><th>Method</th><th>Dose levels</th><th>Observations and remarks
(effects of major toxicological significance)</th></tr>
<tr><td>Two-generation study
Asulam sodium (purity 99%)
Dietary administration
CD rats
F_0- 12 ♂ / 24 ♀ /group
F_1- 16 ♂ / 32 ♀/ group
Gross necropsy was performed on F_0 parents, non-selected F_1 pups, non-selected F_1 parents and all F_2 pups
Histopathology of testes in F_0 examined
No guideline stated, but similar to OECD 416. Pre-GLP

Reference: [40], 1981 (DAR 6.6.1)</td><td>0, 1000, 5000, 25 000 ppm</td><td>Limitations of the study-
1. data on implantations were only recorded for decedents or dams without litters post expected parturition date
2. corpora lutea are only referenced for one dam.
3. sperm parameters were not investigated.
4. litters were not standardised on lactation day 4.
5. historical control data is not available.
6. the top dose of 25 000 ppm in this two-generation reproductive study exceeds the limit dose in F_0 and F_1 animals (>1000 mg/kg bw/d).

Parental effects
<table>
<tr><td></td><td></td><td colspan="8">Dose level (ppm)</td></tr>
<tr><td></td><td></td><td colspan="4">♂</td><td colspan="4">♀</td></tr>
<tr><td></td><td></td><td>0</td><td>1000</td><td>5000</td><td>25000</td><td>0</td><td>1000</td><td>5000</td><td>25000</td></tr>
<tr><td>Pre-mating (mg/kg bw/d)</td><td></td><td>0</td><td>124</td><td>568</td><td>3070</td><td>0</td><td>119</td><td>612</td><td>3409</td></tr>
<tr><td>Post-mating (mg/kg bw/d)</td><td></td><td>0</td><td>46</td><td>224</td><td>1162</td><td>0</td><td>58</td><td>278</td><td>1531</td></tr>
<tr><td colspan="10">Organ weights (F_1)</td></tr>
<tr><td>Liver</td><td>(g)</td><td>26.3</td><td>26.6</td><td>25.6</td><td>254</td><td>13.5</td><td>12.0*</td><td>13.3</td><td>11.4**</td></tr>
<tr><td></td><td>rel</td><td>3.78</td><td>3.77</td><td>3.68</td><td>3.55</td><td>3.57</td><td>3.45</td><td>3.54</td><td>3.27**</td></tr>
<tr><td>Thyroid</td><td>(mg)</td><td>31.6</td><td>35.3</td><td>34.2</td><td>39.4**</td><td>29.4</td><td>26.9</td><td>29.1</td><td>29.0</td></tr>
<tr><td></td><td>rel</td><td>4.6</td><td>5.2</td><td>5.0</td><td>5.5*</td><td>7.8</td><td>7.7</td><td>7.7</td><td>8.2</td></tr>
</table>

Reproductive effects
<table>
<tr><td>ppm</td><td>0</td><td>1000</td><td>5000</td><td>25000</td></tr>
<tr><td colspan="5">F_1</td></tr>
<tr><td>Litter size day 0 (total)</td><td>12.4</td><td>11.1</td><td>9.6**</td><td>9.3**</td></tr>
<tr><td>Litter size day 30</td><td>11.2</td><td>10.5</td><td>9.1*</td><td>8.7**</td></tr>
<tr><td>Stillborn pups (absolute no)</td><td>6</td><td>4</td><td>1</td><td>0</td></tr>
<tr><td>Survival (%) day 4</td><td>93</td><td>95</td><td>98</td><td>94</td></tr>
<tr><td>Survival % day 30</td><td>92</td><td>91</td><td>95</td><td>94</td></tr>
<tr><td>Fertility Index (%)[#]</td><td>91</td><td>97</td><td>86</td><td>87</td></tr>
<tr><td>Viability index (%)</td><td>93</td><td>95</td><td>98</td><td>94</td></tr>
<tr><td>Lactation index (%)</td><td>93</td><td>91</td><td>97</td><td>99</td></tr>
</table></td></tr>
</table>

Method	Dose levels	Observations and remarks (effects of major toxicological significance)

F_2				
Litter size day 0 (total)	11.0	10.1	8.0*	9.4
Litter size at day 30	9.3	9.8	7.2	7.8
Stillborn pups (absolute no)	6	5	1	0
Survival (%) day 4	88	92	88	82
Survival % day 30	87	91	82	82
Fertility Index (%)	83	83	62	74
Viability index (%)	88	92	88	82
Lactation index (%)	98	99	93	100

* significantly different from controls ($p<0.05$); ** $p<0.01$, # fertility index= (No. of pregnant females/ No. of females which mated)*100

Offspring effects

Organ weights		Dose level (ppm)							
		♂				♀			
		0	1000	5000	25000	0	1000	5000	25000
F_1									
Pituitary	(mg)	3.8	5.3*	6.0**	4.6	5.5	4.9	6.0	3.8
	(rel)	3.9	5.1	5.5*	4.6	5.9	4.9	5.8	4.5
Liver	(g)	6.15	5.96	5.99	5.63	5.92	5.56	5.64	4.66**
	(rel)	6.17	5.75	5.31	5.58	6.41	5.59*	5.49*	5.55*
Ovary	(mg)					55.5	47.9	54.7	38.3*
	(rel)					58.6	48.1	54.3	47.0
F_2									
Pituitary	(mg)	10.3	9.8	11.2	10.9	10.3	10.2	11.7	13.2*
	(rel)	3.1	3.1	3.4	3.3	4.9	5.0	5.5	6.3*
Kidney	(g)	2.82	2.76	3.01	3.27*	2.02	1.93	2.02	1.99
	(rel)	0.84	0.87	0.92*	0.99**	0.94	0.93	0.94	0.95
Thyroid	(mg)	22.2	21.1	23.5	30.0*	17.8	21.6	17.9	24.2*
	(rel)	6.9	6.6	7.1	9.1	8.5	10.3	8.5	11.6*

4.11.1.1 Non-human information

The effects of asulam sodium on fertility have been investigated in a multi-generation study [40] in CD rats of Sprague Dawley origin. However, this study has limitations, *e.g.* lack of information on implantations, which make the findings difficult to interpret, particularly in conjunction with the limited developmental toxicity studies (refer to Section 4.11.2.1).

Parental toxicity manifested as a decrease in body weight and a variation in organ weights at 25000ppm (3409 mg/kgbw/day during pre-mating, 1531 mg/kg/day post-mating). In the F_0 parents, top dose males had a slightly lower mean body weight compared with controls but this was not statistically significant and no dose response was observed. In contrast, body weights were affected in females only in F_1 parents at the top dose and a reduced weight gain at mating (10%) was reported. In the F_1 parents, mean absolute and relative liver weights were slightly (but significantly) lower in top dose females; thyroid weights were significantly higher in males at the top dose level.

No effects were seen on fertility index, gestation, viability or survival index in F_1 or F_2 litters. A decrease was seen in litter size in F_1 pups across all treated groups which attained statistical significance at ≥5000 ppm but no dose response was observed as a 5-fold increase in dose to 25000 ppm only produced a decrease in litter size from 9.6 to 9.3. Litter size was reduced across treated groups in the F_2 generation; however, this was only statistically significant in the mid-dose group and did not demonstrate a dose–response relationship. Pup body weight at birth was not affected in the F_1 or F_2 generation.

In the offspring, effects were only reported in organ weights, *e.g.* ovary, kidney, thyroid and pituitary at the top dose; however, there was a lack of adverse histopathology to accompany these changes, a dose-response relationship was absent and the effects reported were not consistent across generations or sexes.

Overall, in a limited two-generation rat study performed with asulam sodium, the only effect was a reduction in litter size in the F_1 and F_2 generation in the groups exposed to ≥5000 ppm. The underlying reason for this effect was unclear.

4.11.1.2 Human information

No data available

4.11.2 Developmental toxicity

Table 20: Summary table of relevant reproductive toxicity studies - Development

<table>
<tr><th>Method</th><th>Dose levels</th><th>Observations and remarks
(effects of major toxicological significance)</th></tr>
<tr><td>Developmental study in rats
Asulam sodium (purity 98.6%)
CD rats
20/group
Oral gavage on days 6 to 15 of gestation
No guideline stated, but similar to early version of OECD 414. Pre-GLP

Reference: [41], 1981 (DAR 6.6.2)</td><td>0, 500, 1000, 2000 mg/kg bw/d</td><td>
<u>Maternal toxicity:</u>
There were no reported clinical signs or effects on body weight, food consumption, effects in dams at any dose level. At necropsy, each animal was examined macroscopically and specimens considered to be abnormal were retained. No maternal necropsy findings were found in the original study report.
<u>Developmental effects</u>
No test item effects were reported in litter parameters at 500, 1000 or 2000 mg/kg bw/d.

Litter data
<table>
<tr><th colspan="2" rowspan="2">Parameter</th><th colspan="4">Dose level (mg/kg bw/d)</th><th rowspan="2">Historical control mean (range)</th></tr>
<tr><th>0</th><th>500</th><th>1000</th><th>2000</th></tr>
<tr><td colspan="2">Pregnant</td><td>20</td><td>20</td><td>20</td><td>20</td><td></td></tr>
<tr><td colspan="2">Corpora lutea</td><td>16.9 ±2.8</td><td>16.7 ±3.3</td><td>17.6 ±3.8</td><td>17.6 ±2.6</td><td>15.9 (14.0-18.3)</td></tr>
<tr><td colspan="2">Implantations</td><td>14.7 ±1.5</td><td>14.3 ±1.9</td><td>14.6 ±2.2</td><td>15.2 ±1.4</td><td>14.2 (11.6-16.5)</td></tr>
<tr><td rowspan="3">Viable foetuses</td><td>♂</td><td>6.2 ±2.1</td><td>6.4 ±2.2</td><td>7.1 ±2.2</td><td>7.6 ±1.8</td><td>6.8 (5.0-8.0)</td></tr>
<tr><td>♀</td><td>7.4 ±2.1</td><td>7.2 ±2.1</td><td>6.8 ±1.7</td><td>7.2 ±2.0</td><td>6.7 (5.5-8.4)</td></tr>
<tr><td>Total</td><td>13.5 ±2.9</td><td>13.6 ±2.2</td><td>13.9 ±2.3</td><td>14.7 ±1.7</td><td>13.5 (10.9-15.9)</td></tr>
<tr><td rowspan="3">Resorptions</td><td>Early</td><td>0.95 ±0.97</td><td>0.60 ±0.77</td><td>0.50 ±0.71</td><td>0.30 ±0.55</td><td>0.55 (0.08-1.53)</td></tr>
<tr><td>Late</td><td>0.20 ±0.45</td><td>0.10 ±0.32</td><td>0.25 ±0.50</td><td>0.15 ±0.39</td><td>0.13 (0.00-1.45)</td></tr>
<tr><td>Total</td><td>1.15 ±1.07</td><td>0.70 ±0.84</td><td>0.75 ±0.87</td><td>0.45 ±0.67</td><td>0.68 (0.07-1.91)</td></tr>
<tr><td colspan="2">Pre-implantation loss</td><td>13.3%</td><td>14.7%</td><td>17.0%</td><td>13.9%</td><td>11.0 (2.6-20.9)</td></tr>
<tr><td colspan="2">Post-implantation loss</td><td>7.8%</td><td>4.9%</td><td>5.1%</td><td>3.0%</td><td>4.8 (0.5-14.0)</td></tr>
<tr><td colspan="2">Foetal weight (g)</td><td>3.32 ±0.07</td><td>3.39 ±0.06</td><td>3.38 ±0.07</td><td>3.31 ±0.07</td><td>3.69 (3.5-4.04)</td></tr>
<tr><td colspan="2">Placental weight (g)</td><td>0.46 ±0.02</td><td>0.47 ±0.02</td><td>0.48 ±0.02</td><td>0.47 ±0.01</td><td>0.50 (0.45-4.04)</td></tr>
</table>

Findings at necropsy were reported only in fetuses at 2000 mg/kg bw/d (summarised in the table below).
</td></tr>
</table>

<table>
<tr><th>Method</th><th>Dose levels</th><th>Observations and remarks
(effects of major toxicological significance)</th></tr>
<tr><td></td><td></td><td>
Fetal data at necropsy
<table>
<tr><th colspan="2" rowspan="2">Parameter</th><th colspan="4">Dose (ppm)</th><th rowspan="2">Historical control</th></tr>
<tr><th>0</th><th>500</th><th>1000</th><th>2000</th></tr>
<tr><td colspan="2">No of fetuses examined (litters)</td><td>176 (20)</td><td>177 (20)</td><td>181 (20)</td><td>197 (20)</td><td>10954[a]
Mean % fetuses (range)</td></tr>
<tr><td rowspan="2">↓ossification; cranium</td><td>No. of fetuses/%</td><td>0/0</td><td>0/0</td><td>0/0</td><td>1/0.5</td><td>-</td></tr>
<tr><td>Litter incidence/ %</td><td>0/0</td><td>0/0</td><td>0/0</td><td>1/5.0</td><td>-</td></tr>
<tr><td rowspan="2">13th rib(s) short/ absent</td><td>No. of fetuses/%</td><td>2/1.1</td><td>-</td><td>-</td><td>8/4.1</td><td>0.07
(0.0- 1.7)</td></tr>
<tr><td>Litter incidence/ %</td><td>2.0/10</td><td>-</td><td>-</td><td>3.0/15</td><td>-</td></tr>
<tr><td>Short</td><td>No./litter incidence</td><td>1/1</td><td></td><td></td><td>5/2</td><td></td></tr>
<tr><td>Absent</td><td>No./litter incidence</td><td>1/1</td><td></td><td></td><td>3/2</td><td></td></tr>
<tr><td rowspan="2">Fused cervical arches</td><td>No. of fetuses/%</td><td>0/0</td><td>0/0</td><td>0/0</td><td>1/0.5</td><td>-</td></tr>
<tr><td>Litter incidence/ %</td><td>0/0</td><td>0/0</td><td>0/0</td><td>1/5.0</td><td>-</td></tr>
<tr><td rowspan="2">↓ossification; caudal vertebrae</td><td>No. of fetuses/%</td><td>2/1.1</td><td>3/1.7</td><td>2/1.1</td><td>5/2.5</td><td>1.01
(0.6- 1.2)</td></tr>
<tr><td>Litter incidence/ %</td><td>1.0/5.0</td><td>3.0/15</td><td>2.0/10</td><td>5.0/25</td><td>-</td></tr>
</table>
a from 74 studies
</td></tr>
<tr><td>Developmental study in rabbits
Asulam sodium (purity 98%)
New Zealand white rabbits
15/group of Asulam
11 positive controls
Oral gavage on days 5-20 of gestation
Uterine contents examined on day 29
No guideline stated, but similar to early version of OECD 414. Pre-GLP</td><td>0, 150, 300, 750 mg/kg bw/d
Positive control- 150 mg/kg bw/d thalidomide</td><td>
Limitations of the study:
Fetuses- external examination for abnormalities and visceral abnormalities by dissection
Fetal brains investigated by serial sectioning
Skeletal findings investigated following Alizarin red staining

Maternal effects

Deaths occurred in 10/30, 9/28, 5/23, 5/23 dams at 0, 150, 300, 750 mg/kg bw/d.
These were attributed to mishandling, dosing error or infection and not treatment-related.
The group dosed with 1500 mg/kg bw/d was terminated early owing to mortality and toxicity “similar to starvation”
No test item related effects were reported in dams at 150 and 300 mg/kg bw/d.
At 750 mg/kg bw/d, bodyweight gain was reduced during the treatment phase (5-21d ↓ 35%) and reduced feed consumption was reported at 5-9d (5%), 9-13d (5%) and 13-17d (17%).
</td></tr>
</table>

<table>
<tr><th>Method</th><th>Dose levels</th><th>Observations and remarks
(effects of major toxicological significance)</th></tr>
<tr><td>Reference: [42], 1980 (DAR 6.6.3)</td><td></td><td>
<table>
<tr><th rowspan="2">Parameter</th><th colspan="5">Dose level (mg/kg bw/d)</th></tr>
<tr><th>0</th><th>150</th><th>300</th><th>750</th><th>+ve control</th></tr>
<tr><td>Mated</td><td>30</td><td>28</td><td>23</td><td>23</td><td>14</td></tr>
<tr><td>Not pregnant</td><td>4</td><td>1</td><td>2</td><td>4</td><td>2</td></tr>
<tr><td>Total deaths</td><td>10</td><td>9</td><td>5</td><td>5</td><td>1</td></tr>
<tr><td>Total resorption</td><td>-</td><td>-</td><td>-</td><td>-</td><td>2</td></tr>
<tr><td>Litters</td><td>16</td><td>18</td><td>16</td><td>14</td><td>8</td></tr>
</table>
<u>Developmental effects</u>
There were no effects reported on litter parameters at any dose level.
<table>
<tr><th rowspan="2">Parameter</th><th colspan="5">Dose level (mg/kg bw/d)</th></tr>
<tr><th>0</th><th>150</th><th>300</th><th>750</th><th>+ve control</th></tr>
<tr><td>Mean no. corpora lutea / dam</td><td>10.4</td><td>10.3</td><td>11.4</td><td>10.9</td><td>10.8</td></tr>
<tr><td>Mean no. implantations/dam</td><td>9.3</td><td>8.7</td><td>10.6</td><td>9.1</td><td>8.7</td></tr>
<tr><td>Pre-implantation loss/animal (%)</td><td>10.2</td><td>15.8</td><td>8.3</td><td>15.4</td><td>18.8</td></tr>
<tr><td>Post-implantation loss/animal (%)</td><td>4.8</td><td>7.1</td><td>12.1</td><td>9.5</td><td>59.5</td></tr>
<tr><td>Total no. of live pups</td><td>143</td><td>145</td><td>147</td><td>115</td><td>35</td></tr>
<tr><td>Fetal death</td><td>6</td><td>11</td><td>22</td><td>12</td><td>52</td></tr>
<tr><td>Early</td><td>4</td><td>6</td><td>11</td><td>1</td><td>18</td></tr>
<tr><td>Late</td><td>2</td><td>5</td><td>11</td><td>11</td><td>34</td></tr>
<tr><td>Litter size (#)</td><td>8.9</td><td>8.1</td><td>9.2</td><td>8.2</td><td>3.5</td></tr>
<tr><td>Fetal weight (g)</td><td>35.5</td><td>38.7</td><td>36.1</td><td>36.6</td><td>35.1</td></tr>
</table>
</td></tr>
</table>

4.11.2.1 Non-human information

The potential for asulam-sodium to cause developmental toxicity has been investigated in rats and rabbits in two developmental toxicity studies and one multi-generation study in rats (refer to Section 4.11.1). The developmental toxicity study in the rabbits is compromised, however, by the high mortality rate and consequently the low number of litters available for evaluation.

Rats

Asulam was administered by gavage to groups of 20 female CD rats from days 6 to 15 of gestation to investigate the effects on dams and embryo-fetal development [41]. Animals were exposed to asulam sodium at 500, 1000 or 2000 mg/kg bw/day. There was no evidence of toxicity at any dose level in the dams. Effects in the fetuses were reported only at the top dose and comprised slight increases in the incidences of short/ absent 13th rib, and decreased ossification of caudal vertebrae (without a clear dose-response relationship). None of these findings were statistically significantly increased when compared to the control group.

Decreased ossification of the cranium and fused cervical arches were reported in single pups in the high-dose group.

Rabbits

Asulam was administered by gavage to groups of 15 female New Zealand white rabbits from days 5 to 20 of gestation to investigate the effects on dams and embryo-fetal development [42]. Animals were exposed to asulam sodium at 0, 150, 300, 750 or 1500 mg/kg bw/day. Severe toxicity was observed in the top dose group and, therefore, these animals were sacrificed before the end of the study. At 750 mg/kg bw/day, bodyweight gain during the treatment phase was reduced and feed consumption declined during days 5-17. There was no evidence of toxicity in dams at 300 mg/kg bw/day or lower.

There were no biologically significant and consistent findings in the foetuses at any dose level.

4.11.2.2 Human information

No data available.

4.11.3 Other relevant information

No data available.

4.11.4 Summary and discussion of reproductive toxicity

Fertility

In a limited two-generation rat study performed with asulam sodium, the only effect was a reduction in litter size in the F_1 and F_2 generation in the groups exposed to ≥5000 ppm. The underlying reason for this effect was unclear as there was no difference in the number of fetuses dead at birth across the treated groups compared with the controls. Furthermore, this effect was not dose responsive as a 5-fold increase in asulam resulted in only a further 0.3 reduction in litter loss in the F_1 generation and a similar lack of dose response was seen in the F_2 generation. As there was no effect on the fertility index in the study this could be considered a developmental effect. However, in the developmental studies in rats and rabbits, there were no adverse effects on later stages of reproduction, including post-implantation loss, resorptions or a decrease in viable foetuses. This suggests that the reduced litter loss was a chance finding not related to treatment.

