Asulam sodium

Annex B (Volume 3)
B.6A Toxicology and metabolism

Version history

When	What
2016-04-18	Initial DAR

B.6 TOXICOLOGY AND METABOLISM

Asulam was reviewed in 2010 by the UK regulatory authority. The recommendations were considered at peer review and an EFSA conclusion was published on the active substance (EFSA Journal 2010; 8(12):1822). Asulam did not gain approval for use as a pesticide active substance in 2010; thus the applicant for Asulam has submitted this dossier for consideration as a new active substance under Regulation 1107/2009 with the intention of addressing the reasons for non-approval. The RMS has assessed the new submission in accordance with Regulation (EC) 544/2011 as the dossier was submitted prior to 1st January 2014 and presented the data accordingly. Statements preceding all studies have been included to communicate whether the study has been evaluated previously by the RMS. No changes have been made to the conclusions of the studies that have been previously evaluated i.e. NOAELs.

Asulam is a carbamate herbicide first reported in 1965 by May and Baker, with the initial development code M&B 9057. With changes in company name and ownership, other codes were used: an overview of the current and previous codes is given below. Asulam is a molecule that contains both basic and acidic groups: it readily forms salts and the supported formulation is an aqueous solution of the sodium salt of asulam (asulam sodium). Toxicity studies were performed using asulam or asulam sodium but these are considered to be representative of the material as manufactured i.e. asulam-sodium and historically the terminology has been used interchangeably: the results of toxicity studies performed with either material are therefore considered to be acceptable for the purposes of this review. The batches tested in the toxicology studies are acceptable to support the new specification and the approval of asulam (see Vol 4 for more details).

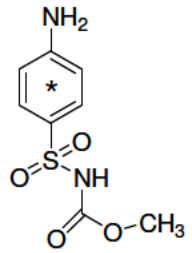
	Asulam sodium	**Asulam**
Current codes:	AE F102789	AE F074383
Previous codes:	M&B 9057, M&B-9057, MB009057* RPA413636 Hoe 102789 AE C518360	M&B 9057, M&B-9057, MB009057* RPA096215, RPA590048, Hoe 074383 AE B106159 XN-36111

**previously asulam and asulam sodium were not differentiated, and the same code may have been used*

B.6.1 Absorption, distribution, metabolism and excretion (toxicokinetics) (IIA 5.1)

Two studies have been submitted to address the ADME and kinetic profile of Asulam. The study by [redacted] addressed the absorption, distribution, metabolism, excretion and pharmacokinetics of Asulam following single and repeat dosing in rats (section 6.1.1 A and 6.1.2). The second study by [redacted] reported on the toxicokinetic profile and tissue distribution of Asulam (6.1.1 B).

Figure B.6.2 Structure of asulam showing the radiolabel position



B.6.1.1 Absorption, distribution and excretion

A This study has been evaluated previously by the RMS; the outcomes presented are consistent with those following peer review and published in the EFSA conclusion.

Study	Asulam Biokinetics and Metabolism in the Male and Female Rat
Reference	[redacted] 1989a
Date performed	May 1987 - November 1989
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	USEPA 85-1
Deviations from the guideline	None
GLP	Yes
Test material	>99% purity (unlabelled, batch LOP 4650); (^{14}C-phenyl)asulam >98% radiopurity, 0.29-0.38mCi/mMol
Study acceptable	Yes

Groups of CD rats (5/sex) were gavaged with a single dose of ^{14}C-phenyl radiolabelled asulam, or with a single dose of ^{14}C-phenyl radiolabelled material following 14 consecutive daily doses of unlabelled material, as detailed in Table B.6.1. The test material was dissolved in water containing an equivalent amount of sodium hydroxide in order to achieve neutral pH. Samples of excreta, blood and tissues were collected as detailed below. Radioactivity was measured by LSC (urine) or LSC following methanol extraction (faeces), combustion (tissues) or solubilisation (blood). The pharmacokinetic or absorption, distribution and excretion phases of this study are reported below; the metabolism phase of the study is reported at Section B.6.1.2.

