Committee for Risk Assessment

RAC

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

Adopted

9 December 2016

9 December 2017

CLH-O-0000001412-86-138/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name:	**sodium methyl [(4-aminophenyl)sulphonyl]carbamate; sodium methyl (*EZ*)-sulfanilylcarbonimidate; asulam-sodium**
EC Number:	**218-953-8**
CAS Number:	**2302-17-2**

The proposal was submitted by the **United Kingdom** and received by RAC on **13 May 2016.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

The United Kingdom has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **31 May 2016**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **15 July 2016**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC:	**Bogusław BARAŃSKI**
Co-Rapporteur, appointed by RAC:	**Žilvinas UŽOMECKAS**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **9 December 2016** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	607-RST-VW-Y	sodium methyl [(4-aminophenyl)sulphonyl]carbamate; sodium methyl (*EZ*)-sulfanilylcarbonimidate; asulam-sodium	218-953-8	2302-17-2	Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410	GHS07 GHS09 Wng	H317 H410		M=1 M=1	
RAC opinion	607-RST-VW-Y	sodium methyl [(4-aminophenyl)sulphonyl]carbamate; sodium methyl (*EZ*)-sulfanilylcarbonimidate; asulam-sodium	218-953-8	2302-17-2	Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410	GHS07 GHS09 Wng	H317 H410		M=1 M=1	
Resulting Annex VI entry if agreed by COM	607-RST-VW-Y	sodium methyl [(4-aminophenyl)sulphonyl]carbamate; sodium methyl (*EZ*)-sulfanilylcarbonimidate; asulam-sodium	218-953-8	2302-17-2	Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410	GHS07 GHS09 Wng	H317 H410		M=1 M=1	

GROUNDS FOR ADOPTION OF THE OPINION

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.**

HUMAN HEALTH HAZARD EVALUATION

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.

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**.

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**.

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**.

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**.

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).

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. **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/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,	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. **Effects at Doses ≥ guidance value for classification:** **25000 ppm - 953 mg/kg bw/d (♂), 1280 mg/kg bw/d (♀):** **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.

Method	Dose Levels	Observations and Remarks[†‡]
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*		**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.

Method	Dose Levels	Observations and Remarks†‡
		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**.

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.**

<table>
<tr><th>Method</th><th>Organism/strain</th><th>Concentrations tested</th><th>Result</th></tr>
<tr><td>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)</td><td>S. Typhimurium - TA98, TA100, TA102, TA1535, TA1537</td><td>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</td><td>Cytotoxicity was reported at 1667 µg/plate
Vehicle and positive controls valid
Asulam-sodium was negative +/-S9.</td></tr>
<tr><td>3rd study
Mouse Lymphoma assay
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>L5178Y mouse lymphoma cells</td><td><u>-S9;</u>
4000 - 5250 µg/mL

<u>+ S9;</u>
4400 - 5200 µg/mL</td><td><u>-S9</u>

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 (/10⁶ 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 (/10⁶ 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</td></tr>
</table>

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

Reference: Report V7903/02, 2009 (DAR B.6.4.1 D)</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>
<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></td></tr>
</table>

<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><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 C030509, 1982 (DAR B.6.4.1 E)</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>

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

Reference: Report C032928, 2004 (DAR B.6.4.2)</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>
<tr><td>12</td><td>1152 (63%)</td><td>3.5</td><td>4</td></tr>
<tr><td>14</td><td>1997 (50%)</td><td>1.0</td><td>4</td></tr>
<tr><td>24</td><td>1693 (54%)</td><td>3.5</td><td></td></tr>
<tr><td>Mean ± SD</td><td>2036 ± 690 (50%)</td><td>1.9 ± 1.6</td><td>3.8 ±0.4</td></tr>
</table>
1000 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>9</td><td>2064 (49%)</td><td>1.0</td><td>3</td></tr>
<tr><td>13</td><td>2357 (46%)</td><td>0.8</td><td>6</td></tr>
<tr><td>17</td><td>3794 (35%)</td><td>2.1</td><td>2</td></tr>
<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</td></tr>
</table>

Method	Organism /strain	Concentrations tested	Result

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.

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)	<u>♂ Non-neoplastic findings</u> **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). <u>♀ Non-neoplastic findings</u> **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%) <u>Males - Neoplastic findings</u> 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. <u>♀- Neoplastic findings</u> No test item-related effects
Two-year carcinogenicity study in mice Asulam-sodium (purity 88%) CD-1 mice 75/sex/group	0, 500, 5000 and 50 000 ppm ♂: 0, 74, 730, 8040	<u>♂- Non-neoplastic findings</u> **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.

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
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)	mg/kg bw/d) ♀: 0, 95, 938, 10353 mg/kg bw/d)	<u>♀- Non-neoplastic findings</u> **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. <u>♂- Neoplastic findings</u> 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%* <u>♀- Neoplastic findings</u> 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.**

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

<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 TG 416. Pre-GLP

Reference: Report C015412, 1981 (DAR 6.6.1)</td><td>0, 1000, 5000, 25000 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 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
<table>
<tr><th rowspan="3" colspan="2"></th><th colspan="8">Dose level (ppm)</th></tr>
<tr><th colspan="4">♂</th><th colspan="4">♀</th></tr>
<tr><th>0</th><th>1000</th><th>5000</th><th>25000</th><th>0</th><th>1000</th><th>5000</th><th>25000</th></tr>
<tr><td colspan="2">Pre-mating (mg/kg bw/d)</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 colspan="2">Post-mating (mg/kg bw/d)</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 rowspan="2">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>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 rowspan="2">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>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><th>ppm</th><th>0</th><th>1000</th><th>5000</th><th>25000</th></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>
<tr><td colspan="5">F_2</td></tr>
<tr><td>Litter size day 0 (total)</td><td>11.0</td><td>10.1</td><td>8.0*</td><td>9.4</td></tr>
<tr><td>Litter size at day 30</td><td>9.3</td><td>9.8</td><td>7.2</td><td>7.8</td></tr>
<tr><td>Stillborn pups (absolute no)</td><td>6</td><td>5</td><td>1</td><td>0</td></tr>
<tr><td>Survival (%) day 4</td><td>88</td><td>92</td><td>88</td><td>82</td></tr>
<tr><td>Survival % day 30</td><td>87</td><td>91</td><td>82</td><td>82</td></tr>
<tr><td>Fertility Index (%)</td><td>83</td><td>83</td><td>62</td><td>74</td></tr>
<tr><td>Viability index (%)</td><td>88</td><td>92</td><td>88</td><td>82</td></tr>
<tr><td>Lactation index (%)</td><td>98</td><td>99</td><td>93</td><td>100</td></tr>
</table></td></tr>
</table>

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

* 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
<table>
<tr><th 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>No of rat foetuses examined (litters)</td><td>176 (20)</td><td>177 (20)</td><td>181 (20)</td><td>197 (20)</td><td>10954[a]
Mean % foetuses (range)</td></tr>
</table>
</td></tr>
</table>

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

↓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

<table>
<tr><th>Method</th><th>Dose levels</th><th>Observations and remarks
(effects of major toxicological significance)</th></tr>
<tr><td colspan="3">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</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 TG 414. Pre-GLP

Reference: Report R001248, 1981 (DAR 6.6.3)</td><td>0, 150, 300, 750 mg/kg bw/d

Positive control- 150 mg/kg bw/d thalidomide</td><td>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%).
<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>positive 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>
Developmental effects
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>positive 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>Foetal 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>Foetal 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>

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**.

ENVIRONMENTAL HAZARD EVALUATION

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.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).