In addition, there were no effects on reproductive organ weights or macroscopic findings in these organs in parental animals or offspring. Repeat dose studies in the rat, mouse and dog did not record any alterations in reproductive organs and support the results of the fertility study.

Overall, it is most likely that these are chance findings, but the quality of the study is insufficient to allow for a conclusion on classification to be drawn.

Developmental toxicity

In the rat developmental study, decreased ossification of the cranium and caudal vertebrae, fused cervical arches and short or absent 13^{th} rib were reported; the combined incidence of absent/short 13^{th} rib and decreased ossification of caudal vertebrae were outside the

historical control data provided but the increases were not statistically significant compared with the controls. Decreased ossification of the cranium and fused cervical arches were each only reported in one pup and so do not provide evidence of a specific effect on development. The decreased ossification of caudal vertebrae did not demonstrate a clear dose-response relationship; furthermore, reduced ossification is generally considered to be a developmental delay and not sufficient to support classification. The combined incidence of short/ absent 13th rib in rats was outside the historical control data and appeared to be treatment-related. The short rib is seen in concurrent control animals (1 fetus) and in the highest dose group, which was above the limit dose (5 fetuses from 2 litters). In accordance with the ECETOC monograph 31[43], this effect is a variation of low-moderate concern only. The absence of the 13th rib was observed in the control group (1 fetus) and in the highest dose group (3 fetuses from 2 litters). Although the absence of ribs is viewed as a malformation in the ECETOC monograph, overall there was a very slight increase at the top dose (in excess of the limit dose) and the presence of this finding in isolation from other malformations of the ossification system is considered insufficient for classification.

In the rabbit developmental study reduced weight gain and decreased feed consumption were observed in the females during the treatment period at 750 mg/kg bw/day. There were no developmental findings at any dose.

4.11.5 Comparison with criteria

Fertility:

Whilst a reduction in litter size was observed in the F_1 and F_2 generation, the underlying reason for this effect was unclear. There was no difference in the number of fetuses dead at birth across the treated groups compared with the controls and this effect was not dose responsive. In the developmental studies in rats and rabbits, there were no adverse effects on later stages of reproduction, including post-implantation loss, resorptions or a decrease in viable fetuses. This suggests that the reduced litter loss is not due to adverse effects on development.

In addition, there were no effects on reproductive organ weights or macroscopic findings in these organs in parental animals or offspring. Repeat dose studies in the rat, mouse and dog did not record any alterations in reproductive organs and support the results of the fertility study.

Overall, it is most likely that these are chance findings, but the quality of the study is insufficient to allow for a conclusion on classification to be drawn.

Development:

Category 1A (known human reproductive toxicant) is not appropriate as *there is no human evidence establishing a causal relationship* between exposure to asulam sodium and an adverse effect on development. Likewise, Category 1B is not appropriate as *there is no* clear evidence of an adverse effect on development in experimental animals.

There was a slight increased incidence of absent 13th rib in the rat developmental study in the absence of maternal toxicity. Overall, there was a very slight increase at the top dose (in excess of the limit dose) and the presence of this finding in isolation from other malformations of the ossification system is considered insufficient for classification in Category 2.

There were no developmental findings at any dose in the rabbit.

4.11.6 Conclusions on classification and labelling

Fertility; Not classified - inconclusive*, data not sufficient for classification

*** the quality of the 2-generation study is considered to be insufficient to allow for a conclusion on classification to be drawn**

Development: Not classified – conclusive but not sufficient for classification

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility and sexual function

No effects of asulam-sodium on reproductive performance and fertility have been observed in a non guideline compliant two-generation study (similar to OECD TG 416) in rats (Report C015412, 1981). Therefore, according to the DS, the available evidence shows that asulam-sodium has no effects on reproductive performance and fertility, therefore classification is not justified.

Development

Based on the results, lack of dose response relationship for the findings, in a non guideline compliant developmental toxicity study in rats (Report R001256, 1982) and absence of biologically significant and consistent findings in rabbits (Report R001248, 1981) (both similar to OECD TG 414), the DS concluded that asulam-sodium does not require classification for developmental toxicity.

Comments received during public consultation

Three MSCAs agreed with the health hazard classification of asulam-sodium proposed by Dossier the DS, although one MSCA suggested improvement of data presentation and interpretation.

Assessment and comparison with the classification criteria

Adverse effect on fertility and sexual function and adverse effects on or via lactation

A two-generation reproduction study in rats was performed with asulam-sodium (Report C015412, 1981) to investigate fertility effects. The study was performed before GLP requirements and technical guidance was introduced by OECD.

Summary table of reproductive toxicity studies – Fertility

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Two-generation study Asulam-sodium (purity 99%) Dietary administration CD rats F_0- 12 ♂ / 24 ♀ /group F_1- 16 ♂ / 32 ♀/ group Gross necropsy was performed on F_0 parents, non-selected F_1 pups, non-selected F_1 parents and all F_2 pups Histopathology of testes in F_0 examined No guideline stated, but similar to OECD TG 416. Pre-GLP Reference: Report C015412, 1981 (DAR 6.6.1)	0, 1000, 5000, 25000 ppm	Limitations of the study- 1. data on implantations were only recorded for decedents or dams without litters post expected parturition date 2. *corpora lutea* are only referenced for one dam 3. sperm parameters were not investigated 4. litters were not standardised on lactation day 4 5. historical control data is not available 6. the top dose of 25000 ppm in this two-generation reproductive study exceeds the limit dose in F_0 and F_1 animals (> 1000 mg/kg bw/d)

Parental effects

		Dose level (ppm)							
		♂				♀			
		0	1000	5000	25000	0	1000	5000	25000
Pre-mating (mg/kg bw/d)		0	124	568	3070	0	119	612	3409
Post-mating (mg/kg bw/d)		0	46	224	1162	0	58	278	1531
Organ weights (F_1)									
Liver	(g)	26.3	26.6	25.6	254	13.5	12.0*	13.3	11.4**
	rel	3.78	3.77	3.68	3.55	3.57	3.45	3.54	3.27**
Thyroid	(mg)	31.6	35.3	34.2	39.4**	29.4	26.9	29.1	29.0
	rel	4.6	5.2	5.0	5.5*	7.8	7.7	7.7	8.2

Reproductive effects

ppm	0	1000	5000	25000
F_1				
Litter size day 0 (total)	12.4	11.1	9.6**	9.3**
Litter size day 30	11.2	10.5	9.1*	8.7**
Stillborn pups (absolute no)	6	4	1	0
Survival (%) day 4	93	95	98	94
Survival % day 30	92	91	95	94
Fertility Index (%)[#]	91	97	86	87
Viability index (%)	93	95	98	94
Lactation index (%)	93	91	97	99
F_2				
Litter size day 0 (total)	11.0	10.1	8.0*	9.4
Litter size at day 30	9.3	9.8	7.2	7.8
Stillborn pups (absolute no)	6	5	1	0
Survival (%) day 4	88	92	88	82
Survival % day 30	87	91	82	82
Fertility Index (%)	83	83	62	74

Viability index (%)	88	92	88	82
Lactation index (%)	98	99	93	100

* significantly different from controls ($p < 0.05$); ** $p < 0.01$, # fertility index = (No. of pregnant females/ No. of females which mated)*100

Offspring effects

Organ weights		Dose level (ppm)							
		♂				♀			
		0	1000	5000	25000	0	1000	5000	25000
F_1									
Pituitary	(mg)	3.8	5.3*	6.0**	4.6	5.5	4.9	6.0	3.8
	(rel)	3.9	5.1	5.5*	4.6	5.9	4.9	5.8	4.5
Liver	(g)	6.15	5.96	5.99	5.63	5.92	5.56	5.64	4.66**
	(rel)	6.17	5.75	5.31	5.58	6.41	5.59*	5.49*	5.55*
Ovary	(mg)					55.5	47.9	54.7	38.3*
	(rel)					58.6	48.1	54.3	47.0
F_2									
Pituitary	(mg)	10.3	9.8	11.2	10.9	10.3	10.2	11.7	13.2*
	(rel)	3.1	3.1	3.4	3.3	4.9	5.0	5.5	6.3*
Kidney	(g)	2.82	2.76	3.01	3.27*	2.02	1.93	2.02	1.99
	(rel)	0.84	0.87	0.92*	0.99**	0.94	0.93	0.94	0.95
Thyroid	(mg)	22.2	21.1	23.5	30.0*	17.8	21.6	17.9	24.2*
	(rel)	6.9	6.6	7.1	9.1	8.5	10.3	8.5	11.6*

Parental toxicity was manifested as a decrease in body weight and a variation in organ weight at 25000 ppm. In the F0 parents, top dose males had a slightly lower mean body weight compared to controls. In contrast, body weights were affected in females only in F1 parents at the top dose and a reduced weight gain at mating (10%) was reported. In the F1 parents, mean absolute and relative liver weights were slightly (but significantly) lower in top dose females; thyroid weights were significantly higher in males at the top dose level.

The two-generation rat study had a number of limitations as indicated in table above (e.g., lack of information on implantations) which make the findings difficult to interpret. It is considered that there were no effects on the fertility, gestation, viability or survival index in the F1 or F2 litters. The fertility index was reported to be 91, 97, 86 and 87% in the F1 generation and 83, 83, 62 and 74% in the F2 generation at 0, 1000, 5000 and 25000 ppm respectively. There was no dose response relationship and the value at the top dose was not significantly different to that in controls. Survival to day 30 was reported to be 87, 91, 82 and 82% in the F2 generation at 0, 1000, 5000 and 25000 ppm respectively. Again, there was no significant difference between the value at the top dose and that in controls.

A decrease was seen in litter size in F1 pups across all treated groups, which attained statistical significance at ≥ 5000 ppm. However, no dose response relationship was observed and a 5-fold increase in dose from pm 5000 ppm to 25000 ppm only produced a non significant decrease in litter size from 9.6 to 9.3.

In the F2 generation litter size was reduced across treated groups but this was only statistically significant in the mid-dose group and did not demonstrate a dose–response relationship. Pup body weight at birth was not affected in the F1 or F2 generation.

There were no effects on reproductive organ weights or macroscopic findings in these organs in parental animals or offspring in this study. The repeat dose toxicity studies in the rat, mouse and dog did not record any alterations in reproductive organs.

Further to this, there were no effects on the later stages of reproduction (including post-implantation loss, resorptions or a decrease in viable foetuses) in the developmental studies. This supports the fact that the decreased litter size in the two-generation study was a chance finding. However, the limitations of the two-generation study make it difficult to fully interpret these findings.

Comparison with the criteria

According to CLP criteria the classification of a substance in Category 1B is largely based on data from animal studies. Substances are classified in Category 2 when there is some evidence from humans or experimental animals, and where the evidence is not sufficiently convincing to place the substance in Category 1B. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

In the reliable 2-generation reproduction study, none of fertility indices were convincingly affected by the tested substance. No evidence of adverse effects were found in the reproductive organs in the repeated dose toxicity studies. The litter size was slightly reduced in F1 generation at both higher doses but without any dose response in spite of high increment between medium and high dose. In F2 generation, the litter size was reduced at medium dose, but not significantly reduced at the high dose (5 times higher), which indicates that this reduction is probably not treatment related. In addition, no effect on number of viable foetuses, resorptions or pre- and post-implantation losses was found in the developmental toxicity studies in rats and rabbits at doses comparable with the top dose used in the 2-generation reproduction study.

Using a weight of evidence approach, RAC is of the opinion that asulam-sodium **does not warrant classification as a substance affecting fertility and sexual function or as a substance causing effects on or via lactation**.

Adverse effects on development of the offspring

The potential for asulam-sodium to cause developmental toxicity has been investigated in rats and rabbits in two developmental toxicity studies and one multi-generation study in rats. The developmental toxicity study in the rabbits is compromised by the high mortality rate and consequently the low number of litters available for evaluation.

<u>Rats</u>

Asulam-sodium was administered by gavage to groups of 20 female CD rats from days 6 to 15 of gestation to investigate the effects on dams and embryo-foetal development (Report R001256, 1982). Animals were exposed to asulam-sodium at 500, 1000 or 2000 mg/kg bw/d.

Mated female CD rats (20/group) were gavaged with asulam-sodium at dose levels of 0, 500, 1000 or 2000 mg/kg bw/d. Animals were examined daily for clinical signs; food intake and bodyweights were measured on days 1, 3, 6-15, 18 and 21. Dams were killed on day 21 and the uterine contents investigated. All foetuses were investigated for external abnormalities. Approximately two thirds of the foetuses were examined for visceral findings by dissection and

for skeletal findings following staining with Alizarin Red. The remaining foetuses were examined by serial sectioning.

Summary table of development toxicity study in rats

<table>
<tr><th>Method</th><th>Dose levels</th><th>Observations and remarks
(effects of major toxicological significance)</th></tr>
<tr><td>Developmental study in rats

Asulam-sodium (purity 98.6%)
CD rats
20/group
Oral gavage on days 6 to 15 of gestation

No guideline stated, but similar to early version of OECD TG 414. Pre-GLP

Reference: Report R001256, 1982 (DAR 6.6.2)</td><td>0, 500, 1000, 2000 mg/kg bw/d</td><td>
<u>Maternal toxicity:</u>
There were no reported clinical signs or effects on body weight, food consumption, effects in dams at any dose level. At necropsy, each animal was examined macroscopically and specimens considered to be abnormal were retained. No maternal necropsy findings were found in the original study report.
<u>Developmental effects</u>
No test item effects were reported in litter parameters at 500, 1000 or 2000 mg/kg bw/d.
<u>Litter data</u>
<table>
<tr><th colspan="2" rowspan="2">Parameter</th><th colspan="4">Dose level (mg/kg bw/d)</th><th rowspan="2">Historical control mean (range)</th></tr>
<tr><th>0</th><th>500</th><th>1000</th><th>2000</th></tr>
<tr><td colspan="2">Pregnant</td><td>20</td><td>20</td><td>20</td><td>20</td><td></td></tr>
<tr><td colspan="2">Corpora lutea</td><td>16.9 ± 2.8</td><td>16.7 ± 3.3</td><td>17.6 ± 3.8</td><td>17.6 ± 2.6</td><td>15.9 (14.0-18.3)</td></tr>
<tr><td colspan="2">Implantations</td><td>14.7 ± 1.5</td><td>14.3 ± 1.9</td><td>14.6 ± 2.2</td><td>15.2 ± 1.4</td><td>14.2 (11.6-16.5)</td></tr>
<tr><td rowspan="3">Viable foetuses</td><td>♂</td><td>6.2 ± 2.1</td><td>6.4 ± 2.2</td><td>7.1 ± 2.2</td><td>7.6 ± 1.8</td><td>6.8 (5.0-8.0)</td></tr>
<tr><td>♀</td><td>7.4 ± 2.1</td><td>7.2 ± 2.1</td><td>6.8 ± 1.7</td><td>7.2 ± 2.0</td><td>6.7 (5.5-8.4)</td></tr>
<tr><td>Total</td><td>13.5 ± 2.9</td><td>13.6 ± 2.2</td><td>13.9 ± 2.3</td><td>14.7 ± 1.7</td><td>13.5 (10.9-15.9)</td></tr>
<tr><td rowspan="3">Resorptions</td><td>Early</td><td>0.95 ± 0.97</td><td>0.60 ± 0.77</td><td>0.50 ± 0.71</td><td>0.30 ± 0.55</td><td>0.55 (0.08-1.53)</td></tr>
<tr><td>Late</td><td>0.20 ± 0.45</td><td>0.10 ± 0.32</td><td>0.25 ± 0.50</td><td>0.15 ± 0.39</td><td>0.13 (0.00-1.45)</td></tr>
<tr><td>Total</td><td>1.15 ± 1.07</td><td>0.70 ± 0.84</td><td>0.75 ± 0.87</td><td>0.45 ± 0.67</td><td>0.68 (0.07-1.91)</td></tr>
<tr><td colspan="2">Pre-implantation loss</td><td>13.3%</td><td>14.7%</td><td>17.0%</td><td>13.9%</td><td>11.0 (2.6-20.9)</td></tr>
<tr><td colspan="2">Post-implantation loss</td><td>7.8%</td><td>4.9%</td><td>5.1%</td><td>3.0%</td><td>4.8 (0.5-14.0)</td></tr>
<tr><td colspan="2">Foetal weight (g)</td><td>3.32 ± 0.07</td><td>3.39 ± 0.06</td><td>3.38 ± 0.07</td><td>3.31 ± 0.07</td><td>3.69 (3.5-4.04)</td></tr>
<tr><td colspan="2">Placental weight (g)</td><td>0.46 ± 0.02</td><td>0.47 ± 0.02</td><td>0.48 ± 0.02</td><td>0.47 ± 0.01</td><td>0.50 (0.45-4.04)</td></tr>
</table>
Findings at necropsy were reported only in foetuses at 2000 mg/kg bw/d (summarised in the table below).
Foetal data at necropsy
</td></tr>
</table>

Parameter		Dose (ppm)				Historical control
		0	500	1000	2000	
No of rat foetuses examined (litters)		176 (20)	177 (20)	181 (20)	197 (20)	10954[a] Mean % foetuses (range)
↓ossification; cranium	No. of foetuses/%	0/0	0/0	0/0	1/0.5	-
	No. of litters / %	0/0	0/0	0/0	1/5.0	-
13th rib(s) short/ absent	No. of foetuses/%	2/1.1	-	-	8/4.1	0.07 (0.0-1.7)
	No. of litters / %	2.0/10	-	-	3.0/15	-
Short	No.foetuses/no. litters	1/1			5/2	
Absent	No.foetuses/no. litters	1/1			3/2	
Ribs 13/14	Foetal incidence %/litter incidence %	-	1.7/10	3.3/20	4.6/35	5.4 (0.6-20.5)
Ribs 14/14	Foetal incidence %/litter incidence %	-	-	1.1/5	3.0/25	3.82 (0.0-17.9)
Fused cervical arches	No. of foetuses/%	0/0	0/0	0/0	1/0.5	-
	No. of litters / %	0/0	0/0	0/0	1/5.0	-
↓ossification; caudal vertebrae	No. of foetuses/%	2/1.1	3/1.7	2/1.1	5/2.5	1.01 (0.6-1.2)
	No. of litters / %	1.0/5.0	3.0/15	2.0/10	5.0/25	-

[a] from 74 studies

There was no evidence of toxicity at any dose level in the dams. Effects in the foetuses were reported only at the top dose and comprised slight increases in the incidences of short/ absent 13th rib, and decreased ossification of caudal vertebrae (without a clear dose-response relationship). None of these findings were statistically significantly increased when compared to the concurrent control group. Decreased ossification of the cranium and fused cervical arches were reported in single pups in the high-dose group. While no extra rib(s) (the 14th) were found in the concurrent control group, there was an increase in the incidence in all treated groups, which was dose-related. However all values were below the historical mean values (and within the historical control range) .

Consequently, a developmental NOAEL of 1000 mg/kg bw/d could be determined, based on the foetal skeletal effects mainly observed at the top dose level of 2000 mg/kg/day (Report R001256, 1982).

Rabbits

Asulam was administered by gavage to groups of 15 female NZW rabbits from days 5 to 20 of gestation to investigate the effects on dams and embryo-foetal development (Report

R001248, 1981). Animals were exposed to asulam-sodium at 0, 150, 300, 750 or 1500 mg/kg bw/d. Mortality and signs of toxicity 'similar to those of starvation' were observed in animals administered 1500 mg/kg bw/d asulam; this group was therefore terminated early. A large number of deaths occurred in the remaining treated and control groups; these deaths are largely attributed to dosing error, mishandling or infection and are not considered to be treatment-related. Terminal body weights of 750 mg/kg bw/d animals were lower than controls; more marked effects on body weight in this group (including weight stasis) were seen during the treatment phase. Food consumption was also reduced at 750 mg/kg bw/d during the treatment phase, most notably between days 13 - 17. There was no evidence of toxicity in dams at 300 mg/kg bw/d or lower. There were no biologically significant or consistent findings in the foetuses at any dose level.