Table B.6.1.1-1 Toxicokinetics study: design

Dose Type	Dose level (mg/kg bw)	Experiment Type	Dose route	Group size	Samples	Time
Low	30	ADE Metabolism	Oral	5M, 5F	CO_2, urine, faeces, blood	0-72h
High	300	ADE Metabolism				
Multiple	30	ADE Metabolism			CO_2, urine, faeces	
Low	30	PK			Blood	0-168h
High	300	PK				
Low	30	DME	Intravenous		CO_2, urine, faeces, blood	0-72h

Excretion

Following a single low dose, excretion of radioactivity was rapid (largely within 24 hours) and predominantly (82.0-83.5%) in the urine. The excretion profile was similar in both sexes. At the high dose level, urinary excretion was slightly higher (91.5-92.5%) and faecal excretion lower in both sexes; findings may reflect the saturation of conjugation or biliary excretion in this group. In the multiple-dose group, urinary excretion was slightly higher in males only (comparable to the high dose group). Following intravenous administration, urinary excretion (93.0-95.5%) was more extensive than seen in orally-dosed animals. Some faecal radioactivity (3.3-4.6%) was detected in this group, consistent with limited biliary excretion. Radioactivity excreted as CO_2 was undetectable in all groups and residual tissue radioactivity was low in all groups. The total recovery of radioactivity was acceptable in all groups. The results of this study indicate that the absorption of asulam following oral administration is at least 85-90%, based on urinary excretion at the low dose level and faecal (assumed to be biliary) excretion in intravenously dose animals.

Table B.6.1.1-2 Excretion of radioactivity

Sample	Time	**Sample radioactivity** (% administered)							
		30 mg/kg bw		**300 mg/kg bw**		**30 mg/kg bw** (rpt)		**30 mg/kg bw** (iv)	
		M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Urine	**0-24h**	77.99	80.01	89.00	87.60	87.94	82.89	90.91	92.14
	0-48h	81.28	83.32	91.27	91.79	90.90	85.53	92.17	94.54
	0-72h	81.98	83.55	91.50	92.27	91.32	86.12	92.76	95.45
Faeces	**0-24h**	13.64	13.30	7.85	5.33	5.69	10.61	4.08	2.69
	0-48h	16.25	14.75	9.46	7.61	7.98	12.52	4.31	3.04
	0-72h	17.32	14.98	9.86	7.92	8.83	13.44	4.57	3.30
Cagewash	**0-72h**	1.74	0.29	0.41	0.47	0.79	0.86	2.49	2.92
Total excreted		**101.0**	**98.8**	**101.8**	**100.7**	**100.9**	**100.4**	**99.8**	**101.7**
Tissues		0.31	0.23	0.23	0.42	0.49	0.81	0.48	0.70
Recovery		**101.4**	**99.1**	**102.0**	**101.1**	**101.4**	**101.2**	**100.3**	**102.4**

Distribution

Levels of radioactivity at 72 hours following administration were low in all tissues. Residues at the low dose level were highest in the gastrointestinal tract and skin/fur. Residues at the high dose level were highest in the gastrointestinal tract, skin/fur (both sexes), lung and carcass (females); the high mean lung value in high dose females is largely attributable to one outlier (3.66 µg/g) and the high carcass value may reflect incomplete excretion at this relatively early time point. Highest tissue levels following intravenous dosing were seen in the kidney and skin/fur. Levels of radioactivity in repeat dosed rats are broadly comparable to those following a single low dose with the exception of higher values for the ovaries and gastrointestinal tract. Highest levels in intravenously dosed rats were measured in the skin/fur and kidney.

Table B.6.1.1-3 Residual tissue radioactivity

Tissue	Tissue radioactivity at 72 hours (µg eq/g)							
	30 mg/kg bw		300 mg/kg bw		30 mg/kg bw (rpt)		30 mg/kg bw (iv)	
	M	F	M	F	M	F	M	F
Liver	nd	nd	0.08	0.09	0.01	0.01	0.05	0.05
Kidney	0.01	0.02	0.10	0.13	0.02	0.03	0.17	0.16
Heart	nd	0.01	0.01	0.06	0.02	nd	0.03	0.02
Lung	nd	nd	0.06	0.80	nd	nd	0.04	nd
Brain	nd	nd	nd	nd	nd	nd	0.01	nd
Spleen	0.04	nd	0.02	0.02	nd	0.02	0.07	0.07
Muscle	0.01	nd	0.06	0.08	nd	0.12	0.01	0.02
Fat	nd	nd	0.04	0.10	0.04	0.07	nd	nd
Ovaries		nd		0.05		0.21		nd
Testes	nd		0.04		nd		0.01	
GIT & contents	0.28	0.07	0.40	0.49	1.02	0.49	0.05	0.07
Bone & marrow	0.01	nd	0.04	0.05	<0.01	0.03	0.01	0.06
Adrenals	nd	nd	nd	nd	nd	nd	nd	nd
Skin & fur	0.21	0.31	1.35	1.38	0.29	0.27	0.52	0.84
Pancreas	nd	nd	0.14	0.10	0.09	0.07	0.04	0.01
Thyroid	nd	nd.	nd	nd	nd	nd	nd	nd
Carcass	0.05	0.02	0.69	1.71	nd	nd	n.d.	nd
Blood	nd	nd	0.17	0.19	nd	nd	nd	nd
Plasma	nd	nd	nd	nd	nd	nd	nd	nd
Uterus		nd		0.17		0.04		nd