Summary table of development toxicity study in rabbits

Developmental study in rabbits Asulam-sodium (purity 98%) New Zealand white rabbits 15/group of Asulam 11 positive controls Oral gavage on days 5-20 of gestation Uterine contents examined on day 29 No guideline stated, but similar to early version of OECD TG 414. Pre-GLP Reference: Report R001248, 1981 (DAR 6.6.3)	0, 150, 300, 750 mg/kg bw/d Positive control-150 mg/kg bw/d thalidomide	Limitations of the study: Foetuses - external examination for abnormalities and visceral abnormalities by dissection Foetal brains investigated by serial sectioning Skeletal findings investigated following Alizarin red staining ***Maternal effects in rabbits*** Deaths occurred in 10/30, 9/28, 5/23, 5/23 dams at 0, 150, 300, 750 mg/kg bw/d. These were attributed to mishandling, dosing error or infection and were not treatment-related. The group dosed with 1500 mg/kg bw/d was terminated early owing to mortality and toxicity described as "similar to starvation". No test item related effects were reported in dams at 150 and 300 mg/kg bw/d. At 750 mg/kg bw/d, bodyweight gain was reduced during the treatment phase (5-21d ↓ 35%) and reduced feed consumption was reported at 5-9d (5%), 9-13d (5%) and 13-17d (17%).

Parameter	Dose level (mg/kg bw/d)				
	0	150	300	750	positive control
Mated	30	28	23	23	14
Not pregnant	4	1	2	4	2
Total deaths	10	9	5	5	1
Total resorption	-	-	-	-	2
Litters	16	18	16	14	8

Developmental effects

There were no effects reported on litter parameters at any dose level.

Parameter	Dose level (mg/kg bw/d)				
	0	150	300	750	positive control
Mean no. *corpora lutea* / dam	10.4	10.3	11.4	10.9	10.8
Mean no. implantations/dam	9.3	8.7	10.6	9.1	8.7
Pre-implantation loss/animal (%)	10.2	15.8	8.3	15.4	18.8
Post-implantation loss/animal (%)	4.8	7.1	12.1	9.5	59.5
Total no. of live pups	143	145	147	115	35
Foetal death	6	11	22	12	52

		Early	4	6	11	1	18
		Late	2	5	11	11	34
		Litter size (#)	8.9	8.1	9.2	8.2	3.5
		Foetal weight (g)	35.5	38.7	36.1	36.6	35.1

There were no biologically significant and consistent findings in the rabbit foetuses at any dose level.

In conclusion, taking into account available data from the developmental toxicity studies in rats and rabbits RAC is of the opinion that asulam-sodium **does not warrant classification as a developmental toxicant**.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Asulam sodium is a carbamate herbicide, with a specific mode of action *via* 7,8-dihydropteroate synthase inhibition. There is no known equivalent mammalian target. No significant inhibition of brain cholinesterase activity was observed in 8-week mouse, 6 month dog studies by the oral route or in the 21-day rabbit dermal study. No other effects indicative of neurotoxicity were observed in any of the available studies.

4.12.1.2 Immunotoxicity

No specific studies of immunotoxicity are available for asulam sodium. No effects indicative of neurotoxicity were observed in any of the available studies.

4.12.1.3 Specific investigations: other studies

No data available.

4.12.1.4 Human information

No data available.

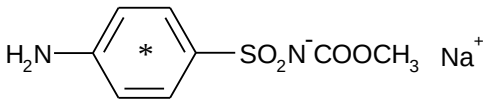
5 ENVIRONMENTAL HAZARD ASSESSMENT

The key information pertinent to determining the environmental hazard classification for asulam sodium is presented below. Unless otherwise stated, these studies were conducted in accordance with GLP and the validity criteria of the respective test guideline. They are considered reliable (Klimisch score 1 or 2) and suitable for use in hazard classification.

References are taken from the Draft Assessment Report (DAR) - Asulam-sodium - Annex B (Volume 3) B.8 Environmental fate and behaviour and B.9 Ecotoxicology.

The majority of radiolabelled studies used ^{14}C-asulam sodium (methyl sulfanilylcarbamate monosodium salt) labelled in the aromatic ring (see Figure 1 for radiolabel position). Radiochemical purity and specific activity are reported in each radiolabelled study. Some studies have been conducted with technical asulam. In solution asulam sodium will dissociate (pK_a1, 1.25; pK_a2, 4.68) and the ionised and unionised forms will be in equilibrium which is dependent on the pH of the compound's environment. Solubility is also pH-dependant (5.5 g/L at pH 4, 962 g/L at pH 8). At environmentally relevant pH, the substance will exist primarily in the ionised form and be readily soluble. The amounts of asulam used in the tests themselves were not sufficient to affect the pH and therefore would not affect the equilibrium, nor was the aqueous solubility of asulam exceeded in any of the tests. In the opinion of the UK CA, asulam and asulam sodium can therefore be considered equivalent and the form of the compound applied will not influence the results of the test.

Figure 1: Position of radiolabel (*) in ^{14}C-asulam sodium



5.1 Degradation

Table 21: Summary of relevant information on abiotic and biotic degradation of asulam (tested as ^{14}C asulam or its sodium salt)

Method	Results	Remarks	Reference
Sterile aqueous hydrolysis at pH 5, 7 and 9 to EPA guideline N 161-1	Stable to hydrolysis (<10%) at all pH tested over 31 days; insufficient degradation to calculate a DT50	Valid study to GLP	Gohdes, 1989a [44] DAR B.8.4.1
Sterile aqueous photolysis at pH 4 and 9 to EPA 161-2, EU 94/37 and SETAC guidelines - plus subsequent technical analyses/modelling of photolytic half-lives at different solar light intensities and depths	DT50 in test at pH9 = 0.87 days (≡ 1.56 days under natural summer sunlight at 52°N) DT50 in test at pH4 = 0.44 days (≡ 0.781 days under natural summer sunlight at 52°N) Quantum yield calculated to be 0.114 at pH4 and 0.157 at pH 9	Valid study to GLP	Mills and Simmonds, 2003a [45]; Lowden, 2004 a&b [46] DAR B.8.4.2

Aqueous photolysis in UK natural water to Japanese MAFF guideline 2-6-2.	Photolytic half-life in test at pH 7.8 = 0.84 days (≡ 4.21 days under natural spring sunlight at 35°N	Valid study to GLP	Mills and Caine, 2004a [47] DAR B.8.4.2
Sterile aqueous photolysis at pH 4 and 9 to EU 94/37 - plus subsequent technical analyses/modelling of photolytic half-lives at different solar light intensities and water depths	DT50 in test at pH9 = 0.863 days (≡ 1.64 days under natural summer sunlight at 52°N) DT50 in test at pH4 = 0.284 days (≡ 0.537 days under natural summer sunlight at 52°N) Quantum yield calculated to be 0.168 at pH4 and 0.0612 at pH 9 See study [74] for details of DT50 changes over different seasons and depths	Valid study to GLP	Mills, 2007a [73]; Lowden, 2007 a&b [74] DAR B.8.4.2
Ready biodegradation test to OECD guideline 301/B	Asulam incubated in activated sewage sludge at 21°C for 29 days showed 51% degradation - 'not readily biodegradable'	Valid study to GLP	Mead, 1999a [48] DAR B.8.4.3
Ready biodegradation test to OECD guideline 301 F	Asulam incubated in activated sewage sludge at 22°C for 28 days showed 21% degradation - 'not readily biodegradable'	Valid study to GLP	Feil, 2008 [75] DAR B.8.4.3
Aerobic water/sediment degradation simulation test study to BBA IV: 5-1 guideline and subsequent kinetic analysis acc. to FOCUS procedures	Studied in two systems at 20±2°C in the dark for 153 days. Whole system DT50 = 65.6-78.8 days (mean 71.9, with 3-13.9% mineralisation	Valid studies, experimental phase to GLP	Purser, 1998a [49]; Hardy and Patel, 2008c [50] DAR B.8.4.4
Aerobic water/sediment degradation simulation test study to BBA IV: 5-1 guideline and subsequent kinetic analysis acc. to FOCUS procedures	Studied in two systems at 20±2°C in the dark for 120 days. Whole system DT50 = 61.9-776.2 days. < 2% of asulam was mineralized in both systems	Valid studies, experimental phase to GLP	Willems, 1997a [51]; Hardy, 2011a [51] DAR B.8.4.4

5.1.1 Stability

Aqueous hydrolysis

A sterile aqueous hydrolysis study was conducted with ^{14}C-asulam in accordance with EPA guideline N 161-1 (1982) and to GLP [44].

Sterile aqueous buffer solutions (pH 5, 7 and 9) were prepared containing ^{14}C-asulam (radiochemical purity 94.7%; specific activity 10.64 mCi/mmol) at a concentration of 4.9µg/mL. Samples of each treated buffer were incubated at 24 to 26°C in the dark in sealed vials (12 for analysis and 4 for pH determination). Duplicate samples were analysed by high-performance liquid chromatography (HPLC) and liquid scintillation counting (LSC) at days 0, 6, 12, (day 13 for pH9), 20, 24 and 31. The recovery of radioactivity applied to the samples relative to day 0 throughout the 31 day period for all pH's was acceptable and in the range 100.8-106.0%. The pH of samples throughout the study period did not vary by more than 0.06 pH units.

HPLC analysis of the buffer solutions showed ^{14}C-asulam as the major component plus 4 minor components designated as regions 1 to 4 in the chromatograph. Throughout the 31 day period, the mean radioactivity for region 1 reached a maximum of 4.7% in pH 5 samples but was not detected for pH 7 and 9 samples and for HPLC regions 2, 3 and 4, the mean radioactivity did not exceed 1.4% throughout the study at all pH's. The mean HPLC results are presented in Table 22. These results indicated that asulam was stable to hydrolysis at all pH values tested over 31 days (based on levels not declining by more than 10% over the study duration). There was insufficient degradation to calculate a DT_{50}.

Table 22: Mean distribution of radioactivity expressed as percentage of applied radioactivity in buffer solutions at pH 5, 7 and 9 containing ^{14}C-asulam

Time (days)	Region 1	Asulam	Region 2	Region 3	Region 4
pH 5					
0	nd	96.8	nd	0.8	0.3
6	1.2	94.5	0.3	0.9	nd
12	1.8	94.4	0.6	0.6	nd
20	3.2	93.9	nd	0.6	nd
24	3.6	90.9	0.3	0.3	0.3
31	4.7	92.2	1.0	0.5	0.6
pH 7					
0	nd	93.8	nd	0.9	0.7
6	nd	94.7	0.3	1.3	nd
12	nd	94.8	0.4	1.1	0.7
20	nd	93.7	nd	0.9	0.5
24	nd	92.8	nd	0.7	0.5
31	nd	94.5	nd	0.9	0.5
pH 9					
0	nd	95.9	nd	0.9	0.5
6	nd	96.9	0.4	1.2	nd
13	nd	96.1	0.2	1.3	nd
20	nd	93.1	nd	1.1	0.6
24	nd	96.1	nd	1.0	0.5
31	nd	95.1	nd	1.1	0.2

nd: not detected

Aqueous photolysis

The aqueous photolysis of asulam was studied according to EPA 161-2 (1998), EU 94/37 and OECD (Draft 2000), and SETAC (1995) guidelines as well as to GLP [45].

Sterile aqueous buffer solutions at pH 9 and pH 4 were prepared containing ^{14}C-asulam (radiochemical purity >98%, specific activity 5.617 MBq/mg) at concentrations of 0.607 µg a.s./mL and 0.604 µg a.s./mL respectively. Aliquots (25 ml) were incubated at 25±2°C in glass photolysis vessels fitted with traps for organic volatiles (ethylene glycol) and CO_2 (2M KOH). Single vessels were either kept in the dark or irradiated continuously with artificial light from a xenon arc lamp (wavelengths filtered below 290 nm) for up to 54 hours (pH 9) and up to 26 hours (pH 4). The intensity of light was compared to natural sunlight and 1 day (24h) of artificial was reported to be

equivalent to 1.79 days (pH 9) and 1.78 days (pH 4) of summer sunlight at 50°N in the UK. Samples were taken at the start and at representative time points up to 54 hours for pH 9 and 26 hours for pH 4. Radioactivity was quantified directly by LSC and analysed by HPLC with confirmatory analysis of metabolites by liquid chromatography-mass spectrometry (LC-MS) and thin-layer chromatography (TLC). The photolysis of an actinometer solution consisting of 4-nitroacetophenone incubated in the presence of pyridine was also investigated under identical conditions in order to calculate the quantum yield of asulam.

Mean recoveries were acceptable. At pH 9, recoveries were 98.7% in irradiated samples and 98.5% in dark controls. At pH 4, mean recoveries in irradiated samples was 100.2% and 99.7% in dark controls. The results of analysis for the pH 9 samples are summarised in Table 22 and for the pH 4 samples in Table 23.

In the irradiated samples at pH 9, levels of asulam declined to 17.8% AR in 51.8 hours (equivalent to 3.9 days of natural UK summer sunlight). A major photodegradate reached a maximum of 24.2% after 46 hours and was identified by LC-MS as N-(4-aminophenyl)formamide. One other photodegradate reached 9-11.9% AR (depending on the method of quantification) and was tentatively identified by LC-MS as (4(4-methoxycarbonylaminophenyl)aminophenyl)carbamic acid. The remaining degradation products were all below 10% AR. No significant degradation of asulam occurred in the pH 9 dark control samples.

In the irradiated samples at pH 4, levels of asulam declined to a minimum of 21.0% AR in 25.8 hours (equivalent to 1.9 days natural UK summer sunlight). One major photodegradate reached 55.5% AR after 20.1 hours and was identified by LC-MS and confirmed by TLC as sulphanilic acid. The remaining degradation products were all below 10% AR. One minor degradation product was observed in pH 4 dark controls as <1% AR.

No significant quantities of volatile products were collected in the irradiated samples or the dark controls at either pH. At both pH values, the DT_{50} and DT_{90} values for the photolytic degradation of asulam were calculated using Microsoft Excel assuming single first order kinetics and are summarised in Table 23. The quantum yield of asulam was calculated to be 0.114 at pH 4 and 0.157 at pH 9.

Table 23: Calculated DT_{50} values for asulam in irradiated aqueous solution at pH 9 and pH 4 assuming single first order kinetics

System	Irradiation in test	Natural sunlight equivalent*
	DT_{50}	**DT_{50}**
Irradiated pH 9 buffer	20.9 hours, 0.870 days	1.56 days
Irradiated pH 4 buffer	10.6 hours, 0.440 days	0.781 days

* 1 day in test = 1.79 summer sunlight days 52°N at pH 9
= 1.78 summer sunlight days 52°N at pH 4

Additional photodegradation calculations based on Mills and Simmonds 2003a [45]

Two further analyses (Lowden 2004 a&b) [56] are available in the asulam-sodium DAR which calculate environmental photolytic half-lives for asulam at different solar light intensities from the Quantum Yield and light absorbance data determined for asulam in the above aqueous photolysis study [45]. The Lowden 2004a [56] analysis was conducted according to the method described in ECETOC Technical Report No. 12(1984), UBA Test Guidelines (1990) and the Spectral irradiance

data of Frank and Kopffler (1988). This estimated the half-life for asulam when dissolved in the top few millimetres of a natural aquatic system using solar irradiance data appropriate to central European latitudes (52°N). Based on these assumptions, the half-life values for asulam for each month at pH 4 and pH 9 are summarised in Table 24.

Table 24: Photolytic half-lives of asulam sodium at pH 4 and pH 9 in the top millimetres of natural aquatic systems (Central European latitude 52°N)

Photolytic half-lives of asulam sodium in hours, by month					
Month	**pH 4**	**pH 9**	**Month**	**pH 4**	**pH 9**
Jan	76	135	Jul	8	13
Feb	38	65	Aug	8	13
Mar	19	31	Sep	13	21
Apr	11	18	Oct	25	41
May	8	14	Nov	61	106
Jun	7	12	Dec	119	213

The Lowden 2004b [56] analysis used the GCSOLAR program of Zepp and Cline to generate half-life data at 40°, 50° and 60°N by season, at the surface and at depths of 30 and 100 cm in the water body. This program has the capability to determine realistic half-lives according to the properties of the water (refractive index, light absorption and depth). The program does not account for cloud cover, concentration of pollutants in air, or suspended solids and vegetation cover in the water. The results for photolytic half-lives for asulam at different latitudes are summarised for pH 4 in Table 25 and for pH 9 in Table 26.

Table 25: Photolytic half-lives of asulam (pH 4) at the surface and at depths of 30 cm and 100 cm depth at various European latitudes

Latitude	**Season**	**Half-life (days) in water body depth of:**		
		0 cm	**30 cm**	**100 cm**
40°N	Spring	0.431	1.94	6.13
	Summer	0.307	1.45	4.60
	Autumn	0.785	3.56	11.3
	Winter	1.660	6.77	21.3
50°N	Spring	0.586	2.49	7.84
	Summer	0.362	1.66	5.24
	Autumn	1.450	6.04	19.0
	Winter	4.090	14.9	46.1
60°N	Spring	0.823	3.32	10.4
	Summer	0.444	1.96	6.19
	Autumn	3.29	12.3	38.5
	Winter	13.6	44.0	135

Table 26: Photolytic half-lives of asulam (pH 9) at the surface and at depths of 30 cm and 100 cm depth at various European latitudes

Latitude	Season	Half-life (days) in water body depth of:		
		0 cm	30 cm	100 cm
40°N	Spring	0.703	3.34	10.7
	Summer	0.501	2.48	7.96
	Autumn	1.29	6.18	19.8
	Winter	2.78	12.1	38.5
50°N	Spring	0.965	4.36	13.9
	Summer	0.591	2.85	9.13
	Autumn	2.42	10.7	34.2
	Winter	7.19	27.5	86.8
60°N	Spring	1.38	5.92	18.9
	Summer	0.728	3.40	10.9
	Autumn	5.68	22.6	71.5
	Winter	24.9	84.0	261

ii) The aqueous photolysis of asulam was studied according to Japanese J MAFF 2-6-2 (2001) guidelines, followed guidance notification13 Seisan No. 3986 (2001) and to GLP [47].

The study was conducted in natural water (UK reservoir pond, pH 7.8) which was sterilised by filtration through a sterile 0.22µm membrane filter and dosed with ^{14}C-asulam (radiochemical purity >98%, specific activity 5.16 MBq/mg) at a nominal concentration of 1 µg a.s./mL. Aliquots (35 ml) were incubated at 25±2°C in sealed glass photolysis vessels. Single vessels were either kept in the dark or irradiated continuously with artificial light from a xenon arc lamp (wavelengths filtered to remove wavelengths below 290 nm). The intensity of light was compared to natural sunlight and 1 d (24 h) of artificial was reported to be equivalent to 5.01 d of natural spring sunlight at 35°N in Tokyo, Japan which is comparable with Southern Europe (e.g. Athens 37°N). Samples were taken at the start and at intervals up to 145.7 h for irradiated samples (equivalent to 30.43 days natural Japan spring sunlight) and up to 169.6 h for non-irradiated samples. Radioactivity was quantified by LSC and analysed by HPLC with confirmatory analysis by HPLC and TLC.

Mean recoveries were acceptable at 100% in the irradiated samples and 103% in dark controls. For the irradiated samples carbon dioxide levels determined from the loss in dissolved radioactivity after precipitation with barium chloride, ranged up to 27.4% AR and 1.1% in the dark controls. After precipitation of CO_2 with barium chloride, the remaining radioactivity in the samples was analysed by HPLC with selected samples analysed by HPLC and TLC as confirmation. The levels of asulam declined rapidly reaching <1% AR at the end of the irradiation period. Many photodegradates (59 in total) were formed but each was individually less than 10% AR (maximum 6.4%). No significant degradation of asulam was found in the dark control samples.

The formation of only minor metabolites (<10% AR) in this study was noted by the pesticides Rapporteur to be in contrast to the study performed in sterile buffer where a number of major metabolites were formed in the presence of light (see [45] above). The Notifier proposed that the results of this study performed in natural water were more relevant since the effects of indirect phototransformation (where the substance is transformed through reaction with other photochemically-formed reactive/energetic molecules, particularly various forms of oxygen) are considered alongside those of direct phototransformation in natural water.

The half-life for the photolytic degradation of asulam was calculated assuming single first order kinetics and was determined to be 0.84 experimental days, equivalent to 4.21 environmental days (1 day suntest = 5.01 days sunlight in Japan, comparable with Southern Europe, e.g. Athens at 37°N). The correlation coefficient for the experimental data was 0.997.

iii) The aqueous photolysis of asulam was studied according to the guideline EU 94/37 but not to GLP (not required) [73].

The study was conducted, under sterile conditions at 25 ± 2°C, with continuous illumination under artificial sunlight (< 290 nm). Aqueous solutions at pH 4 and at pH 9 were dosed with ^{14}C-asulam at a nominal concentration of 0.6 mg a.s./ L. Illumination was continued for just greater than two half-lives at each pH. Dark (non-irradiated controls) were also run. The solutions were dispensed into photolysis vessels that were connected to traps for volatile compounds and carbon dioxide. Samples were taken at various intervals from 1 hour to 72 hours incubation. The samples were examined by HPLC system and some solutions from each pH were selected for concentration and liquid chromatography-mass spectrometry (LC/MS) analysis.

At pH 4 the irradiation continued for 25 hours which is equivalent to 2.0 days of natural summer sunlight (N EU). The radioactivity recovered ranged from 96.8% to 107.5% with a mean of 102.6% of applied radioactivity (AR) for irradiated samples and from 100.1% to 108.8% with a mean of 104.3% AR for non-irradiated samples. No significant quantities of volatile products were collected in either the irradiated experiment or the non-irradiated experiment. HPLC analysis showed that the amount of asulam present in the irradiated samples declined to a minimum of 13.5% AR during 25 hours irradiation.

At pH 9 the irradiation continued for 47 hours which is equivalent to 3.7 days of natural summer sunlight (N EU). For the irradiated samples recoveries ranged from 99.9% to 107.6% with a mean of 104.1% AR; for non-irradiated samples recoveries ranged from 100.2% to 106.7% with a mean of 104.0%AR. No significant volatiles were collected in either the irradiated or non-irradiated experiment. HPLC analysis showed that the amount of asulam present in the irradiated samples declined to 24.0% AR after 47 hours irradiation.

Details of photodegradates are given in the 2016 DAR, these are not considered further for classification of asulam-sodium. No significant degradation of asulam was observed in the non-irradiated system. At both pH values, the half-life values for the decline of asulam in the irradiated experiment were determined assuming first order kinetics. The photolytic DT_{50} at pH 9 was calculated to be 20.7 hours or 0.863 experimental days, equivalent to 1.64 environmental days (1 day Suntest = 1.89 summer sunlight days 52°N at pH 9 (latitude comparable with Northern Europe, e.g. London). The photolytic DT_{50} at pH 4 was calculated to be 6.8 hours or 0.284 experimental days, equivalent to 0.537 environmental days (1 day Suntest = 1.9 summer sunlight days 52°N at pH 4. The quantum yield for asulam was calculated as 0.168 at pH 4 and 0.0612 at pH 9. The RMS considered the study acceptable and the DT_{50} values are comparable with the ones in Mills and Simmonds 2003a (44).

Additional photodegradation calculations based on Mills (2007a) [73]

Two further analyses (Lowden 2007 a&b) [74] are available in the asulam-sodium DAR which model environmental photolytic half-lives for asulam at different solar light intensities from the Quantum Yield and light absorbance data determined for asulam in the above aqueous photolysis

study [73]. The Lowden 2007a analysis used a method by Frank and Klöpffer to reflect the climatic situation in Central Europe and the adjacent North Sea. The calculations showed that the half-lives in this region would vary with pH and according to the time of year, ranging from 4 to 5 hours in June up to 29 to 38 hours in December. The Lowden 2007b analysis used a GC Solar program of Zepp & Cline to determine the theoretical lifetime in the top layer of aqueous systems and the real lifetime of asulam in natural aquatic systems.

The results indicate that asulam in the top few millimetres of aquatic systems will undergo degradation by direct photolytic processes under the conditions prevailing in Central Europe, with half-lives of <0.5 days in summer, regardless of latitude or pH. The half-lives in spring will be < 0.5 days and those in Autumn < 1.0 days except for at latitude 60° N at which they may increase to just under 1.5 days. Real half-lives in natural aquatic systems will vary according to the depth of the water body. For water bodies of 30 cm depth the half-life will be up to 0.6 days in summer and up to 1 day in spring. Autumn half-lives would vary from about a day up to about 3 days, depending on latitude. For water bodies with a depth of 100 cm the half-life will be up to 2 days in summer and up to 2½ days in spring. Autumn half-lives would vary from about 1½ days up to just over a week, depending on latitude and pH.

Aqueous photolysis conclusions

It is concluded that, although asulam-sodium will be rapidly degraded by light in the top few millimetres of an aquatic system, the degradation will be slower in natural water bodies, throughout which it will readily dissolve. In water bodies of modest depth (30 cm, 100 cm) the half-lives will range from about half a day in summer to just over a week in autumn. This is not considered sufficient to meet CLP ‘rapidly degradable’ criteria.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Not conducted for this substance

5.1.2.2 Screening tests

i) Ready biodegradation of asulam was studied according to OECD guidelines (No. 301B, 1992) and to GLP (OECD 1997) [48].

UK activated domestic sewage sludge (30 mg suspended solids/L) was added to a standard mineral solution and this inoculum was preincubated for 24 hours at 21°C. Duplicate samples of inoculum (3 litres) were then treated with unlabelled asulam (24 mg/L; equivalent to 10 mg ThOC/L), sodium benzoate (17.1 mg/L equivalent to 10 DOC/L) or both. Further samples of untreated inoculum were also prepared. All samples were then incubated at 21°C for 29 days in flasks fitted with traps for CO_2 (sodium hydroxide).

For all treatments, evolved CO_2 was determined by carbon analysis at day 0 and at sixteen further sampling intervals. At study termination, 74% conversion of sodium benzoate to CO_2 was observed, confirming the suitability of the inoculum and test conditions. Evolved CO_2 from the asulam samples showed 51% degradation. Hence, asulam was considered to be ‘not readily

biodegradable'. The asulam plus sodium benzoate toxicity control attained 46% degradation after 28 days and sodium benzoate alone achieved 74% degradation. This indicated that asulam was not itself toxic to the sewage treatment micro-organisms.

ii) A ready biodegradability on asulam technical was conducted in a manometric respirometry test (OECD Guideline for Testing of Chemicals No. 301 F) as well as to GLP [75].

Aerobic activated sludge was supplied by the domestic sewage works in Germany. Asulam technical (97.45% pure) was investigated for its ready biodegradability in a manometric respirometry test over a period of 28 days. The study was carried out at 22°C, in the dark. The pH- of test flasks at the end of the test was within the pH range 6.0 to 8.5 as required by the test guideline.

Evolved carbon dioxide was absorbed in gas traps and the consumption of oxygen was determined by measuring the change of pressure in the flasks. Biodegradation was determined by the oxygen uptake of the micro-organisms during exposure. Sodium benzoate was tested as a reference item. The degradation rate of asulam was calculated by the oxygen consumption of the aerobic activated sludge microorganisms after 28 days of incubation. The concentration of asulam was 105 mg/L corresponding to an oxygen demand of about 124 mg/L ($ThOD_{NH4}$) and 182 mg/L ($ThOD_{NO3}$). The oxygen demand in the abiotic control was zero.

After 28 days of incubation the mean biodegradation of asulam technical was 21% ($ThOD_{NO3}$); the 10 day window failed. For N-containing test items such as asulam, a correction for a potential uptake of oxygen by nitrification was made. Partial nitrification occurred and the degradation rate of asulam when considering nitrification did not reach 60% within the 10-day window and after 28 days of incubation, therefore, asulam technical is considered not to be readily biodegradable, but an inherent biodegradation potential was found. The reference item sodium benzoate was sufficiently degraded to a mean of 104% after 14 days and to a mean of 110% after 28 days of incubation, thus confirming the suitability of the aerobic activated sludge inoculum used. In the toxicity control containing both, the test item and the reference item sodium benzoate, a mean of 47% biodegradation was noted within 14 days and 50% biodegradation after 28 days of incubation (based on $ThOD_{NH4}$). Thus, the test item can be assumed to be not inhibitory to the aerobic activated sludge micro organisms.

In conclusion, the degradation rate of asulam technical did not reach 60% within the 10-day window and after 28 days of incubation. Therefore, asulam was considered not to be readily biodegradable under the conditions of this test, but an inherent biodegradation potential was found.

5.1.2.3 Simulation tests

i) An aerobic water-sediment study was conducted according to BBA IV: 5-1 (1990) guidelines and to GLP [49]. A subsequent kinetic evaluation in the DAR Additional report (2009) was conducted in accordance with EU/95/36EC, FOCUS (2006) procedures using the computer program KinGUI - and to Good Modelling Practice [50].

In the original study by Purser [49] samples of two natural water/sediment systems were equilibrated in borosilicate glass cylinders for 82 days at 20±2°C in the dark. Sediment (2 mm sieved) was added to each replicate unit to a 2.5 cm depth and surface water was added to achieve a 6 cm depth. The water phase was agitated slightly on an orbital shaker and moistened air was

drawn over the surface. Water and sediment characteristics are presented in Table 27. Following equilibration ^{14}C-asulam (radiochemical purity 98.7%, specific activity 73.6 µCi/mg), dissolved in sodium hydroxide solution (0.025 M) to form the sodium salt, was added at 142.3 µg or 10.47 µCi. Treated flasks were purged with CO_2 free air and fitted with traps for polar organic volatiles (ethanediol) and for CO_2 (2M NaOH). All units were then incubated at 20±2°C in the dark for up to 153 days. Measurement of the redox potential and dissolved oxygen indicated that the water phase remained aerobic and the sediment phase remained anaerobic throughout.

Table 27: Characterisation of sediment and associated overlying water

Name	**System 1 Mill Stream Pond**	**System 2 Emperor Lake**
Sediment		
% Sand (63 µm to 2 mm)	37.10	29.68
% Silt (2 µm to 63 µm)	39.37	53.94
% Clay (< 2 µm)	23.53	16.08
BBA Classification	Medium sandy loam	Medium clay silt
ADAS (UK) Classification	Clay loam	Sandy silt loam
Dry mass (%)	24.7	42.2
% Organic carbon	5.8	3.4
% Organic matter	10.0	5.8
pH: H_2O	7.8	5.8
pH: KCl	7.5	4.7
Maximum water holding capacity (%)	124.4	86.8
Cation exchange capacity (me/100 g)	33.7	26.9
Total nitrogen (mg/kg)	5817	3038
Total phosphorus (%)	1761	695
Biomass Start	2148.8	293.5
Biomass End	818.1	481.3
Water		
At collection:		
pH	8.29	6.01
Temperature (ºC)	15.3	11.6
% Oxygen content (just below surface)	122	94
% Oxygen content (5 cm above sediment)	122	90
Laboratory measurements:		
% Organic carbon	35.0	33.7
Hardness (CaCO3, mg/L)	204	49.0
Total nitrogen (mg/kg)	2.8	<0.05
Total phosphorus (mg/kg)	0.2	0.1

Duplicate units were analysed at day 0 and at 1, 3, 7, 14, 30, 62, 104 and 153 days after application. Radioactivity in the water was quantified by LSC and HPLC. Confirmatory analysis for asulam and its metabolites was by LC-MS. Sediment was extracted with acetone:water (50:50 v/v) and radioactivity in extracts quantified by HPLC. One extracted sediment sample from each aquatic system at 62 and 104 days was re-extracted with sodium hydroxide solution (0.5 M) at 50 °C followed by centrifugation.

Radioactivity in the extract and residue (humin fraction) were determined by LSC. The sodium hydroxide extract was acidified (pH 2) and radioactivity in the supernatant (fulvic acid fraction) was

determined following centrifugation and neutralisation. The solid residue was reconstituted in sodium hydroxide (0.5M) and again the radioactivity determined (fulvic acid fraction). One extracted soil sample from each aquatic system at 153 days were re-Soxhlet extracted with acetonitrile:water (80:20 v/v). Radioactivity in the volatile traps was determined by LSC.

Mean recoveries were acceptable for both systems with an overall mean recovery 96.6% AR. The pattern of degradation of asulam was comparable in both water/sediment systems. Soxhlet extraction performed on single replicates for the 153 day timepoint sediments only liberated a further 5 to 6% of AR. Bound residue fractionation performed on 62 day and 104 day samples showed fairly even distribution of radioactivity between fulvic acid, humic acid and humin fractions. It was suggested by the Notifier that radioactivity associated with the sediment fractions may be tightly bound asulam or its degradates or incorporated breakdown products (including carbon dioxide). Up to 14% and 3% AR was recovered from sodium hydroxide traps in Emperor Lake and Mill Stream Pond systems respectively, representing limited mineralisation to CO_2. Only negligible amounts of AR (<0.1%) were retained in the ethanediol traps indicating minimal volatile organic compounds (VOCs). Full details of the recovery and characterisation of radioactivity in water, sediment and total system are presented in Vol. 3, Section B.8.4.4 of the asulam DAR (2016). The results for the whole systems only (from the original Purser, 1998a study [49]) are presented in Table 28.

Table 28: Percent of applied radioactivity present as asulam and its degradation products (determined by HPLC) extracted from two natural water-sediment systems (total system) following ^{14}C-asulam application (means of duplicate samples)

Time (days)	Asulam	Acetyl asulam	Sulfanilic acid	Sulfanil -amide	Acetyl Sulfanil -amide	Total Unknowns	Unresolved background	Total allocated
System 1: Mill Stream Pond								
0.007	95.5	nd	nd	0.2	nd	nd	0.7	96.3
1	93.5	nd	nd	0.5	nd	nd	0.5	94.4
3	90.2	nd	nd	0.8	nd	0.4	0.5	92.0
7	86.2	0.2	nd	0.7	nd	1.8	0.5	89.3
14	75.0	1.3	nd	0.8	nd	4.1	0.5	81.6
30	68.4	1.0	0.2	0.3	0.1	1.1	0.8	71.8
62	54.5	0.1	nd	1.0	nd	5.0	0.4	60.9
104	37.5	nd	nd	0.9	0.2	1.3	0.2	40.1
153	26.3	0.1	0.1	1.5	0.3	2.5	0.1	30.9
System 2: Emperor Lake								
0.007	97.0	nd	nd	nd	nd	nd	0.2	97.2
1	96.1	nd	nd	0.5	nd	0.5	0.6	97.6
3	86.9	0.4	nd	0.8	nd	0.5	0.6	89.1
7	86.2	0.3	nd	0.8	nd	2.2	0.6	90.0
14	71.4	0.4	0.1	2.0	nd	4.1	0.3	78.3
30	65.3	0.3	0.1	1.9	0.2	3.0	0.7	71.4
62	53.1	0.6	nd	3.3	nd	2.5	0.4	59.8
104	31.9	nd	nd	3.0	0,2	2.5	0.3	37.8
153	16.7	0,8	nd	3.4	1.0	1.8	0.2	23.7

nd = not detected

Asulam was a major component in all surface water and sediment samples analysed. Summation of the separate phases to consider the aquatic system as a whole showed a steady decline in the amount of asulam present down to 26 and 17% AR after 153 days. Four degradation products were identified by co-chromatography with analytical standards, acetyl asulam, sulphanilic acid, sulphanilamide and acetyl sulphanilamide (none greater than 3.8% AR in any phase). Seven other unknown metabolites also occurred. No unidentified metabolite exceeded 2.9% AR.

Data for the water phase and total system were initially calculated using first order kinetics and a 'double first-order in parallel' (DFOP) model. DT_{50} values were calculated for the water phase, but these represented dissipation rather than degradation rates since they include loss due to partitioning to sediment; this process was relatively slow (dissipation DT_{50} 58 to 68 days). For the sediment phase an enhanced symmetry decay curve with an accumulation phase was fitted using Microsoft Excel software. However, this approach was not considered appropriate by the pesticides Rapporteur and the results were excluded from the DAR. Bound residues not extracted from the sediment represented 56-58% AR at the end of the study.

In subsequent kinetic analysis presented in the DAR Additional Report (2009) (Hardy and Patel, 2008c) [50] the values for the total system were entered into the KinGUI SFO (single first-order) kinetic model for the whole system and optimisations carried out for M0 and rate constant k. The goodness of fit was assessed visually and by Chi^2 (χ^2) error <15% and t-test >99%. The parameters were validated by the pesticide RMS using Microsoft Excel and considered acceptable. The degradation DT_{50} results from the subsequent kinetic re-analysis for the whole system (accepted in the previous EFSA peer review) are presented in Table 29.

Table 29: Degradation rates of ^{14}C-asulam for the whole water-sediment system calculated using first order kinetics

Phase	**Total system**			
System	**DT_{50} (days)**	**Min χ^2 error**	**t-test**	**Mineralization at end of study**
Mill Stream Pond	78.8	3.6	3.8E-10, >99%	3.0% after 153 d
Emperor Lake	65.6	4.8	3.7E-11, >99%	14% after 153 d

ii) An aerobic water-sediment study was conducted according to EU Guideline 95/36/EC and to GLP [51]. A subsequent kinetic evaluation in the 2016 DAR was conducted in accordance with FOCUS (2006) procedures and to Good Modelling Practice [see also 51].

This water-sediment study by Willems(1997a) is a late addition to the dossier and is not evaluated in full here - further details of it and the kinetic reanalysis by Hardy (2008c) are available in the latest verion of the asulam-sodium DAR (2016), section B.8.4.4(c).

Radiolabelled [^{14}C]asulam (purity 95.4%) was aerobically incubated in two different uncontaminated water/sediment systems in the dark at 20°C ± 2°C. The two systems were from 'Oostvaardersplassen' (OVP) which is a diked area from lake Ijssel, about 30 kilometres north east of Amsterdam - and 'Schoonrewoerdsewiel' (SW) which is a pool near Leerdam, approximately 60 kilometres south of Amsterdam. The properties and characteristics of the water and sediment systems are tabulated in the 2016 DAR.

The incubation system consisted of 1L dark brown glass metabolism flasks. The solid sediment content for the SW system was 7.5-8.5% and 9.0-9.2% for the OVP system and the sediment layer

thicknesses were 2.5 cm and 2.0 cm for the SW and OVP systems, respectively. After a 10.5 week pre-incubation period, asulam was applied at a rate of 1.4-1.9 mg a.s./L in the water layer. Activity was fractionated into $^{14}CO_2$, volatile compounds, activity in the water layer, sediment extractable residues (methanol extraction) and sediment unextractable residues. Full methodological and analytical details are given in the 2016 DAR.

According to the original study report, asulam degraded in both water/sediment systems with a DT_{50} of 59 days in the OVP system and 17 days in the SW system. Degradation did not follow first order degradation kinetics and slowed down with time. Considering only the water layer, the DT_{50} values were 34 and 10 days in the OVP and SW systems, respectively. These DT_{50}s are further analysed bekol. Any declines, in the absence of mineralization and volatilization, were primarily the result of the transfer of radioactive residues from the water layer to the sediment. Sediment extractable residues reached a maximum at 7 days and remained fairly level up to day 14 in the OVP sediment and up to day 58 in the SW sediment. After this period, the extractable residues decreased to approximately 7 %. The decrease in extractable residues is the result of increased unextractability which is most likely caused by bound residue formation. Unextractable residues increased continuously with incubation time and reached 58.0 % and 73.2 % after 120 days of incubation.

Mass balances averaged 106.5 % (±2.8 sd) and 100.5 % (±5.4 sd) for the OVP and SW systems, respectively. Individual mass balances were always between 90% and 110 %. Complete mineralization of the aromatic moiety of asulam was minimal in both water/sediment systems. After 120 d of incubation less than 2% of asulam was mineralized in both systems. No volatile organics were formed in both water/sediment systems.
No degradants occurring at levels above 10 % were observed in water phases or in sediments after 120 days of incubation or at any other time during incubation. The remaining activity at 120 d was for the largest part recovered as asulam in the water layer (6-30%).

The original DT_{50} values from the study report were not considered appropriate by the RMS and have been re-calculated in accordance with current FOCUS kinetics (2006) in a re-evalution by Hardy (2011a) [also 51] - see 2016 DAR, section B.8.4.4(c).
Various best fit kinetic models were considered and the most appropriate Single First Order (SFO) whole system degradation DT_{50} for the OVP system was 76.2 days. The best whole system degradation DT_{50} for the SW system followed a Hockey Stick (HS) decline model (using the slow phase k_2 rate constant) and was 61.9 days.

Conclusion from both sediment-water studies

Considering both systems from both simulation studies by Purser (1998a) and Willems (1997a), along with their respective kinetic reanalyses, the RMS has calculated an overall geometric mean whole system DT_{50} for asulam of 70.3 days. This is not sufficient to meet CLP criteria for ‘rapid degradation’.

5.1.3 Summary and discussion of degradation

Asulam is stable to hydrolysis at all pH values (pH 5, 7 and 9) over 31 days. There was insufficient degradation to calculate degradation half lives.