nd: not detected

Kinetics

Detectable levels of radioactivity in whole blood were rapidly measured following the oral administration of a single low dose; T_{max} was 0.5 hours. Levels of radioactivity subsequently declined rapidly and were not detectable at 72 hours following administration. A similar profile was seen at the high dose level, with T_{max} at 0.5 hours and radioactivity not detectable at 144 hours. C_{max} values are slightly higher in females at both dose levels, however values in both sexes are approximately proportional to dose level. Elimination half-lives were calculated to be 6.4h and 6.7h at the low dose level and 12.9h and 13.4h at the high dose level for males and females respectively.

Table B.6.1.1-4 Blood levels of radioactivity

Time	Whole blood radioactivity (µg eq/g)			
	30 mg/kg bw		300 mg/kg bw	
	M	F	M	F
0.25h	14.82 ±4.11	17.91 ±5.04	131.63 ±10.77	133.51 ±14.22
0.5h	17.94 ±2.43	21.61 ±4.68	167.32 ±28.08	164.20 ±25.71
1h	11.18 ±1.63	14.91 ±1.83	125.85 ±22.83	142.67 ±22.35
2h	2.61 ±0.94	4.04 ±1.02	33.57 ±19.68	62.16 ±18.42
3h	1.68 ±0.64	2.52 ±1.15	11.27 ±1.72	16.47 ±3.75
5h	1.09 ±0.49	1.02 ±0.34	7.83 ±0.76	8.75 ±1.33
7h	0.82 ±0.35	0.76 ±0.26	5.67 ±1.26	6.44 ±1.52
24h	0.20 ±0.17	0.20 ±0.01	1.03 ±0.23	1.45 ±0.59
48h	0.04 ±0.08	0.06 ±0.09	0.65 ±0.23	0.95 ±0.51
72h	nd	nd	0.26 ±0.11	0.67 ±0.54
96h	nd	nd	0.23 ±0.11	0.35 ±0.34
120h	nd	nd	0.07 ±0.10	0.09 ±0.12
144h	nd	nd	nd	nd
168h	nd	nd	nd	nd

nd: not detected

The results of this study indicate that asulam is rapidly absorbed (T_{max} =0.5h), cleared and excreted following oral administration. Kinetic parameters are broadly similar in both sexes and between dose groups, although faecal excretion was slightly reduced at the high dose level and (in males) following the administration of multiple doses. Oral absorption following a single low dose is estimated to be at least 85-90%. There is no evidence for bioaccumulation, but some evidence for preferential binding to blood cells.

[redacted] 1989a

B This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report.

Study	Asulam - Tissue distribution and kinetic study in the rat
Reference	[redacted] 1995a
Date performed	4 July - 22 September 1995
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	JMAFF Nohsan 4200
Deviations from the guideline	None
GLP	Yes
Test material	>99% purity; >98% radiopurity, 18.61 mCi/mMol
Study acceptable	Yes

Sprague-Dawley CD rats were gavaged with single oral doses of ^{14}C-phenyl radiolabelled asulam (dissolved in water and neutralised with sodium hydroxide) at dose levels of 30 or 300 mg/kg bw. Tissue radioactivity was measured using LSC or LSC following combustion. In the toxicokinetics phase of this study, blood samples were taken from groups of 4 rats/sex at intervals up to 72 hours following dosing. In the tissue kinetics phase, groups of 4 rats/sex were sacrificed at time points corresponding to T_{max} and ½T_{max} (elimination) and at 6 hours post dosing.

Toxicokinetics phase

Blood radioactivity was rapidly detectable (within 15 minutes) in all groups. T_{max} values were calculated to be ~0.4h in both sexes at the low dose level and ~0.5h for both sexes at the high dose level. C_{max} values are approximately proportional to the dose level. Levels of blood radioactivity decreased rapidly following C_{max} and were either very low or not detectable at 72 hours following administration. During the elimination phase, ½C_{max} was estimated to be attained at 0.85h and 0.98h at the low dose level; and at 1.13h and 1.42h at the high dose level in males and females respectively.