Asulam is not readily biodegradable since only 52% biodegradation occurred over 28 days based on biochemical oxygen demand in an OECD 301 B study and 21% based on theoretical oxygen demand in an OECD 301 F study.

An aqueous photolysis study was performed with asulam sodium in sterile buffer solutions at pH 4 and pH 9 at 25°C. DT_{50} values for asulam were calculated assuming single first order kinetics to be 0.44 days (pH 4) and 0.87 days (pH 9) following artificial illumination (equivalent to 0.78 and 1.56 days of 52°N summer sunlight respectively). Estimated photolytic half lives of asulam in natural surface waters, calculated from the quantum yield, ranged from 7 to 119 hours at pH 4 and 8 to 135 hours at pH 9 in central European latitudes (52°N). Three major (i.e. >10% AR) photo-degradation products were formed and identified as sulfanilic acid, AP formamide and MCAPAP carbamate.

A further aqueous photolysis study was performed with asulam sodium in sterile natural water at 25°C. The DT_{50} was calculated using first order kinetics to be 0.84 days (equivalent to 4.21 days spring sunlight at 35°N in Japan, which is comparable to Athens, Southern Europe). Many minor photodegradates were formed, all <10% AR and none of the major metabolites identified in the sterile buffered photolysis study were formed in significant amounts.

In another aqueous photolysis study using artificial illumination equivalent to 0.54 days (pH 4) and 1.64 days (pH 9) in natural sunlight at 52°N at 25°C - the DT_{50} values calculated using SFO kinetics were comparable with the original study above at 0.28 days (pH 4) and 0.86 days (pH 9) respectively. The modelled half-life of asulam in the top few millimetres ranged from 0.183 days in summer at 40° N to 5 days in winter at 60° N and at pH 9 it ranged from 0.25 days in summer at 40° N to 5.1 days in winter at 60° N. Similar levels of photodegradates were produced. No significant degradation of asulam was observed in a non-irradiated system.

It is noted from dark water/sediment studies (below) that the partitioning of asulam from the water phase to sediment was relatively slow (DT_{50} 58 to 68 days) compared to the photolytic half-life, suggesting that asulam might be available in the surface water for photolysis to occur. However, although photolytic degradation may be rapid in the top few millimeters of an aquatic system, this degradation will reduce in deeper natural water bodies where calculated half-lives range from about half a day in summer to just over a week in autumn. In typical turbid European natural surface waters, particularly at higher latitudes, photolysis is not considered to be a significant enough route of degradation for asulam-sodium to be considered ‘rapidly degradable’.

In laboratory incubations in aerobic natural water-sediment systems (in the dark at 20°C), asulam was relatively persistent (SFO DT_{50}: 66-79 days). Partitioning of asulam to the sediment was relatively slow and moderate. No major metabolites were formed. Mineralisation to carbon dioxide accounted for 3-13.9% AR, whilst sediment bound residues represented 56-58% AR at the end of the study. In another study [51] whole system DT_{50}s ranged were a similar at 61.9 to 76.2 days. Considering all of the water-sediment systems from both simulation studies, along with their respective kinetic reanalyses, an overall geometric mean whole system DT_{50} for asulam of 70.3 days has been calculated. This is also not sufficient to meet CLP criteria for ‘rapid degradation’.

Overall, although rapid photolytic degradation may occur under certain aquatic conditions, the available abiotic and biotic degradation information does not indicate that asulam is ultimately degraded (>70%) within 28 days (equivalent to a half-life < 16 days) or transformed to entirely non-classifiable degradants. Consequently asulam is considered ‘not rapidly degradable’ for the purposes of classification under the CLP Regulation.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

i) A batch equilibrium adsorption/desorption study was conducted with asulam in accordance with OECD guideline 106 (1981), EC Directive 95/36/EC and EPA guidelines N, 163-1, (1982) and to GLP (Lewis, 1999a) [76]. The definitive study was conducted in 3 UK soils (two sandy loams and a clay loam), 2 US soils (a sand and sandy silt loam) and a UK sediment (a sandy clay loam). The pH of these ranged from 5.5-7.5 and organic matter content from 0.2-10.5%. The full characteristics of each soil are presented in Vol. 3, Section B.8.2.1(a) of the asulam sodim DAR (2016).

Stock solutions of ring labelled ^{14}C-asulam (radiochemical purity >97%, specific activity 5.617 MBq/mg) were initially prepared in an equimolar aqueous sodium hydroxide solution and further diluted to concentrations of 5, 1, 0.2 and 0.04 µg/mL with 0.01M $CaCl_2$ solution prior to addition to the soils and sediment. Preliminary studies were performed to establish adsorption and desorption equilibrium times, soil:solution ratio and to obtain preliminary solubility and stability data. Equilibrium time and the adsorption and desorption studies were conducted in the dark at 20±2°C. The stability of asulam in the 24 h adsorption was verified by HPLC analysis. Degradation products were <5% of radioactivity in solution.

The determination of isotherms was performed by addition of 10 mL portions of ^{14}C-asulam solutions to duplicate 10 g dry weight equivalent samples of soil which had been air dried and equilibrated for 24 h by shaking with twice its weight in water. One adsorption and 2 desorption steps were performed. After each step, samples were centrifuged and the supernatants removed. The soils and sediment treated with the highest concentration (5 µg/mL) were extracted with acetonitrile after the second desorption stage. The extracted soils and sediment were allowed to air dry. The radioactivity was determined in the adsorption and desorption solutions (and extracts) by liquid scintillation counting (LSC). The mean mass balance data was acceptable (96.0 to 99.3%).

Freundlich adsorption coefficients (K_f, K_{foc} and K_{fom}) and values of 1/n which have been validated by the pesticides Rapporteur, are shown in Table 30. Arithmetic mean values for K_{foc} and 1/n were calculated to be 20 and 0.75 respectively. Only the four soils were included in the mean calculation. The US sand soil LA 98-983 (with very low carbon content) and the UK Emperor lake sediment (not a soil) were excluded. With the exception of the sand, variation between K_{foc} values was less than between K_f values, indicating that organic carbon of soil content is an important factor in determining adsorption. Adsorption was not noted to correlate with pH or any other soil parameter.

Table 30(a): Freundlich constants determined for ^{14}C-asulam sodium in soils and sediment

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

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

A batch equilibrium adsorption/desorption study was conducted with asulam in accordance with OECD Guideline No. 106 and EC Directive 95/36/EC and to GLP (Völkel. 2011a) [77]. This is a late addition to the dossier and is not evaluated in full here - further details are available in the latest version of the asulam-sodium DAR (2016), section B.8.2.1.

The adsorption/desorption behaviour of the test item ^{14}C-asulam on soil was determined with five soils. these were a silt loam, a loam, a loamy sand, a silty clay and a clay. Full characteristics of each soil are presented in the asulam-sodium DAR (2016). The soils showed a range of different characteristics important for adsorption i.e. organic carbon content (OC), cation exchange capacity, pH and clay content.

Screening results showed that no adsorption equilibrium was reached after 44 hours. After 44 hours of adsorption, 41.2%, 78.4%, 20.4%, 42.9% and 18.7% of the applied amount was absorbed to each soil, respectively. All applied radioactivity was recovered in the control samples (96.5% to 100.6%) and remained constant throughout the incubation time. The amount of test item desorbed did not reach an equilibrium after 44 hours of desorption.

Degradation of the test item was observed in two of the soils during the adsorption phase. Therefore, the radioactivity measured in the supernatant solutions was corrected by the amount of asulam as analysed by HPLC. The mean values for the adsorption and desorption coefficients Kd

and Kdes were 1.1 mL/g and 3.8 mL/g. The mean values for the adsorption and desorption coefficients related to the organic carbon content of the soils K_{OC} and $Kdes_{,OC}$ for soils were 35 mL/g and 129 mL/g, respectively. The higher coefficients for desorption indicated a partially irreversible sorption process.

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

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

The adsorption/desorption parameters resulting from the Freundlich isotherms are presented in Table 30(b) below.

Table 30(b): Asulam sodium Freundlich adsorption constants and K_{OC} values in four soils

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

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

iii) A further adsorption/desorption study is reported in the 2016 asulam-sodium DAR - Lowden and Mahay (1999a) [77].

This batch equilibrium aged desorption study was conducted with asulam sodium in accordance with EC Directive 96/36/EC and OECD guideline 106 (1981) and to GLP. ^{14}C-Asulam (radiochemical purity >98.1%, specific activity 740 MBq/mmole), as the sodium salt, in 75 mL 0.01M $CaCl_2$ was added to duplicate samples of 4 UK soils (15 g dry weight) at equivalent concentrations of 5, 1, 0.2 and 0.04 mg a.s./L. Soil characteristics are presented in section B.8.2.1 of the DAR (**Error! Reference source not found.**) . The desorption of ^{14}C-asulam was investigated ollowing aging of the samples for periods of 0, 2 and 7 days. At the end of each aging period the soil samples were subjected to five successive desorption cycles (each of 24 h duration) with 0.01M $CaCl_2$ solution. Following the final desorption cycle one replicate from each soil at the highest

concentration was extracted with acetonitrile:water (1:1 v/v) and aliquots removed for liquid scintillation counting (LSC).

The stability of asulam sodium during the desorption period was demonstrated by HPLC in which asulam sodium was the only significant peak and it represented all the desorbable material at all time points. Overall the recovery of radioactivity was considered by the RMS as acceptable, in the mean range 88.1 to 99.5%. There were some individual outliers in the lowest concentration group but these were considered not to affect the validity of the study.

The Freundlich desorption coefficients $K_{f\ des}$ and $K_{foc\ des}$ were calculated for each desorption cycle. The values of $K_{f\ des}$ and $K_{foc\ des}$ were shown to increase with each successive desorption cycle from the first to the fifth for each aging period in all soils. It was also observed for Day 0, Day 2 and Day 7 aging phases that the 1/n values for $K_{f\ des}$ varied significantly from 1 for initial desorption but became closer to 1 for successive cycles. The increases in $K_{f\ des}$ and $K_{foc\ des}$ values with each desorption cycle, particularly at seven days, indicated that there may be different adsorption mechanisms at work, some of which resulted in very strong adsorption of some of the asulam applied to the soil. The effect of the aging period indicated that the potential mobility of asulam may be significantly reduced with time.

Table 30(c): Summary of Freundlich desorption coefficients in four soils after periods of aging for ^{14}C-asulam sodium in the first desorption cycle

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

5.2.2 Volatilisation

No specific aqueous volatility studies are available, however, asulam has a low potential for volatilization with a vapour pressure of 5 x 10^{-7} Pa at 45°C and a Henry's law constant of 3 x 10^{-10} Pa m^3 mol^{-1}. Its atmospheric half-life estimated according to the Atkinson calculation is 0.4 days (ref. Section 1.3).

5.2.3 Distribution modelling

None submitted.

5.2.4 Summary of likely environmental distribution

Asulam is considered unlikely to volatilise significantly from surface waters. Its mean K_{foc} value of 20 determined from the batch adsorption/desorption study by Lewis (1999a) [76] indicates that asulam is unlikely to partition substantially from the water phase. This was confirmed by the relatively slow movement to sediment seen in the water-sediment studies. In soil asulam would be expected to exhibit very high mobility, this was also confirmed in a column leaching study (Reeves *et al.* 1988a) in four UK soils (pH 7.10-7.26; organic matter 0.84-3.56%) evaluated in more detail at B.8.2.2(a) in Vol. 3 of the asulam-sodium DAR. A study on the effect of aging [77] indicated that the potential mobility of asulam-sodium may be significantly reduced with time.

5.3 Aquatic Bioaccumulation

Table 31: Summary of relevant information on aquatic bioaccumulation of asulam/asulam sodium

Method	Results	Remarks	Reference
Partition coefficient n-octanol/water EEC method A 8, (tested purity: 99.1%)	Log K_{ow} = 0.11 at pH 4 and 25°C Log K_{ow} = 0.15 at pH7 and 25°C Log K_{ow} = 0.77 at pH 9 and 25°C	Valid study to GLP	Francon, 1999c [4] DAR B.2.1
Bioconcentration test on catfish (*Ameirus melas*), exposed for 28 days + 15 days depuration via soil (mixed with water) with asulam at 0.01 and 1.0 µg a.s./g dw. (tested radiochemical purity: 98.5%)	Measured whole fish bioconcentration factor (BCF): 0.1 -1.4 Clearance time for 90% of the substance (CT_{90}): < 7 days	Non-standard study, no measurement of exposure concentrations, no GLP but otherwise valid study	1981a [52] DAR B.9.2.1.4

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

No specific bioaccumulation modelling has been conducted, however a measured fish bioconcentration study is available and this is evaluated below.

5.3.1.2 Measured bioaccumulation data

A bioconcentration test was conducted on catfish (*Ameirus melas*) using radiolabelled ^{14}C-asulam, with a radiochemical purity of 98.5% (1981a)[52]. The exposure phase lasted for 28 days and there was a 15 day de-puration phase. The study was conducted prior to implementation of GLP and was not conducted to standard guidelines but to internal company protocols. There was no measurement of concentrations in the supernatant water as there would be in a standard OECD 305 test and the study is of uncertain relevance for classification purposes. Other studies suggest that asulam is likely to have been relatively stable during the course of this test and to have equilibrated with the water phase or remained partially bound to the soil, the particular dynamics of this system are unclear however. Catfish live and feed predominantly in the bottom sediments of the tank. Although not required due to the low log K_{ow} of asulam, the study was therefore considered reliable and of some use in regulatory assessment during the pesticide review under Dir. 91/414/EEC and more recent assessment under 1107/2009. A summary is provided below for completeness.

Samples of loam soil were treated with ^{14}C-asulam to give concentrations of 0.01 and 1.0 µg a.s./g on air-dried basis. After aerobic incubation at ambient temperature for 35 days the soils were placed in aquaria and the water and catfish were added. Deionised water of total hardness 20 mg/L as $CaCO_3$ was used. 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 1.0 mg/g concentration group. Sixty fish (5-6 cm length, weighing 2-3 g) were assigned to each aquarium. The fish were fed daily throughout the study but the concentrations of asulam moving to food and taken up by dietary exposure were not considered separately.

Samples of soil, water and fish were taken at day 1, 3, 7, 10, 14, 21 and 28 to measure the distribution of radioactivity. The fish were then removed into three other soil-free aquaria in order to measure the depletion/depuration of radioactivity. During the 15-day depuration 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 phase.

Some of the radioactivity became bound to the soil during aerobic incubation, and remained bound following transfer to the aquatic systems (approximately 20% of the material applied to the aquarium water). The amounts of the radiolabelled material taken up by the fish were very low. In the aquarium containing soil treated at 1.0 µg/g a maximum ^{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).

Under these study exposure conditions, bioconcentration factors (BCF) 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. No re-calculation based on lipid content or growth of the fish was undertaken. 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. The CT_{90} (time for 90% clearance of the active) was therefore less than 7 days.

Samples of soil, water and fish from the higher treatment rate system were analysed for degradants/metabolites 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, sulfanilic acid and benzenesulfonic acid. Very low levels of methyl benzene-sulfonylcarbamate and benzenesulfonamide were determined in fish.

In conclusion, whole fish bioconcentration factors (BCF) varied from 0.1 to 1.4 but the majority of the results were below 1.0, indicating there was no concentration of residues in catfish exposed under the particular conditions of this study. The lack of confirmation of water concentration makes this non-standard study of limited use for hazard classification purposes, although it does indicate a low bioconcentration potential in benthic fish.

5.3.1.3 Summary and discussion of aquatic bioaccumulation

Asulam has a log K_{ow} at pH 7 of 0.15 which is below the CLP trigger of 4 indicating a low potential for bioaccumulation. A bioconcentration study on catfish is also available; although non-standard and not to GLP, this also indicates a low bioaccumulation potential under the particular condition of the study, with measured whole fish BCF values of 0.1 to 1.4 (i.e. much less than the CLP BCF trigger of 500).

5.4 Aquatic toxicity

The reported toxicity studies on aquatic organisms used either technical asulam or asulam sodium. Study endpoints based on asulam sodium have been converted into pure asulam equivalents (and *vice versa*) using a conversion factor of 0.9128, based on the molecular weight of asulam (230.2) and asulam sodium (252.2).

All tests were conducted without significant deviation from guideline and in accordance with GLP (apart from the re-analysis of algal endpoints by Dorgerloh (2004) [61] [63] [65] which did not require GLP). The studies were reviewed under Directive 91/414/EEC and are considered valid; the endpoints were agreed in the EFSA Conclusion on asulam (2010). Some newer studies have since been submitted during consideration of asulam sodium as a new active substance under Reg.n 1107/2009 and are included in the updated DAR (2016), Vol. 3, Section B.9.2.

A summary of the aquatic toxicity data on asulam/asulam sodium is presented in Table 32. In addition, two studies are available on a main aquatic degradant, sulfanilamide, these are summarised in Table 33. Further detail on the studies considered for classification purposes is given in the following sections.

Table 32: Summary of the acute and chronic toxicity of asulam/asulam sodium to aquatic life

Test species	Test substance and purity	Test type and guideline (to GLP unless stated)	Actual conc.n (% of nominal)	LC or EC_{50}[a] (mg/L)	NOEC[a] (mg/L)	References
Fish (acute)						
Rainbow trout (*Oncorhynchus mykiss*)	Asulam sodium, 88% pure	96-h static test to EPA 72-1	77-88%	LC_{50} >175 [≡ >159.8 pure asulam] mm	175 [≡ 159.8 pure asulam] mm	1988a [53] DAR B.9.2.1.1
Bluegill sunfish (*Lepomis macrochirus*)	Asulam sodium, 81.4% pure	96-h semi-static (renewal at 48 h) test to OECD 203	100-110%	LC_{50} **>100** [≡ >91.3 pure asulam] mm	100 [≡ 91.3 pure asulam] mm	2000 [54] DAR B.9.2.1.1
Fish (prolonged)						
Rainbow trout (*Oncorhynchus mykiss*)	Asulam, 80.6% pure	28-d flow-through juvenile growth test to OECD 215	98-121%	EC_{50} >119.1 (≡ 130.5 mg asulam sodium/L) mm	119.1 (≡ **130.5** mg asulam sodium/L) mm	1997a [55] DAR B.9.2.1.3
Aquatic invertebrates (acute)						
Daphnia magna	Asulam sodium, 88% pure	48-h flow-through test to EPA 72-2	64-75%	EC_{50} = **63.4** [≡ 57.87 pure asulam] mm	25.5 [≡ 23.28 pure asulam] mm	Manning (1988b) [56] DAR B.9.2.2.1

Aquatic invertebrates (chronic)						
Daphnia magna	Asulam, 80.6% pure	21-d semi-static to EPA 72-4	86-117%	EC_{50} (adult survival) = 57.1 (≡ 62.6 mg asulam sodium/L) mm	6.4 (≡ **7.01** mg asulam sodium/L) mm	McElligott (1997b) [57] B.9.2.2.3
Daphnia magna	Asulam, 80% pure	20-d semi-static to OECD 202 II	Not analysed - study not relied on	EC_{50} (repro. rate) = 21.48 (≡ 23.53 mg asulam sodium/L) nom[d]	8.96 (≡ 9.82 mg asulam sodium/L) nom[d]	Herrmann *et al.* (1992a)[d] [58] DAR B.9.2.2.3
Chironomus riparius	Asulam sodium, 82.2% pure	28-d spiked water test to draft OECD 219 (2000) and draft BBA (1995)	Not analysed - study not relied on	>100 [≡ >91.3 pure asulam] nom[d]	100 [≡ 91.3 pure asulam] nom[d]	Heintze (2002)[d] [59] DAR B.9.2.2.3
Algae						
Pseudokirch-neriella subcapitata[b]	Asulam sodium, 89.5% pure	120-h static test to EPA 122-2/123-2 and OECD 201	89-98%	72-h E_rC_{50} = **1.90** [≡ 1.73 pure asulam] mm	72-h NOE_rC = **0.02** [≡ 0.018 pure asulam] mm	Study: Hoberg (1992a) [60] and Reassessment to OECD 201 by Dorgerloh (2004a)[c] [61] DAR 9.2.3.1
Anabaena flos-aquae	Asulam sodium, 89.5% pure	120-h static test to EPA 122-2/123-2 and OECD 201	89-93%	72-h E_rC_{50} >0.72 [≡ >0.66 pure asulam] mm	72-h NOE_rC = 0.19 [≡ 0.17 pure asulam] mm	Study: Hoberg (1992b) [62] and Reassessment to OECD 201 by Dorgerloh (2004b)[c] [63] DAR 9.2.3.1
Skeletonema costatum	Asulam sodium, 89.5% pure	120-h static test to EPA 122-2/123-2 and OECD 201	88-113%	72-h E_rC_{50} >1.8 [≡ >1.64 pure asulam] mm	72-h NOE_rC = 0.33 [≡ 0.3 pure asulam] mm	Study: Hoberg (1992d) [64] and Reassessment to OECD 201 by Dorgerloh (2004c)[c] [65] DAR 9.2.3.1

Navicula pelliculosa	Asulam sodium, 89.5% pure	120-h static test to EPA 122-2/123-2, effects based on cell no./biomass only - not growth rate	82-98%	72-h E_rC_{50}: >4.4 [≡ >4.2 pure asulam] mm	72-h NOE_rC = 0.54 [≡ 0.49 pure asulam] mm	Hoberg (1992c) [66] DAR 9.2.3.1
Aquatic plants						
Lemna gibba	Asulam sodium, 89.5% pure	14-d static test to EPA 122-2/123-2	88-112% initial range (mean 99% in fresh sol.n; mean 15% at study end)	14-d E_rC_{50} = **0.16** [≡ 0.146 pure asulam] mm	14-d NOE_rC = 0.051 [≡ 0.047 pure asulam] mm	Hoberg (1992e) [67] DAR B.9.2.4.1
Lemna gibba	400 g asulam/L SL formulation (simple solution of asulam in water)	7-d static test to OECD 221	88-98% (mean 94%)	7-d E_rC_{50} ≡ 0.845 mg/L pure asulam (≡ 0.926 mg asulam sodium/L) mm	7-d NOE_rC = 0.033 mg/L pure asulam (≡ 0.0362 mg asulam sodium/L) mm	Vinken &. Wydra (2007) [78] DAR B.9.2.4.1
Lemna paucicostata	Asulam, purity not stated	7-d static growth test to ISO/WD 20079 (2001 draft), effects based on frond no. and biomass only - not growth rate. Not to GLP	Not analysed - study not relied on	14-d E_bC_{50} = 93.8 pure asulam (≡ 102.8 mg asulam sodium/L) nom[d]	Not reported[d]	Michel *et al.* (2004)[d] [68] DAR B.9.2.4.1
Five aquatic macrophytes including: *Myriophyllum spicatum*, *Elodea nuttallii*, *Elodea canadensis*, *Ranunculus circinatus* and *Potamogeton crispus*	'Asulox' 400 g asulam/L SL formulation (simple solution of asulam in water)	21-d static growth test to non-standard protocol, effects assessed on shoot length and biomass only - not growth rate. Not to GLP	86-101% at initiation, remained >80% over 21 d	Most sensitive test species *M. spicatum* 21-d E_bC_{50} = 0.0107 pure asulam (≡ 0.0117 mg asulam sodium/L) nom[d]	Not reported[d]	Arts & Belgers (2013)[d] [69] DAR B.9.2.4.1

Myriophyllum spicatum	'Asulox' 400 g asulam/L SL formulation (simple solution of asulam in water)	14-day static growth test to draft OECD test guideline (July 2014), using rooted plants in a water-sediment test system.	Mean 104% at initiation 80% at termination, Overall mean 92%	14-d E_rC_{50} = 5.88 pure asulam/L (≡ 6.44 mg asulam sodium/L) nom	14-d NOE_rC = 0.01 pure asulam/L (≡ **0.011** mg asulam sodium/L) nom	Seeland, Fremer & Wydra (2014) [70] DAR B.9.2.4.1

[a] mm = based on mean measured concentrations; nom = based on nominal concentrations. Endpoints relate to full duration of study unless otherwise stated
[b] Referred to in study by previous name *Selenastrum capricornutum*
[c] Re-assessment of original algal study endpoint (based on cell number) to growth rate, GLP is not applicable
[d] Study of uncertain reliability and not relied on, due either to lack of analysis throughout study, high variability in endpoint, lack of GLP and/or reporting detail
Values in **bold** indicate key acute and chronic classification endpoints for each trophic group

Aquatic toxicity studies on degradants

For completeness, data are summarised below on a main aquatic degradant of asulam, i.e. sulfanilamide. The alga *Pseudokirchneriella subcapitata* [syn. *Selenastrum capricornutum*] and duckweed *Lemna minor* have been tested. The studies were conducted in accordance with the test guideline(s) and to GLP; brief details only are included in the following Table.

In terms of hazard classification, the proposal is based on asulam/asulam sodium alone. This is because asulam is 'not rapidly degradable' and any degradants are mostly minor (none >3.8% applied radioactivity (AR) in any phase of a water-sediment simulation study and they are likely to be less toxic than the parent substance, as the data below indicate.

Table 33: Acute/Short-term toxicity of the degradant sulfanilamide to aquatic life

Test species	**Test substance and purity**	**Test type and guideline**	**Actual conc.n (% of nominal)**	**E_rC_{50} (mg/L)**	**NOE_rC (mg/L)**	**Reference**
Pseudokirchner-iella subcapitata	Sulfanilamide, 99.9% pure	120-h static test to OECD 201, EEC 92/69/EWG C.3, and EPA J 123-2	66-80%	>21.15 mm[a]	2.78 mm[a]	Gosch & Sowig (2003d) [71] DAR B.9.2.3.2
Lemna minor	Sulfanilamide, 101.4% pure	7-day semi-static test to OECD 221	100-103% in fresh sol.ns, 85-97% in spent soln.s	5.82im[b, c]	0.67 im[b, c]	Juckeland (2011) [72] B.9.2.4.2

[a] Endpoints based on mean measured concentrations of sulphanilamide
[b] Endpoints based on initial measured concentrations of sulphanilamide - acceptable given analytical verification throughout test
[c] Growth rate endpoints based on frond number

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Study i):
The acute toxicity of asulam sodium (purity 88%) was investigated under static conditions in rainbow trout (*Oncorhynchus mykiss* - tested as *Salmo gairdneri*) [53]. The study was conducted to US EPA Guideline 72-1 and to GLP.

Test organisms were 120-day old rainbow trout of 0.42 ± 0.11 g wet weight and 9.9 ± 3.6 mm length. Groups of 10 fish were exposed over a period of 96 hours to a control and 5 nominal concentrations of 26, 43, 72, 120 and 200 mg/L. The test vessels were 19 L glass jars containing 15 L of dilution water or test solution. Dilution water was well water with a pH of 8.1 and a hardness of 250 mg/L as $CaCO_3$. The fish loading was 0.28 g/L. Fish were not fed during the exposure period. Samples were taken from control and each test concentration at test initiation and test termination for determination of actual asulam concentrations.

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 therefore reported as mean measured values of 20, 37.5, 61, 100 and 175 mg/L.

No mortality was observed in the control or at any of the test concentrations. Therefore, based on measured concentrations, the 96-hour LC_{50} was determined to be greater than 175 mg/L (equivalent to 159.8 mg pure asulam/L), the highest dose tested. The 96-hour mean measured NOEC was 175 mg/L asulam sodium based on the lack of mortality or any other treatment-related effects at this concentration.

Study ii):
The acute toxicity of asulam sodium (purity stated to be equivalent to 814 g/kg asulam) was investigated under semi-static conditions in bluegill sunfish (*Lepomis macrochirus*) [54]. The study was conducted to OECD 203, 1992 and to GLP.
Bluegill sunfish with a mean wet weight of 0.65 g and a mean total length of 36 mm were used for the test. Groups of 10 fish were exposed to a control and nominal concentrations of 48, 58, 69, 83 and 100 mg/L over a period of 96 hours with renewal of test solutions at 48 hours. The test was performed in 19.5 L glass aquaria, each containing 15 L of test solution or dilution water (control). The biological loading was 0.43 g/L/day. Dilution water was well water of pH 7.1-7.2 and total hardness of 32-36 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, 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 HPLC.

During the study pH ranged from 6.6-8.2; dissolved oxygen ranged from 4.4-8.2 mg/L (48-94% saturation) and temperature was 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 exposure under semi-static conditions, no mortality or adverse effects were observed amongst fish exposed to any treatment level tested or in the control. The 96-hour LC_{50} for asulam sodium to *Lepomis macrochirus* was therefore greater than a mean measured 100 mg/L (equivalent to 91.3 mg pure asulam/L), the highest concentration tested. The 96-hour mean measured NOEC was 100 mg asulam sodium/L.

5.4.1.2 Long-term toxicity to fish

A 28-day flow-through juvenile fish growth test with rainbow trout (*Oncorhynchus mykiss*) was reported using technical asulam (purity 80.6%) [55]. The study was conducted under flow-through conditions according to test guideline OECD 215 (draft 1994).

Groups of 16 fish were exposed in 57 litre tanks to nominal concentrations of 4.7, 10.3, 22.7, 50 and 110 mg asulam/L (one group/tank per concentration). There was also a dilution water control. Fish were fed with a commercial fish food during the exposure period. Weights of fish were determined at test initiation and also on test days 14 and 28. Mortalities, abnormal behaviour or appearance of fish was recorded on each working day. Samples of each test concentration and control were analysed by HPLC at least three times during the first week of testing and once a week thereafter for asulam concentrations.

The pH ranged from 7.0-8.0, dissolved oxygen ranged from 8.1-9.5 mg O_2/L and temperature from 14.0-14.5°C. Measured concentrations ranged from 98-121% of nominal during the test period and were reported as 4.9, 10.8, 23.5, 52.3 and 119.1 mg/L. Results were expressed in terms of mean measured concentrations of asulam.

No mortalities and no sub-lethal toxicity were observed during the 28 days of exposure. The percentage difference in growth rate for each test concentration compared to the control was less than 20% (actual range -3.15 to +4.30% relative to control with no clear trend). The 28-day mean measured EC_{50} was therefore >119.1 mg asulam/L, the highest concentration tested. The 28-day mean measured NOEC was 119.1 mg asulam/L. Based on molar weight this would be approximately equivalent to 130.5 mg asulam sodium/L).

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

The acute toxicity of asulam sodium (purity 88%) to the water flea (*Daphnia magna*) was investigated over 48 hours under flow-through conditions [56]. The study was conducted to US EPA guideline 72-2, 1987 and to GLP.

Twenty daphnids (2 replicates of 10 per concentration) less than 24-hours old, were exposed to nominal concentrations of 0 (water control), 14, 24, 40, 66 and 110 mg asulam/L for a period of 48 hours. 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 and a hardness of 235 mg/L as CaCO3. Observations of dead animals were made at 24 and 48 hour of exposure. The daily volume turnover was approximately 6.4. One water sample was collected from each control and test solution at test initiation and termination to monitor actual exposure concentrations of asulam.

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 acute 48-hour mean measured EC_{50} for *Daphnia magna* exposed to asulam sodium was determined to be 63.4 mg/L (95% CL: 51.0-89.8 mg/L), equivalent to 57.87 mg pure asulam/L. The 48-hour mean measured NOEC was 25.5 mg/L (equivalent to 23.28 mg asulam/L) based on immobilisation occurring at 48.5 and 83 mg/L.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Study i):
A 21-day study on the reproductive toxicity of technical asulam (purity 80.6%) to *Daphnia magna* was reported [57]. The study was conducted under semi-static conditions in accordance with OECD 211, 1997 and EPA 72-4, 1987.

There were ten, individually held, neonates (<24 hours old at the start of the study) per concentration. The nominal test concentrations were 2.6, 6.4, 16.0, 40.0 and 100 mg asulam/L. A dilution water control was also included. Test solutions were renewed three times a week and test organisms were observed and fed three times a week. Biological observations 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. Test conditions and concentrations of asulam were measured in fresh and old solutions at each media renewal. Quantification was performed by HPLC.

Measured test concentrations were found to be 86-117% of nominal during the test period and mean values were 2.6, 6.4, 16.1, 39.9 and 99.0 mg asulam/L. Temperature was 20.0-21.2°C, dissolved oxygen was ≥7.6 mg/L, total hardness ranged from 163-178 mg/L as $CaCO_3$ and pH was 7.3-8.4.

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 measured 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.

Based on mean measured concentrations of pure asulam, the 21-day EC_{50} (adult survival) in *Daphnia magna* was estimated to be 57.1 mg asulam/L. Based on the statistical analysis of the test data, the 21-day mean measured NOEC was found to be 6.4 mg pure asulam/L. Based on molar weight this would be approximately equivalent to 7.01 mg asulam sodium/L).

Study ii):
A second 20-day study on the reproductive toxicity of technical asulam (purity 80%) to *Daphnia magna* was reported [58]. The study was conducted under semi-static conditions in accordance with OECD 202, 1984.

There were 4 groups of 10 neonates (<24 hours old at the start of the study) per concentration. A dilution water control (containing 4 groups of 10 neonates) was also included. The nominal test concentrations were 3.58, 8.96, 22.4, 56, 140 and 350 mg asulam/L. Test solutions were renewed three times a week and test organisms were fed three times a week. 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 chemical analysis was conducted for asulam and few other methodological details are provided.

Immobility in controls was <10%. No treatment-related effects were observed at 3.58 and 8.96 mg asulam/L. Survival then decreased to 0% at ≥140 mg/L, there was not a clear concentration-related effect on reproduction at the lower concentrations but this also decreased to 0% at ≥56 mg/L. Based on nominal concentrations (expressed as pure active substance), the 20-day EC_{50} for reproduction rate was stated to be 21.48 mg asulam/L and the 20-day EC_{50} for immobilisation was stated to be 45.6 mg asulam/L. The 20-day nominal NOEC was 8.96 mg pure asulam/L. Based on molar weight this would be approximately equivalent to 9.82 mg asulam sodium/L).

The EC_{50} for reproduction should be viewed with caution (considered Klimisch 3) due to limited methodological reporting, a lack of clear concentration-related reproductive response and all values from this study are of uncertain reliability due to the lack of analysis of test concentrations. Its endpoint will not be used in preference to the study [57] above.

5.4.2.3 Algae and aquatic plants

Studies on algae

Study i(a):
A study is available on the toxicity of asulam sodium purity (89.5%) to the freshwater green alga *Pseudokirchneriella subcapitata* (tested as S*elenastrum capricornutum*) [60]. The study was conducted under static conditions to US EPA/FIFRA guidelines122-2 and 123-2, 1982.

The 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 24-25°C) and continuous shaking (100 rpm) and illumination. The initial cell density in each test level was 0.3×10^4 cells/mL. 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 HPLC.

Temperature ranged from 24-25°C and pH ranged from 7.3-7.5 at test initiation, increasing to 9.7-10.8 at test termination. 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.

Based on mean measured concentrations, the 120-hour EC_{50} (cell density) for *Pseudokirchneriella subcapitata* exposed to asulam sodium was calculated to be 0.19 mg/L, equivalent to 0.17 mg pure asulam/L, with 95% confidence limits of 0.071-0.49 mg/L. The 120-hour mean measured NOEC (cell density) was determined to be 0.020 mg/L (0.018 mg pure asulam/L) based on significant ($p \leq 0.05$) inhibition of cell growth seen at concentrations of 0.049 mg/L and above.

i(b): A non-GLP 'recalculation report' on the influence of asulam sodium on growth of the green alga, *Pseudokirchneriella subcapitata* was subsequently submitted by the Applicant during pesticide registration [61]. This reconsidered the effects seen in the above study [60] and recalculated the cell density endpoint in terms of growth rate at 72 hours, according to OECD 201 requirements.

The recalculated mean measured 72-hour E_rC_{50} for *Pseudokirchneriella subcapitata* was 1.90 mg asulam sodium/L (equivalent to 1.73 mg pure asulam/L). The recalculated mean measured 72-hour NOE_rC was determined to be 0.02 mg asulam sodium/L (equivalent to 0.018 mg pure asulam/L).

Study ii(a):
A study is available on the toxicity of asulam sodium purity (89.5%) to the freshwater alga, *Anabaena flos-aquae* [62]. The study was conducted under static conditions to US EPA/FIFRA guidelines122-2 and 123-2, 1982.

The 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 1.0×10^4 cells/mL. 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 HPLC.

Temperature ranged from 24-25°C and pH ranged from 7.4-7.5 at test initiation, increasing to 9.9-10.2 at test termination. 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.

Based on mean measured concentrations, the 120-hour EC_{50} (cell density) for *Anabaena flos-aquae* exposed to asulam sodium was calculated to be >0.72 mg/L, equivalent to 0.66 mg pure asulam/L, with 95% confidence limits of 0.43-1.2 mg/L. The 120-hour mean measured NOEC (cell density) was determined to be 0.19 mg/L (0.17 mg pure asulam/L), based on significant ($p \leq 0.05$) inhibition of cell growth seen at concentrations of 0.37 and 0.72 mg/L.

ii(b): A non-GLP 'recalculation report' on the influence of asulam sodium on growth of the alga, *Anabaena flos-aquae* was subsequently submitted by the Applicant during pesticide registration [63]. This reconsidered the effects seen in the above study [62] and recalculated the cell density endpoint in terms of growth rate at 72 hours, according to OECD 201 requirements.

The recalculated mean measured 72-hour E_rC_{50} for *Anabaena flos-aquae* remained at >0.72 mg asulam sodium/L (equivalent to >0.66 mg pure asulam/L). The recalculated mean measured 72-hour NOE_rC was determined to be 0.19 mg asulam sodium/L (equivalent to 0.17 mg pure asulam/L).

Study iii(a):
A study is available on the toxicity of asulam sodium purity (89.5%) to the marine diatom, *Skeletonema costatum* [64]. The study was conducted under static conditions to US EPA/FIFRA guidelines122-2 and 123-2, 1982.

The 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. 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 HPLC.

Temperature ranged from 20-21°C and pH ranged from 8.1 at test initiation, increasing to 8.8-9.0 at test termination. 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.

Based on mean measured concentrations, the 120-hour EC_{50} (cell density) for *Skeletonema costatum* exposed to asulam sodium was calculated to be 0.43 mg/L, equivalent to 0.39 mg pure asulam/L, with 95% confidence limits of 0.15-1.2 mg/L. The 120-hour mean measured NOEC (cell density) was determined to be 0.022 mg/L (0.020 mg pure asulam/L), based on the significant ($p \leq 0.05$) inhibition of cell growth seen at concentrations of 0.058 mg/L and above.

iii(b): A non-GLP 'recalculation report' on the influence of asulam sodium on growth of the diatom, *Skeletonema costatum* was subsequently submitted by the Applicant during pesticide registration [65]. This reconsidered the effects seen in the above study [64] and recalculated the cell density endpoint in terms of growth rate at 72 hours, according to OECD 201 requirements.

The recalculated mean measured 72-hour E_rC_{50} for *Skeletonema costatum* was >1.8 mg asulam sodium/L (equivalent to >1.64 mg pure asulam/L). The recalculated mean measured 72-hour NOE_rC was considered to be 0.33 mg asulam sodium/L (equivalent to 0.3 mg pure asulam/L).

Study iv):
A study is available on the toxicity of asulam sodium purity (89.5%) to the freshwater diatom, *Navicula pelliculosa* [66]. The study was conducted under static conditions to US EPA/FIFRA guidelines122-2 and 123-2, 1982.

The 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 x 10^4 cells/mL. 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 HPLC.

Temperature ranged from 24-25°C and pH ranged from 7.4-7.6 at test initiation, increasing to 8.0-9.2 at test termination. 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. Concerns are raised in the

DAR over the slow increase in cell numbers over the first 24 hours, however this is considered not uncommon for *Navicula* and not to invalidate the test.

Based on mean measured concentrations, the 120-hour EC_{50} (cell density) for *Navicula pelliculosa* exposed to asulam sodium was calculated to be 2.3 mg/L, equivalent to 2.1 mg pure asulam/L, with 95% confidence limits of 1.2-4.7 mg/L. The 120-hour mean measured NOEC (cell density) was determined to be 0.15 mg/L (0.14 mg pure asulam/L), based on significant ($p \leq 0.05$) inhibition of cell growth and effects on algal cells (cultures containing cell fragments and bloated cells) seen at mean measured concentrations of 0.30 mg/L and above.