Table B.6..1.1-5 Levels of radioactivity in whole blood following a single oral dose

Time	Radioactivity in whole blood (µg/g)			
	30 mg/kg bw		300 mg/kg bw	
	M	F	M	F
0 h	nd	nd	nd	0.21 ±0.42
0.25 h	37.17 ±26.95	30.13 ±7.61	171.84 ±30.59	214.28 ±13.15
0.50 h	29.50 ±3.97	29.60 ±6.49	250.97 ±24.32	287.04 ±45.59
1 h	16.46 ±3.72	14.93 ±2.44	140.01 ±5.41	205.08 ±25.67
2 h	5.33 ±1.16	4.00 ±1.68	25.53 ±3.15	57.39 ±9.99
3 h	2.34 ±0.40	1.75 ±0.67	8.81 ±1.32	21.37 ±3.79
6 h	1.69 ±0.23	1.10 ±0.30	7.13 ±1.26	9.39 ±1.44
8 h	1.12 ±0.32	0.83 ±0.30	4.58 ±0.72	6.49 ±0.65
24 h	0.35 ±0.09	0.21 ±0.06	1.66 ±0.55	2.85 ±1.71
32 h	0.17 ±0.03	0.08 ±0.01	1.45 ±0.15	1.49 ±0.09
48 h	0.08 ±0.02	nd	nd	0.31 ±0.37
56 h	0.03 ±0.04	nd	nd	0.14 ±0.28
72 h	0.01 ±0.03	nd	nd	0.36 ±0.44

nd: not detected

The results of this phase indicate that asulam (and/or its metabolites) is rapidly cleared following the administration of a single low or high oral dose.

Tissue kinetics phase

At the low dose level at the earlier time points, concentrations of radioactivity were highest in the kidneys, stomach and intestines (with contents); findings reflect the ongoing absorption and excretion of the test material. At the second time point (corresponding to ½T_{max}), levels of radioactivity had decreased in the majority of tissues, but were higher in muscle, carcass (both sexes), skin/fur and uterus (females). At the final time point, levels were highest in the intestines and kidneys, reflecting incomplete excretion. Levels in the other tissues at the final time point are low and are broadly comparable, with the exception of levels in the skin/fur of females which remained relatively high. There was some evidence for binding to blood cells.

Table B.6.1.1-6 Levels of tissue radioactivity at the low dose level

Tissue	Tissue radioactivity (µg eq/g)					
	M			F		
	0.43h	**0.85h**	**6h**	**0.38h**	**0.98h**	**6h**
Liver	17.81	13.62	0.80	21.72	12.24	0.34
Kidneys	113.97	113.97	13.55	117.46	84.86	1.08
Heart	10.83	10.00	0.29	13.29	8.08	0.23
Lungs	13.32	12.14	0.43	17.94	10.79	0.40
Brain	0.84	0.80	0.06	1.20	0.73	0.05
Spleen	14.62	7.48	0.76	16.11	7.73	0.40
Pancreas	8.09	6.66	1.41	11.15	7.59	0.30
Muscle	6.45	11.94	0.34	7.88	21.38	0.34
Fat	4.52	4.11	0.10	4.95	5.02	0.10
Gonads	7.47	13.05	0.39	17.44	13.88	0.30
Stomach + contents	606.42	346.78	0.59	246.54	157.76	0.88
Intestine + contents	171.54	105.67	43.58	184.21	114.16	34.22
Bone & marrow	6.75	6.65	0.21	7.77	6.67	0.18
Skin & fur	9.29	8.66	0.97	14.67	22.71	2.22
Adrenals	3.22	2.28	0.44	11.38	8.21	0.15

Uterus				24.05	34.59	0.24
Blood	23.02	19.29	1.07	31.31	16.71	0.89
Plasma	31.91	28.80	0.28	41.78	22.37	0.23
Thyroids	37.04	31.08	nd	12.76	10.83	n.d.
Residual carcass	12.86	21.73	1.58	10.18	13.97	0.49

nd: not detected

A similar pattern of distribution was seen at the high dose level with highest levels of radioactivity initially in the kidney and gastrointestinal tract. Levels of radioactivity at the second time point were lower in all tissues with the exception of the carcass (both sexes), uterus and thyroids (females). The highest levels of radioactivity at the final time point were seen in the intestines; relatively high levels were also noted in the adrenals of males.