In the original asulam DAR, the EC_{50} was re-calculated at 72-hours to be 3.4 mg asulam sodium/L, equivalent to 3.1 mg pure asulam/L), it appears this was based on cell number rather than specific growth rate. The NOEC was not recalculated at 72-hours or based on growth rate - but given that it was based on visible cell damage, it was proposed to retain the original 120-h mean measured NOEC value of 0.15 mg asulam sodium/L (0.14 mg pure asulam/L). In the updated DAR (2016) the lower 120-hour E_bC_{50} of 2.3 mg asulam sodium/L (equivalent to 2.1 mg asulam/L) is now used for risk assessment. For hazard classification, the Applicant has since provided recalculated 72-hour E_rC_{50} and NOE_rC values for *N. pelliculosa* based on mean measured concentrations as follows:

- 72-hour E_rC_{50}: >4.4 mg asulam sodium/L (>4.02 mg pure asulam/L)
- 72-hour NOE_rC: 0.54 mg asulam sodium/L (0.49 mg pure asulam/L)

Studies on higher aquatic plants/macrophytes

Study i):
A study is available on the toxicity of asulam sodium purity (89.5%) to duckweed (*Lemna gibba*) [67]. The study was conducted under static conditions to US EPA/FIFRA guidelines122-2 and 123-2, 1982.

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 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). There was good growth throughout the 14-days in controls (meeting validity citeria) indicating no problems with nutrient depletion. 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. At test initiation and termination, a sample of each treatment level was removed for analysis of asulam concentration via HPLC.

During the test, temperature ranged from 24-25°C and pH 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 decreasing to 15% of nominal at test termination. Therefore concentrations used for EC_{50} calculations were originally based on initial measured concentrations - which were found to be 0.035, 0.065, 0.12, 0.26 and 0.44 mg/L. These were subsequently recalculated as mean measured concentrations. The two lowest concentrations could not be verified as they were below the LOD for the study of 0.018 mg/L. Therefore, for the concentration 0.063 mg/L the value 0.018 mg/L was used. For the lowest concentration 0.031 mg/L the LOD/2 was used = 0.009 mg/L. The following

concentration range was derived for the assessment of mean measured endpoints: 0.017, 0.034, 0.051, 0.099 and 0.169 mg/L. The key biological information (frond no. and biomass only) is summarised in Table 34.

Table 34: 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.

Based on initial measured concentrations, the 14-day EC_{50} for *Lemna gibba* with asulam sodium based on frond density was calculated to be 0.30 mg/L (95% confidence limits: 0.020-0.61), equivalent to 0.27 mg pure asulam/L. The initial measured 14-day EC_{50} based on biomass was calculated to be 0.32 mg/L (95% confidence limits: 0.12-0.54), equivalent to 0.29 mg pure asulam/L. The 14-day initial measured NOEC based on both frond density and biomass was determined to be 0.12 mg/L.

Chlorosis was seen in all fronds at an initial measured 0.26 mg/L but the study also reported that all fronds in one replicate at 0.12 mg/L were observed to be slightly chlorotic, with less root formation in comparison to control fronds. A conservative NOEC based on chlorosis would be an initial measured 0.065 mg/L (within the range 0.01 to 0.1 mg/L). However it is standard for hazard classification to use a growth rate endpoint for algae and aquatic plants. Growth rate E_rC_{50} and NOE_rC values have since been recalculated and provided based on mean measured concentrations over the different observation periods in the study (which did not include 7-days). These are as follows:

- 6-day E_rC_{50}: 0.205 mg asulam sodium /L (≡ 0.187 mg pure asulam/L)
- 9-day: E_rC_{50}: 0.186 mg asulam sodium (≡ 0.17 mg pure asulam/L)
- 14 day: E_rC_{50}: 0.160 mg asulam sodium/L (≡ 0.146 mg pure asulam/L)
- 6, 9 and 14 day NOE_rC: 0.051 mg asulam sodium/L (≡ 0.047 mg pure asulam/L)

For hazard classification purposes, it is proposed to use the most sensitive growth rate values derived after 14 days, i.e. a mean measured 14-day E_rC_{50} of 0.16 mg asulam sodium/L and 14-day NOE_rC of 0.051 mg asulam sodium/L. Whichever E_rC_{50} is chosen (6 to 14-days) these lie within the acute classification range 0.1-1.0 mg/L and the 6 to 14-day NOE_rC or chlorosis NOEC are all within the chronic range 0.01 to 0.1 mg/L.

Study ii):
A study is available on the toxicity of a 400g/L asulam sodium SL formulation to duckweed (*Lemna gibba*) [78]. The study was conducted under static conditions according to OECD 221 (with no significant deviations)and to GLP.

Plants were exposed to a control and nominal concentrations of 0.032, 0.1, 0.32, 1.0, 3.2 and 10.0 mg formulation/L under continuous illumination for a period of 7 days. Dilution water + growth medium was used as the control. The number of fronds present in each replicate was counted and observations were recorded over 7 days. The validity criteria for this guideline were 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 use in hazard assessment. Measured concentrations of asulam over the 7 days were 88-98% of nominals, with a mean 94%. Given that analysed concentrations over the study duration were within 90% of the nominal test test concentrations, the endpoints were based on nominal test concentrations.

The specific growth rate E_rC_{50} of 2.56 mg 'Asulam 400g/L SC'/L (based on changed in frond number) for *L. gibba* is considered to be the most relevant classification endpoint - which is equivalent (based on the reported analytical concentration of 389.9 g asulam /L and density of 1.181 kg /L) to 0.845 mg asulam /L, or 0.926 mg asulam sodium/L. The reported NOE_rC of 0.1 mg formulation/L is equivalent (assuming toxicity relates to asulam content) to 0.033 mg asulam/L or 0.0362 mg asulam sodium/L. This formulation therefore seems to be relatively less toxic to *L. gibba* than the above study on the technical material indicates (even accounting for the different durations).

Study iii)
A published study on dose-response relationships between herbicides with different modes of action on growth of *Lemna paucicostata* [68] has been identified as part of a literature search and is evaluated in the updated pesticide DAR (2016) for asulam sodium. The study was conducted under static conditions according to ISO/WD 20079 (2001 draft) but not to GLP.

In this study 26 herbicides (including asulam) with up to 19 different modes of action were tested on leaf area growth of the duckweed *L. paucicostata*. This species was used because it is smaller than *L. minor* and *L. gibba*, thus facilitating the smaller test systems used. However, it was considered that the principles of the study guideline were transferable to other *Lemna* species. The herbicides used were all described as analytical grade (purity unstated) and the solvent used for asulam was acetone (1% by volume). After range-finding, an unstated range of concentrations was tested to derive a growth EC_{50} for asulam. There were three replicates at each concentration within this range and the whole experiment was replicated at least twice.
Tests were conducted in an illuminated incubator and total frond area was recorded by an image analysis system once per day from day 0 to day 7. The total frond area for each day, at days 0-7, were used to calculate the growth rate. E_rC_{50} values were then determined using a logistic regression from dose-response curves based on the average growth rate from days 0 to 7. A NOEC was not reported.

In all tests, untreated control *L. paucicostata* grew exponentially and no differences were seen between negative and solvent controls throughout the experiment. The E_rC_{50} value for asulam was the lowest toxicity value obtained for all the herbicides tested at 407 µM, equivalent to 93.8 mg/L.

(approximately equivalent to 102.8 mg asulam sodium/L). The key regression information is summarised in Table 35.

Table 35: Median effective concentration (EC_{50}) of asulam tested on *L. paucicostata* including regression parameters

Herbicide	Upper level of curve	Lower level of curve	Slope	EC_{50} (µM), 95% CI
Asulam	0.278 ± 0.007	0.001 ± 0.020	2.07 ± 0.444	407.3 ± 50.73 (≡ 93.8 mg/L)

Although not GLP compliant and not conducted to the newer OECD 221 *Lemna* test guideline, the study appears to have been performed to an acceptable standard - apart from the lack of chemical analysis to confirm test substance concentrations. 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 report and hence it is not possible to verify that the study passes the ISO or OECD 221 'validity' criterion of a doubling time of 2.5 days. Overall however, the study was considered by the pesticide RMS and CLH dossier submitter to be 'Reliable with restrictions'.

Study iv):
An unpublished study report was submitted on the toxicity of asulam to aquatic macrophytes [69]. The study was conducted to an in-house methodology and not to GLP. The toxicity tests were performed with five submerged, rooted aquatic macrophytes without a sediment compartment. Species tested included *Myriophyllum spicatum, Elodea nuttallii, Elodea canadensis, Ranunculus circinatus* and *Potamogeton crispus*. The test item, asulam, was applied as a 400 g/L solution in water formulation. The test was static and conducted over 21-days during the spring growing season of the macrophytes.

All tests were performed in a controlled climate room with a constant water temperature 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 with a length of 10 cm.

The tests were conducted in three phases and at different asulam concentrations each time. In 2005, macrophytes were exposed in duplicate to asulam at: 0, 1.4, 5.6, 14, 42, 140, 420 and 1260 µg/L. In 2009 and 2012, macrophytes were exposed in duplicate (2009) or in triplicate (2012) to asulam at: 0, 0.1, 1, 10, 100 and 1000 µg/L. 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 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). Also measured were total wet weight (2005 experiment), total dry weight, length of the main shoot and total length of new shoots. EC_{50} values were based on nominal and time-weighted-average concentrations of the test item. Samples from the experimental systems taken 1 hour post-treatment showed that on average 86 to 101% of the nominal concentrations of asulam were present in the test systems and that concentrations remained stable during the exposure period of 21 days. The pesticide RMS considered that the adequate levels of recovery of asulam (>80% of nominal values) throughout the study supported the reporting of endpoints based on nominal asulam test concentrations (not asulam sodium).

Of all macrophyte species tested, *Myriophyllum spicatum* was the most sensitive species. Its most sensitive endpoint was total dry weight, of which the EC_{50} values were 9.6 and 10.7 µg/L based on time weighted average (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. Based on molar weight the lower nominal EC_{50} of 10.7 µg pure asulam/L would be 11.7 µg asulam sodium/L (≡ 0.0117 mg asulam sodium/L). In the 2012 experiments *Elodea canadensis* was the next most sensitive macrophyte species. Dry weight of new shoots and length of new shoots were its most sensitive endpoints. A full table of results is provided in the asulam DAR (2016).

It is noted that although recently conducted (2013) the study is not performed to any standard guideline, nor was it GLP compliant. Also, 95% confidence intervals for the most sensitive nominal dry weight E_bC_{50} of 10.7 µg/L are large i.e. 2-74 µg/L - indicating uncertainty in the accuracy of this endpoint. Growth rate endpoints and NOECs, normally both used for hazard classification, were also not reported. Given these deficiencies, the results are not relied on and those from Study (iv) below on *Myriophyllum* [70] are considered more reliable for regulatory use by the pesticide RMS and CLH dossier submitter.

Study v):
A study on the toxicity of asulam (tested as a 400 g/L SL formulation) to *Myriophyllum spicatum* was conducted using a draft OECD guideline for testing rooted aquatic macrophyte in a water-sediment system (July, 2013) along with earlier ring test protocols for the same study (2009, 2011). It was also conducted to GLP.

Shoots of *Myriophyllum spicatum* were exposed in a static test to 5 treatment groups (2.56, 0.64, 0.16, 0.04 and 0.01 mg asulam/L) 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 in small 500 ml 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 12 cm. 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 exposed for 14 days under static conditions. The asulam formulation stock solution was dosed directly in to the water phase and mixed gently so as to avoid disturbing the sediment. 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. Water samples collected at start and after 14 days were analysed for asulam by an LC-MS/MS method. The inhibition of yield and growth rate based on total shoot length, wet and dry weight in relation to control cultures were determined over a test period of 14 days.

Water temperature was maintained at 18 - 21 °C; the light regime was: 16 h light : 8 h dark; mean light intensity was: 9207 lux (8320 - 9560 lux); pH values ranged from a minimum 7.8 at test start to 9.8 at the end of the test; oxygen concentrations were variable at 5.8 - 12.6 mg/L. 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 in the water (average of all test concentrations), there was therefore no appreciable degradation or dissipation to sediment. Throughout the test, the plants were exposed to a mean 92% of nominal concentrations; therefore, all reported results refer to nominal concentrations. As the SL formulation is a simple solution in water, the endpoints were directly expressed as concentrations of asulam (asulam sodium was calculated from this based on molar weight). All other validity criteria from the draft guideline were met.

Table 36: Summary of biological results

Parameter	Yield (shoot length) [mg a.s./L]	Specific growth rate (shoot length) [mg a.s./L]	Yield (wet weight) [mg a.s./L]	Specific growth rate (wet weight) [mg a.s./L]	Yield (dry weight) [mg a.s./L]	Specifc growth rate (dry weight) [mg a.s./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_{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

n.d.: could not be determined
Values refer to nominal test concentrations

After static exposure of *Myriophyllum spicatum* to 'Asulam 400 g/L SL' for 14 days, the following endpoints, based on nominal asulam concentrations, were given in the study report:
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 asulam/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 E_rC_{50} of greater than the highest test dose of 2.56 mg a.s./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.

The Applicant has since provided recalculated and more accurate figures (not >) for the growth rate endpoints, these are:

Nominal 14-day growth rate E_rC_{50} = 47.1 mg asulam/L for shoot length and 5.88 mg asulam/L for wet weight respectively. For the most critical parameter (wet weight) the endpoint expressed as asulam sodium is 6.44 mg asulam sodium/L.

Nominal 14-day growth rate NOE_rC: 0.01 mg asulam/L for shoot length and wet weight and 0.04 mg asulam/L for dry weight. For the most critical parameters (shoot length and wet weight) the endpoint expressed as asulam sodium is 0.011 mg asulam sodium/L.

The lowest nominal endpoints for classification purposes were, therefore, the 14-day E_rC_{50} of 6.44 mg asulam sodium/L and the 14-day NOE_rC of 0.011 mg asulam sodium/L).

5.4.3 Other aquatic organisms (including sediment)

A chronic toxicity study on the sediment-dwelling invertebrate *Chironomus riparius* was reported using asulam sodium (purity 82.2%) [59]. The study was conducted in accordance with the OECD draft guideline 219 (using spiked water), 2000 and BBA guideline proposal, 1995.

The test employed nominal concentrations of 25, 50 and 100 mg asulam sodium/L and a control group. Each treatment group and control was tested with 6 replicates containing 25 larvae. The test system employed 2 L glass beakers with a 2-3 cm sediment layer (prepared according to OECD 207) and a supernatant water column of 1.6 L. 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 chironomids were exposed during their subsequent larval development for 28 days or until emergence as adults. There was no chemical analysis for measured asulam concentrations, therefore endpoints are based on nominals. The distribution of asulam between the water and sediment phases is also unknown.

Test parameter included imaginal emergence rate (ER) and larval development rate (DR) of the test organisms. 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).

Following 28 days of exposure to the test substance there was no significant difference between the emergence rate of adult midges or development rate of midges at any of the test concentrations and the control group. The nominal 28-day EC_{50} was therefore estimated to be >100 mg asulam sodium/L and the nominal 28-day NOEC is 100 mg asulam sodium/L, the highest concentration tested. The lack of chemical analysis calls in to question whether this initial nominal concentration was retained in water over the duration of the study. However other water-sediment and distribution fate studies and the rooted macrophyte studies suggest that asulam is likely to remain in the water phase. Overall, the chironomid endpoint (>100 mg/L) is not considered accurate or at the sensitive end of the spectrum, so will not be relied on for aquatic hazard classification.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Abiotic and biotic degradation

Asulam sodium (and asulam) is stable to hydrolysis over 31 days at all pH tested (pH 5, 7 and 9). The substance is also not readily biodegradable as only 21-52% biodegradation occurred over 28/9 days.

Aqueous photolysis studies indicate that under certain environmental condition in shallow surface waters, photolysis of asulam sodium can occur. However, in typical turbid European natural surface waters, particularly at higher latitudes, photolysis is not expected to be a major route of rapid degradation.

In whole aerobic natural water/sediment systems, asulam sodium was relatively persistent (DT_{50} 61.9-79 days - mean 70.3%). No major metabolites were formed. Mineralisation to carbon dioxide accounted for <2-14% AR, whilst sediment bound residues represented 56-58% AR at the end of one study.

Overall, the available abiotic and biotic degradation information does not indicate that asulam sodium (or asulam) is ultimately degraded (>70%) within 28 days (equivalent to a half-life <16 days) or transformed to entirely non-classifiable degradants. Consequently asulam sodium is considered 'not rapidly degradable' for the purposes of classification under the CLP Regulation.

Environmental distribution and bioaccumulation

The chemical properties and available fate data on asulam sodium indicate that it will predominantly remain in the water phase and is unlikely to dissipate rapidly in significant amounts to sediment or soil compartments, although partitioning will occur over time.

Asulam has a log K_{ow} at pH 7 of 0.15 which is below the CLP trigger of 4 indicating a low potential for bioaccumulation. A bioconcentration study on catfish is also available; although non-standard, not well quantified in terms of its exposure and not to GLP, this study provides some further indication of a low bioaccumulation potential with measured whole fish BCF values of 0.1 to 1.4 (less than the CLP BCF trigger of 500).

Aquatic toxicity

Acute aquatic hazard:
Acute aquatic toxicity data are available on asulam/asulam sodium for fish, invertebrates, algae and aquatic plants. Fish and *Daphnia* showed low sensitivity to this herbicide and, as expected, algae and aquatic plants are the most acutely sensitive groups - see Summary Table 32. Algae and plant data are also available on a main (but minor in terms of %AR) degradant of asulam, i.e. sulfonilamide, which indicate that it is less toxic than the parent substance. Therefore degradants are not considered further in relation to the classification of asulam sodium. The non-standard, non-GLP study on five aquatic macrophytes [69] is not considered sufficiently relevant or reliable to use its low derived endpoint on *Myriophyllum* (which is also not based on growth rate). A follow-up GLP study on *Myriophyllum spicatum* to a draft OECD guideline, has indicated that this species is not more sensitive than *Lemna*. The lowest reliable acute/short-term endpoint for classification purposes is therefore, the 14-day mean measured E_rC_{50} for *Lemna gibba* of 0.16 mg asulam sodium/L. This is in the range >0.1 to ≤1.0 mg/L and therefore asulam sodium should be classified as: **Aquatic Acute 1: H400 with an Acute M-factor of 1.**

Chronic aquatic hazard:
In terms of chronic toxicity, a prolonged 28-day juvenile fish growth test is available on rainbow trout, this is considered sufficient instead of a true chronic study given the very low acute sensitivity of fish and log K_{ow} <5. The 28-day mean measured NOEC was 119.1 mg asulam/L (≡ 130.5 mg asulam sodium/L). Data are available from two chronic *Daphnia* studies, the most reliable being the 21-day study [57], which also gave the lowest invertebrate NOEC of 6.4 mg asulam/L (≡ 7.01 mg asulam sodium/L). A water-spiking chronic study on the midge *Chironomus riparius* is also available, although of uncertain relevance from a classification perspective, this gave a 28-day NOEC of 91.3 mg asulam sodium/L. These NOECs all indicate that fish and invertebrates have a low chronic sensitivity to asulam and asulam sodium.

The most chronically sensitive organisms are again algae and aquatic plants - see Summary Table 32. The lowest value is a 14-d nominal NOE_rC of 0.01 pure asulam/L (≡ 0.011 mg asulam sodium/L) for the aquatic macrophyte *Myriophyllum spicatum* from the study by Seeland *et al*, 2014) [70]. For asulam sodium, this is just within the range >0.01 to ≤0.1 mg/L (as were NOECs from the *Lemna* study) and therefore, since the substance is also considered 'not rapidly

degradable', it should be classified as: **Aquatic Chronic category 1: H410 with a Chronic M-factor of 1.**

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Aquatic Acute category 1; H400: Very toxic to aquatic life
Acute M-factor = 1
Aquatic Chronic category 1; H410: Very toxic to aquatic life with long lasting effects
Chronic M-factor = 1

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

In the CLH report, the DS clarified that the majority of radiolabelled studies used 14C-asulam-sodium labelled in the aromatic ring. Radiochemical purity and specific activity is reported in each radiolabelled study. Still, some studies have been conducted with technical asulam. However in solution, asulam-sodium will dissociate and the ionised and unionised forms will be in equilibrium, depending on the pH of the environment. Solubility will also be pH-dependent; at environmentally relevant pHs, the substance will exist primarily in the ionised form and be readily soluble. The amounts of asulam used in the tests themselves were not sufficient to affect the pH and therefore would not affect the equilibrium, nor was the aqueous solubility of asulam exceeded in any of the toxicity tests. Therefore, asulam and asulam-sodium can be considered equivalent and the form of the compound applied will not influence the results of the tests.