Table B.6.7 Levels of tissue radioactivity at the high dose level

Tissue	**Tissue radioactivity** (µg eq/g)					
	M			**F**		
	0.5h	**1.13h**	**6h**	**0.5h**	**1.42h**	**6h**
Liver	220.50	144.84	3.17	214.18	121.53	2.78
Kidneys	1305.68	1044.27	21.77	935.01	603.42	12.84
Heart	148.49	108.35	3.05	139.83	79.13	2.75
Lungs	218.22	116.62	3.23	175.80	96.94	3.91
Brain	10.62	7.76	0.57	11.28	6.88	0.64
Spleen	118.83	82.09	3.47	107.88	72.57	2.98
Pancreas	174.32	72.96	2.44	108.50	59.61	3.63
Muscle	80.21	64.86	1.83	109.23	107.18	2.04
Fat	98.31	32.46	1.96	47.53	27.64	1.44
Gonads	127.63	45.35	6.18	190.28	90.06	2.71
Stomach + contents	3438.46	1825.67	3.40	4079.47	330.83	23.52
Intestine + contents	2024.81	1074.31	482.60	1556.11	1013.36	330.32
Bone & marrow	72.97	61.60	1.74	73.43	48.65	1.99
Skin & fur	122.55	90.19	16.88	170.42	119.96	10.02
Adrenals	147.64	81.98	12.91	142.65	72.73	1.39
Uterus				269.23	407.18	4.40
Cardiac Blood	350.36	203.55	8.82	302.01	145.25	8.29
Plasma	323.01	210.48	2.90	338.93	174.04	3.36
Thyroids	179.78	163.38	nd	250.28	372.49	nd.
Residual carcass	141.64	257.73	4.07	112.83	123.12	2.72

nd: not detected

The results of this study indicate that asulam is widely distributed and rapidly cleared in the rat following oral administration. There is no evidence for bioaccumulation, but some evidence for the preferential binding of radioactivity to the cellular component of blood.

[redacted] 1995a

B.6.1.2 Metabolism

This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report.

Study	Asulam Biokinetics and Metabolism in the Male and Female Rat
Reference	[redacted] 1989a
Date performed	May 1987 - November 1989

Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	USEPA 85-1
Deviations from the guideline	None
GLP	Yes
Test material	>99% purity (unlabelled); (^{14}C-phenyl)asulam >98% radiopurity, 0.29-0.38mCi/mMol
Study acceptable	Yes

Samples of urine and faeces taken from animals in this study (the study design is detailed at Section B.6.1.1A) were analysed for radioactivity by TLC and the metabolites quantified and identified using LC/MS.

Unchanged asulam accounted for the majority of the urinary radioactivity (69.0-80.3% of the administered dose), with acetyl-asulam identified as the major urinary metabolite (2.0-14.9%). Two further minor urinary metabolites were identified as sulphanilamide (0-1.0%) and acetyl-sulphanilamide (0.6-2.8%). Unchanged asulam was also identified as the major radioactive species in faeces (0.9-3.9% of the administered dose), with acetyl-asulam (0.5-2.2%) identified as the only faecal metabolite. The metabolite profiles were broadly similar between dose levels and routes of administration; however metabolism is noted to be slightly less extensive in the high-dose and intravenous-dosed groups.

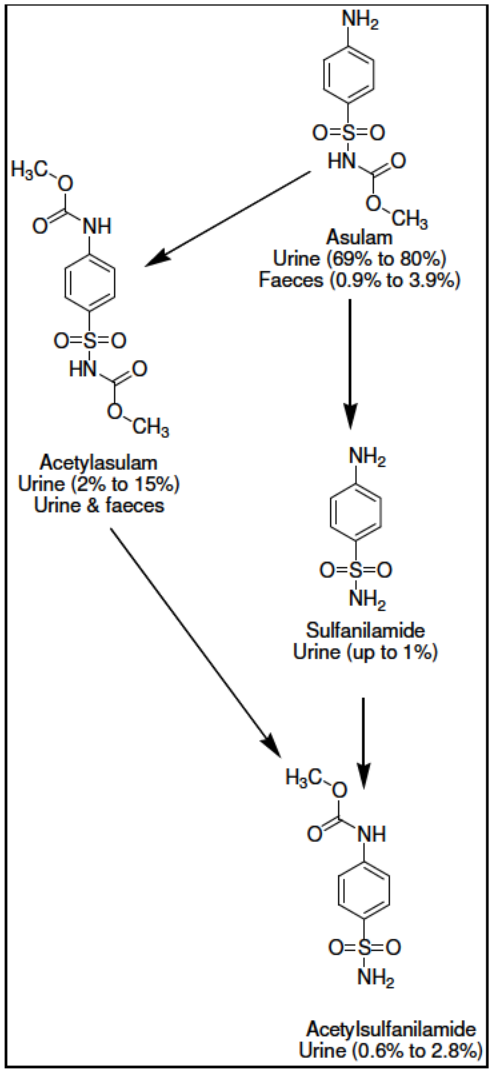
Table B.6.1.2-1 Urinary and faecal metabolites of asulam in the rat

Metabolite	**Radioactivity** (% administered dose)							
	30 mg/kg bw		**300 mg/kg bw**		**30 mg/kg bw (rpt)**		**30 mg/kg bw (iv)**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Urine*								
Asulam	71.3	69.0	76.9	72.4	70.0	74.2	80.3	76.9
Acetyl-asulam	13.1	11.2	7.5	7.6	11.8	16.2	2.0	14.9
Sulphanilamide	1.0	0.3	nd	nd	0.3	nd	0.9	nd
Acetyl-sulphanilamide	1.7	2.4	2.8	2.7	2.8	2.6	1.1	0.6
Total identified	**87.1**	**82.9**	**87.2**	**82.7**	**84.9**	**93.0**	**84.3**	**92.4**
Faeces**								
Asulam	2.5	3.9	1.4	2.9	3.9	2.9	1.5	0.9
Acetyl-asulam	1.2	1.7	1.0	2.2	2.0	1.5	1.0	0.5
Total identified	**3.7**	**5.6**	**2.4**	**5.1**	**5.9**	**4.4**	**2.5**	**1.4**
Total (urine + faeces)	**90.8**	**88.7**	**89.6**	**87.8**	**90.8**	**97.4**	**86.8**	**93.8**

*nd: not detected, *= 0-48h, **=0-24h*

The results of this study indicate that the metabolism of asulam in the rat is limited, with parent largely unchanged and only three metabolites identified. A proposed metabolic pathway is presented below.

Figure B.6.1.2-1 Proposed metabolism of asulam in the rat



[redacted] 1989a

B.6.1.3 Summary of toxicokinetics studies

Absorption

Asulam was found to be rapidly and extensively absorbed in the rat following oral administration. T_{max} values of approximately 30 minutes were calculated for the low dose level (30mg/kg bw) and approximately 1 hour for the high dose level (300 mg/kg bw). The extent of oral absorption was at least 85-90%, based on urinary excretion in orally-dosed animals and faecal excretion (assumed to be biliary in origin) in intravenously dosed animals (reported as complete ≥80% in the EFSA conclusions (2010)). C_{max} values are proportional to the dose level, but are slightly higher in females at both dose levels.

Distribution

Asulam was shown to be rapidly and widely distributed following oral administration. Following T_{max}, the levels of radioactivity decreased in all tissues with the exception of carcass, muscle, skin/fur and uterus. Levels at 72 hours following administration were relatively low in all tissues (including fat), but were slightly higher in skin/fur. There is no evidence for bioaccumulation in fat, but some evidence for the preferential binding to blood cells.

Metabolism

Asulam was found to be excreted largely unchanged in the rat; parent compound in urine accounted for 69.0-80.3% of the administered radioactivity. The major urinary metabolite was acetyl-asulam (2.0-16.2%); two additional urinary metabolites were identified as sulphanilamide (0-1.0%) and acetyl-sulphanilamide (0.6-2.8%). Unchanged asulam was also the major component of faecal radioactivity in all groups, accounting for 0.9-3.9% of the administered dose. The only other faecal metabolite was identified as acetyl-asulam. The metabolic profile was broadly consistent between the sexes and dose groups, however slightly more unchanged parent was excreted by both sexes at the high dose level.

Excretion

Asulam was rapidly (predominantly within 24 hours) excreted in the rat, and largely (82.0-92.3%) in the urine following oral administration. Urinary excretion was slightly higher (and faecal excretion slightly lower) following a single high dose, indicating the saturation of biliary excretion. Following multiple doses, urinary excretion was slightly higher (and faecal excretion slightly lower) in males. Excretion of faecal radioactivity in intravenously-dosed rats (3.3-4.6% of the administered dose) indicates the limited biliary excretion of a conjugate of asulam.