The DS concluded that asulam-sodium is stable to hydrolysis, and that photolysis is not expected to be a major route of degradation. From the available abiotic and biotic degradation information, asulam-sodium is considered not rapidly degradable for the purposes of classification. The log K_{ow} 0.15 (at pH 7) is below the CLP trigger of 4, indicating low potential for bioaccumulation, as are the measured whole fish BCF (0.1 – 1.4) values which are much lower than the trigger value of 500. Acute and chronic toxicity data are available on asulam-sodium for fish, invertebrates, algae and aquatic plants. The lowest reliable acute/short-term endpoint for classification purposes is the E_rC_{50} for *Lemna gibba* of 0.16 mg/L. This is in the range > 0.1 to ≤ 1.0 and therefore asulam-sodium should be classified as Aquatic Acute 1 (H400) with an M-factor of 1. The lowest reliable chronic/long-term endpoint for classification purposes is the NOE_rC for *Myriophyllum spicatum* of 0.011 mg/L. This is in the range > 0.01 to ≤ 0.1 and therefore asulam-sodium should be classified as Aquatic Chronic 1 (H400) with an M-factor of 1.

Degradation

The DS considered asulam to be hydrolytically stable at all environmentally relevant pHs (5, 7, 9) over 31 days. The test was conducted at 24 to 26 °C and results showed less than 10%

hydrolysis over the study duration in all samples (Gohdes, 1989a). Degradation was insufficient to calculate a DT_{50}.

Based on biochemical oxygen demand in an OECD TG 301 B study (Mead, 1999a), and on theoretical oxygen demand in an OECD TG 301 F study (Feil, 2008), only 52% and 21% biodegradation occurred respectively over 28 days, the DS considered asulam as not readily biodegradable.

In two aqueous photolysis studies (Mills and Simmonds, 2003a; Lowden, 2004 a&b) in sterile buffer solutions at pH 4 and 9 at 25 °C , the DT_{50} were 0.44 days at pH 4 and 0.87 days at pH 9 (respectively 0.781 and 1.56 days under natural summer sunlight at 52°N). Estimated photolytic half-life of asulam in natural surface waters was calculated from the quantum yield and ranged from 7 to 119 hours at pH 4 and from 8 to 135 hours at pH 9 in central European latitudes (52°N). Three major (i.e. > 10% AR) photo-degradation products were formed and identified as sulfanilic acid, AP formamide and MCAPAP carbamate. In another aqueous photolysis study in sterile natural water (Mills and Caine, 2004a) at pH 7.8 at 25 °C, the DT_{50} was 0.84 days (4.21 days under natural spring sunlight at 35°N). Many minor photodegradates were formed, all < 10% AR and none of the major metabolites identified in the sterile buffered photolysis study were formed in significant amounts. In other sterile aqueous photolysis studies (Mills, 2007a; Lowden, 2007 a&b) at pH 4 and 9 at 25 °C, the DT_{50} was 0.284 days at pH 4 and 0.863 days at pH 9 (respectively 0.537 and 1.64 days under natural summer sunlight at 52° N). No significant degradation of asulam observed in a non-irradiated system. However, although asulam-sodium will be rapidly degraded by light in the top few millimetres of an aquatic system, the degradation will be slower in natural water bodies, throughout which it will readily dissolve. In water bodies of modest depth (30 cm, 100 cm) the half-lives will range from about half a day in summer to just over a week in autumn. Therefore, the DS concluded that photolysis is not considered to be a significant route of degradation for asulam-sodium.

In laboratory incubations in aerobic water/sediment degradation simulation study (Purser, 1998a; Hardy and Patel, 2008c) systems (at 20 ± 2 °C in the dark for 153 days), asulam was relatively persistent (DT_{50}: 65.6-78.8 days). Partitioning of asulam to the sediment was relatively slow and moderate. No major metabolites were formed. Mineralisation to carbon dioxide accounted for 3-13.9% AR, whilst sediment bound residues represented 56-58% AR at the end of the study. In other studies (Willems, 1997a; Hardy, 2011a), whole system DT_{50} ranges were similar at 61.9 to 76.2 days. Considering all of the water-sediment systems from both simulation studies, along with their respective kinetic re-analyses, an overall geometric mean whole system DT_{50} for asulam of 70.3 days has been calculated. The DS considered that this is also not sufficient to meet CLP criteria for rapid degradation.

Overall, although rapid photolytic degradation may occur under certain aquatic conditions, the available abiotic and biotic degradation information does not indicate that asulam is ultimately degraded (> 70%) within 28 days (equivalent to a half-life < 16 days) or transformed to entirely non-classifiable degradants. Consequently, the DS considered asulam-sodium as not rapidly degradable for the purposes of classification under the CLP Regulation.

Aquatic Bioaccumulation

The log K_{ow} of asulam at 25 °C, pH 7 was 0.15 (pH 4 = 0.11; pH 9 = 0.77) (Francon, 1999c). This value is below the CLP trigger of ≥ 4 and indicating low potential for bioaccumulation. In a non-standard and non GLP compliant study on catfish (*Ameirus melas*), a whole fish BCF of 0.1 – 1.4 (Report R000747, 1981a) was measured, which is much lower than the trigger value of 500. Therefore, the DS proposed not to consider asulam-sodium as bioaccumulative.

Aquatic Toxicity

The ecotoxicological tests results from available acute and chronic studies for all trophic levels of asulam or asulam-sodium are summarised in the following table and sections. Study endpoints based on asulam-sodium have been converted into pure asulam equivalents (and *vice versa*) using a conversion factor of 0.9128, based on the molecular weight of asulam (230.2) and asulam-sodium (252.2). The table contains already recalculated acute and chronic endpoints for asulam-sodium. Study of uncertain reliability and not relied on, due either to lack of analysis throughout study, high variability in endpoint, lack of GLP and/or reporting detail was not provided in the table.

Test organism / guideline	**Test substance and purity /actual conc. n (% of nominal)**	**Short-term result (endpoint)**	**Long-term result (endpoint)**	**References**
Fish				
Rainbow trout (*Oncorhynchus mykiss*) / US EPA 72-1, GLP	Asulam-sodium, 88% pure / 77-88%	96h LC_{50} > 175 mg/L mean measured	96h NOEC = 175 mg/L mean measured	Report R001267, 1988a
Bluegill sunfish (*Lepomis macrochirus*) / OECD TG 203, GLP	Asulam-sodium, 81.4% pure / 100–110%	96h LC_{50} > 100 mg/L mean measured	96h NOEC = 100 mg/L mean measured	Report R006767, 2000
Rainbow trout (*Oncorhynchus mykiss*) / OECD TG 215	Asulam, 80.6% pure / 98-121%	28-d EC_{50} > 130.5 mg/L mean measured	28-d NOEC = 130.5 mg/L mean measured	Report R005641, 1997a
Invertebrates				
Water flea (*Daphnia magna*) / US EPA 72-2, GLP	Asulam-sodium, 88% pure / 64-75%	48h EC_{50} = 63.4 mg/L mean measured	48h NOEC = 25.5 mg/L mean measured	Manning, 1988b
Water flea (*Daphnia magna*) / US EPA 72-4, OECD TG 211	Asulam, 80.6% pure / 86-117%	21d EC_{50} = 62.6 mg/L mean measured	21-d NOEC = 7.01 mg/L mean measured	McElligott, 1997b
Algae				
Freshwater green alga (*Pseudokirchneriella subcapitata*) / US EPA/FIFRA 122-2 and 123-2, OECD TG 201	Asulam-sodium, 89.5% pure / 89-98%	72h E_rC_{50} = 1.90 mg/L mean measured	72h NOE_rC = **0.02** mg/L mean measured	Hoberg, 1992a; Reassessment to OECD TG 201 by Dorgerloh, 2004a
Freshwater alga (*Anabaena flos-aquae*) / US EPA 122-2/123-2; OECD TG 201	Asulam-sodium, 89.5% pure /89-93%	72h E_rC_{50} > 0.72 mg/L mean measured	72h NOE_rC = 0.19 mg/L mean measured	Hoberg, 1992b; Reassessment to OECD TG 201 by Dorgerloh, 2004b
Marine diatom (*Skeletonema costatum*) / US EPA 122-2/123-2, OECD TG 201	Asulam-sodium, 89.5% pure /88-113%	72h E_rC_{50} > 1.8 mg/L mean measured	72h NOE_rC = 0.33 mg/L mean measured	Hoberg, 1992d; Reassessment to OECD TG 201 by Dorgerloh, 2004c
Freshwater diatom (*Navicula pelliculosa*) / US EPA 122-2/123-2	Asulam-sodium, 89.5% pure / 82-98%	72h E_rC_{50} > 4.4 mg/L mean measured	72h NOE_rC = 0.54 mg/L mean measured	Hoberg, 1992c
Aquatic plants				
Duckweed (*Lemna gibba*) / US EPA/FIFRA 122-2/123-2	Asulam-sodium, 89.5% pure / 88-112%	6d E_rC_{50} = **0.205** mg/L 9d E_rC_{50} = **0.186** mg/L 14d E_rC_{50} = **0.16** mg/L all mean measured	14d NOE_rC = **0.051** mg/L mean measured	Hoberg, 1992e
Duckweed (*Lemna gibba*) / OECD TG 221, GLP	400 g asulam/L SL formulation	7d E_rC_{50} = 0.926 mg/L mean measured	7d NOE_rC = 0.0362 mg/L mean measured	Vinken & Wydra, 2007
Myriophyllum spicatum / draft OECD TG test guideline (July 2014), GLP	'Asulox' 400 g asulam/L SL formulation	14d E_rC_{50} = 6.44 mg/L nominal	14d NOE_rC = **0.011** mg/L nominal	Seeland, 2014

Fish and invertebrates showed low sensitivity to asulam-sodium; algae and aquatic plants are the most sensitive groups. Acute/chronic aquatic toxicity data are available on asulam/asulam-sodium for fish, invertebrates, algae and aquatic plants. The DS pointed out that no "true" chronic toxicity study on fish is available, however the available prolonged 28 day juvenile fish growth test are considered sufficient to indicate a low chronic toxicity to fish. Data are available from aquatic plants and algae on the main degradant of asulam (sulfonilamide), which indicate that sulfonilamide is less toxic than the parent substance (Gosch and Sowig, 2003d; Juckeland 2011). However, the degradants are not considered further in relation to the classification of asulam-sodium.

Overall, the DS proposed to classify asulam-sodium as:

Aquatic Acute 1 (H400) based on the mean measured *Lemna gibba* 14 day E_rC_{50} of 0.16 mg/L. As this value is in the range of 0.1 mg/L < $L(E)C_{50}$ ≤ 1 mg/L, the M-factor should be 1.

Aquatic Chronic 1 (H410) based on the mean measured *Myriophyllum spicatum* 14 day NOE_rC of 0.011 mg/L. As this value is in the range of 0.01 mg/L < $L(E)C_{50}$ ≤ 0.1 mg/L, and substance is not rapidly degradable. The M-factor should be 1.

Comments received during public consultation

Three MSCA submitted comments on the environmental part of the DS's proposal. One of them agreed with the proposed classification of asulam-sodium as Aquatic Acute 1 (M=1) and Aquatic Chronic 1 (M=1) without further justification. Another MSCA pointed out that the key study for acute aquatic toxicity with *Lemna gibba* performed according to US EPA/FIFRA 122 and 123-2 determined a 14d E_rC_{50}. Following OECD TG 221 growth inhibition on *Lemna sp.* should be terminated after 7 days. The 7d E_rC_{50} was not calculated but 6d and 9d E_rC_{50}s were in the same range as 14d E_rC_{50}, therefore they supported the proposed classification. The third MSCA agreed with the proposed classification and M-factors but suggested to use for acute aquatic hazard classification the E_rC_{50} (9d) = 0.186 mg/L / E_rC_{50} (6d) = 0.205 mg/L from the *Lemna gibba* study instead of the E_rC_{50} (14d) = 0.16 mg/L. For chronic aquatic hazard classification they proposed to use the NOE_rC (14d) = 0.051 mg/L mean measured derived from the *Lemna* study and the NOE_rC (72h) = 0.02 mg/L mean measured derived from the static study with *Pseudokirchneriella subcapitata* (Hoberg, 1992a and reassessment by Dorgerloh, 2004b) instead of the NOE_rC (14d) = 0.011 mg/L nominal for *Myriophyllum spicatum* (Seeland, 2014), because in this study the test substance was a 400 g/L soluble liquid formulation and not a pure active ingredient like in the key studies or other relevant studies. As the proposed E_rC_{50} (6 and 9 days) values were in the same range as E_rC_{50} 14 days value, that will not change proposed classification or M-factors.

In their answers, DS agreed that for acute aquatic toxicity it may be preferable to use a ≈ 7 days endpoint from *Lemna* studies instead of those at 14 days, particularly when the test substance is not stable throughout the test or there are indications of a reduction in growth by day 14 due to nutrient depletion. However, endpoints at 14 days were based on mean measured concentrations and it was also reported that "*there was good growth throughout the 14 days in controls (meeting validity criteria) indicating no problems with nutrient depletion*". So in this case, the DS felt that the *Lemna* 14 days E_rC_{50} endpoint may be suitable to use for classification (it was also used for risk assessment). For the chronic aquatic hazard classification, the DS replied that the formulation study on *Myriophyllum* (Seeland, 2014) used a simple solution of asulam in water, with no other coformulants or solvents to confound the toxicity, so the DS felt that an endpoint based on the asulam-sodium equivalent concentration

would be suitable to use for classification. However, the DS left the eventual choice of the E_rC_{50} and NOE_rC to the RAC.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS's proposal that asulam-sodium does not meet the criteria for "rapidly degradable" based on current degradation criteria in the CLP Regulation. Based on available hydrolysis, photolytic degradation studies, results obtained in a biodegradation study and aerobic natural water/sediment systems studies RAC agrees with the DS conclusion that available degradation information does not indicate that asulam-sodium is ultimately degraded (> 70%) within 28 days (equivalent to a degradation half-life of < 16 days). Consequently, asulam-sodium is considered to be not rapidly degradable for the purposes of classification under the CLP Regulation.

Aquatic Bioaccumulation

Asulam-sodium has a log K_{OW} of 0.15 (at pH 7) which is less than the CLP trigger of ≥ 4. Additionally, this was confirmed in an non-standard study, with no measurement of exposure concentrations, and which was non GLP compliant but was otherwise considered to be a valid study on catfish *(Ameirus melas),* where the whole fish bioconcentration factor (BCF) was 0.1 - 1.4 and substantially less than the CLP BCF trigger of 500. Therefore, RAC agrees with the DS's conclusion that the substance is not bioaccumulative.

Aquatic Toxicity

RAC agrees that there are reliable acute and chronic aquatic toxicity data for all trophic levels (fish, invertebrates, algae/aquatic plants) and that degradants (sulfonilamide) need not be considered further in relation to the classification of asulam-sodium. RAC agrees that the algae/aquatic plants are the most sensitive groups for the purpose of aquatic acute/chronic classification. RAC notes that the available prolonged 28 days juvenile fish growth test is considered sufficient to indicate a low chronic toxicity to fish.

Acute toxicity

RAC agrees that the lowest acute (short-term) endpoints for aquatic acute classification purposes of asulam-sodium is the aquatic plant (*Lemna gibba*) 6d E_rC_{50} = 0.205 mg/L, 9d E_rC_{50} = 0.186 mg/L and 14d E_rC_{50} = 0.16 mg/L, all based on mean measured concentrations. As the 14d E_rC_{50} = 0.16 mg/L endpoint indicated a good growth throughout the 14 days in controls (meeting validity criteria) indicating no problems with nutrient depletion, RAC considered that it was suitable to use for aquatic acute classification.

Chronic toxicity

RAC agrees that the lowest chronic (long-term) endpoints for aquatic chronic classification purposes of asulam-sodium is the aquatic plant (*Myriophyllum spicatum*) 14d NOE_rC = 0.011 mg/L based on the nominal concentration. However, RAC considers that the 72h NOE_rC = 0.02 mg/L for *Pseudokirchneriella subcapitata* and 14d NOE_rC = 0.051 mg/L for *Lemna gibba*, both mean measured, would be more appropriate for aquatic chronic classification despite the fact that formulation study on *Myriophyllum* (Seeland, 2014) used a simple solution of asulam in water, without other coformulants or solvents.

Conclusion on classification

Asulam-sodium is considered as not rapidly degradable and does not fulfil the criteria for bioaccumulation. Based on the available and most reliable information, RAC is of the opinion that asulam-sodium should be classified as:

Aquatic Acute 1 based on E_rC_{50} = 0.16 mg/L for *Lemna gibba*. As this acute toxicity value falls within the 0.1 < $L(E)C_{50}$ ≤ 1 mg/L range, the **acute M-factor is 1**.

This classification conclusion is supported byother results of the same acute toxicity study for *Lemna gibba* with E_rC_{50} = 0.205 and E_rC_{50} = 0.186 mg/L.

Aquatic Chronic 1 based on NOE_rC =0.02 mg/L for *Pseudokirchneriella subcapitata*. As this chronic toxicity value falls within the 0.01 < NOEC ≤ 0.1 mg/L range, the **chronic M-factor is 1.**

This classification conclusion is supported by two other reliable chronic toxicity studies for *Lemna gibba* (Hoberg, 1992e) with NOE_rC = 0.051 mg/L and *Myriophyllum spicatum* (Seeland, 2014) with NOE_rC = 0.011 mg/L.

6 OTHER INFORMATION

No other relevant information.

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All references are taken from the Draft Assessment Report (DAR) for asulam-sodium

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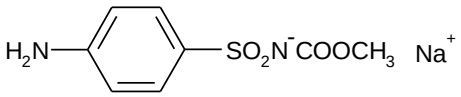
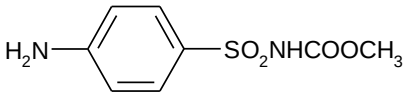
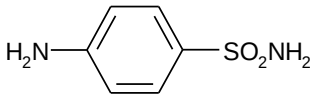
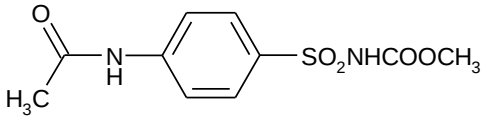
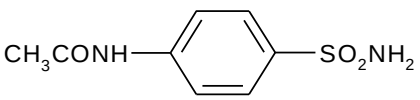
8 ANNEXES

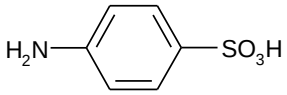
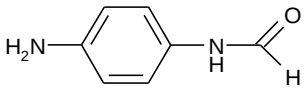
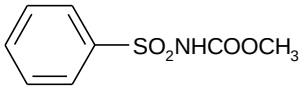
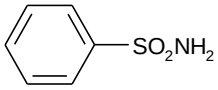
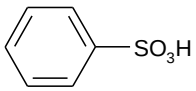
Annex I – Details of asulam sodium metabolites

Annex II – Confidential information on impurities (provided as separate document)

ANNEX 1

Table 1: Details of asulam sodium plus its metabolites and degradation products (found in water, sediment, soil)

Names / codes used	Formula	Occurrence
Asulam-sodium Asulam-natrium Sodium asulam (Asulox) RPA413636 Hoe 102789 AE C518360 CAS RN 2302-17-2	 AE F102789 Sodium methyl [(4-aminophenyl)sulfonyl]carbamate	Active substance
Asulam M&B 9057, MB009057, RPA096215, RPA590048, Hoe 074383 AE B106159 CAS RN 3337-71-1	 AE F074383 Methyl sulfanilylcarbamate	
Sulfanilamide MB000631 MB000863 RP014501 RPA023385 RPA718068 CAS RN 63-74-1	 AE C473799 4-Aminobenzenesulfonamide	Soil Water/sediment
Acetyl asulam MB 9495 MB009495 Hoe 073553 AE C440740 CAS RN 18431-25-9	 AE F073557 Methyl [(4-acetamidophenyl)sulfonyl]carbamate	Soil Water/sediment
Acetyl sulfanilamide MB24805 MB024805 Hoe 073547 AE C418106 AE C426035 AE C500816 CAS RN 121-61-69	 AE F073547 N-(4-Sulfamoylphenyl)acetamide	Soil Water/sediment

Names / codes used	Formula	Occurrence
CAS RN 5661-14-3		
Sulfanilic acid CAS RN 121-57-3	 AE B004107 4-Aminobenzenesulfonic acid	Soil Water/sediment + light
	 N-(4-Aminophenyl)formamide	Water + light
Desamino asulam Mb022232 CZS RN 32324-23-5	 Methyl phenylsulfonylcarbamate (methylbenzenesulfonylcarbamate)	Soil
	 Benzenesulfonamide	Soil
	 Benzenesulfonic acid	Soil