Table 6.1.3-1: Summary of absorption, distribution, metabolism and excretion data

Parameter	Conclusion	Reference
Rate and extent of absorption:	Rapidly and extensively absorbed (T_{max}0.5 h). Oral absorption >80% (estimated from urine, tissues, cage wash and faecal excretion following *iv* administration).	5.1.1/01 [redacted] (1989) 5.1.1/02 [redacted] (1995) 5.1.3/01 [redacted] (1989)
Distribution	Widely distributed in tissues with levels up to 0.8% of the dose in tissues, with predominance in the kidneys (due to renal excretion) and GI tract + contents (probably due to unabsorbed material), skin and fur. Some evidence of binding to blood cells	
Potential for accumulation	Low. Asulam was not retained in tissues following single or repeated oral administration or single *iv* administration.	
Rate and extent of excretion	Rapidly eliminated, with 99-102% of administered radioactivity eliminated within 72 h. Majority eliminated in urine in the first 24 h.	
Metabolism in animals	Biotransformation occurred *via* N-deacetylation or N-acetylation of asulam, and N-acetylation of the metabolite to produce 3 quantifiable metabolites which were acetyl asulam (2-16.2%), acetyl sulphanilamide (0.6-2.8%), sulphanilamide (0-1%)	

B.6.1.4 Comparison of rat metabolism with animal, plant and environmental metabolism

The main metabolites in rats are acetyl-asulam, sulphanilamide and acetyl-sulphanilamide; these are also seen in domestic animals and plants. In addition, conjugates of asulam are observed in plants and domestic animals. These were considered during the review of Asulam in 2010 and no additional toxicological assessment was performed but concerns were raised on the residues of Sulphanilamide in spinach. EFSA concluded that the risk to consumers to Sulphanilamide was a data gap and information to address this issue has been provided and is summarised in section 6.8.

B.6.2 Acute toxicity, irritancy and skin sensitisation studies (IIA 5.2)

B.6.2.1 Acute oral toxicity (IIA 5.2.1)

This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report.

Study	FR 1398/1: Acute oral toxicity study in the rat
Reference	[redacted] 1987a
Date performed	17 November - 2 December 1986
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	OECD 401 (1981)
Deviations from the guideline	None
GLP	Yes
Test material	Asulam sodium; batch NPD W002, 88% purity
Study acceptable	Yes

Fasted CD rats (5/sex) were gavaged with a single oral dose of the test material (as a 25% solution in distilled water) at a dose level of 5000 mg/kg bw and observed for 14 days.

No deaths occurred. 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. Bodyweights were unaffected by treatment. Gross necropsy revealed dark punctate foci on the lungs of one male; this finding is not considered to be related to treatment.

The acute oral LD_{50} of the test material in the rat was found to be >5000 mg/kg bw under the conditions of this study.

[redacted], 1987a

B This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report.

Study	Asulam Sodium Salt - Acute Peroral (Rat) and Percutaneous (Rabbit) Toxicity Studies
Reference	[redacted] 1988a
Date performed	19 September - 3 October 1988
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	USEPA 81-1, 81-2
Deviations from the guideline	None
GLP	Yes
Test material	Asulam sodium; batch XN-36111, 88% purity
Study acceptable	Yes

Fasted Sprague-Dawley rats (5/sex) were gavaged with a single oral dose of the test material (as a 50% solution in distilled water) at a dose level of 5000 mg/kg bw and observed for 14 days.

No deaths occurred. Signs of toxicity were limited to brown perineal staining on the day of dosing; animals appeared normal on Day 2 (females) or Day 3 (males). Bodyweights were unaffected by treatment. Gross necropsy did not reveal any treatment-related findings.

The acute oral LD_{50} of the test material in the rat was found to be >5000 mg/kg bw under the conditions of this study.

[redacted], 1988a

B.6.2.2 Acute dermal toxicity (IIA 5.2.2)

This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report.

Study	FR 1398/1: Acute percutaneous toxicity study in the rat
Reference	[redacted] 1987b
Date performed	18 November - 3 December 1986

Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	OECD 402 (1981)
Deviations from the guideline	None
GLP	Yes
Test material	Asulam-sodium Batch NPD W002; 88% purity
Study acceptable	Yes

The test material was applied for 24 hours under occlusive conditions to the shorn and moistened dorsal skin of CD rats (5/sex) at a dose level of 2000 mg/kg bw. Animals were observed for 14 days.

No deaths occurred and no signs of toxicity or local dermal reactions were observed during the study period. Slight weight loss was noted in one female during the second week, however all animals gained weight over the study period. Gross necropsy did not reveal any treatment-related findings.

The acute dermal LD_{50} of the test material in the rat was found to be >2000 mg/kg bw under the conditions of this study. Under regulation (EC) 1272/2008, Asulam-sodium does not require classification for acute dermal toxicity.

[redacted] 1987b

B This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report.

Study	Asulam Sodium Salt - Acute Peroral (Rat) and Percutaneous (Rabbit) Toxicity Studies
Reference	[redacted] 1988a
Date performed	19 September - 3 October 1988
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	USEPA 81-1, 81-2
Deviations from the guideline	None
GLP	Yes
Test material	Asulam sodium; batch XN-36111, 88% purity
Study acceptable	Yes

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.

Deaths (1M, 1F) occurred at 2000 mg/kg bw; these animals also exhibited abdominal distension and emaciation. Similar signs (and additionally diarrhoea) were also observed in one further male at 2000 mg/kg bw and in one male at 4000 mg/kg bw. Weight loss during the first week of the study was observed in one female at 2000 mg/kg bw and in two males at 4000 mg/kg bw; weight loss over the study period was seen in one male 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 2000 mg/kg bw male. 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.

The acute dermal LD_{50} of the test material in the rabbit was found to be >4000 mg/kg bw under the conditions of this study.

[redacted] 1988a

B.6.2.3 Acute inhalation toxicity (IIA 5.2.3)

This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report.

Study	Asulam (Sodium Salt) - Acute Dust Aerosol Inhalation Toxicity Test in Rats
Reference	[redacted] 1988a
Date performed	8 August - 22 August 1988
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	EPA 81-3
Deviations from the guideline	None
GLP	Yes
Test material	Asulam-sodium Batch XN-36111; 88%purity
Study acceptable	Yes

Sprague-Dawley rats (5/sex) were exposed (whole body) for four hours to an atmosphere containing the test material (ground to dust) and observed for 14 days.

Table B.6.2.3-1 Acute inhalation study: conditions of exposure

Concentration (mg/l)		**MMAD** (µm)	**gsd**
Nominal	**Gravimetric**		
74.5	5.46	6.5* 5.1*	±2.9 ±2.9

*- these are outside the permitted the range as specified in current OECD guideline; however the test article was described as a fine powder and the methodology used for dosing and analysis are acceptable. The exposure conditions in the laboratory will also be difficult to mimic in the actual exposure scenarios for asulam and therefore this study is acceptable for the purposes of toxicology assessment.

No deaths occurred. Observation of the animals during exposure is reported to have been prevented by the dusty atmosphere. 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.

The acute inhalation LC_{50} of the test material in the rat was found to be >5.46 mg/l under the conditions of this study.

[redacted] 1988a

B.6.2.4 Skin irritancy (IIA 5.2.4)

This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report.

Study	FR 1398/1: Acute dermal irritation / corrosion test in the rabbit
Reference	[redacted] 1987a
Date performed	18 November - 21 November 1986
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	OECD 404
Deviations from the guideline	None
GLP	Yes
Test material	Asulam-sodium Batch NPD W002; 88% purity
Study acceptable	Yes

Test material (500 mg moistened with 0.2 ml distilled water) was applied for 4 hours under semi-occlusive conditions to the shorn dorsal skin of three New Zealand White rabbits. Dermal reactions were assessed at up to 72 hours using the Draize scale. Signs of irritation were limited to Grade 1-2 erythema in one rabbit and had resolved by 72 hours following dressing removal.

Table B.6.2.4-1 Individual and Mean scores for dermal irritation

	Erythema						Oedema					
Animal no	1	2	3	4	5	6	1	2	3	4	5	6
1 hr	0	0	0	0	2	0	0	0	0	0	0	0
24 hr	0	0	0	0	2	0	0	0	0	0	0	0
48 hr	0	0	0	0	1	0	0	0	0	0	0	0
72 hr	0	0	0	0	0	0	0	0	0	0	0	0
mean individual score 24-72 hr	0	0	0	0	1.0	0	0	0	0	0	0	0
Mean score 24-72 hr	0.2						0.0					

The test material was found to be a slight skin irritant under the conditions of this study.

[redacted] 1987a

B.6.2.5 Eye irritancy (IIA 5.2.5)

This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report. The tables have been updated to aid classification to

Regulation (EC) 1272/2008 and subsequently classification to this regulation has been completed.

Study	FR 1398/1: Acute eye irritation / corrosion test in the rabbit
Reference	[redacted] 1987b
Date performed	2 February - 10 March 1987
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	OECD 405 (1981)
Deviations from the guideline	None
GLP	Yes
Test material	Asulam- sodium Batch NPD W002; 88% purity
Study acceptable	Yes

Test material (100 mg) was placed into one eye of 12 New Zealand White rabbits. The eyes of six rabbits were washed with tap water at approximately 30 seconds following exposure; the eyes of the other rabbits remained unwashed. Ocular reactions were assessed for up to 15 days following treatment, using the Draize scale.

Signs of irritation in washed eyes were limited to Grade 1-2 conjunctival erythema and chemosis; findings had resolved by 72 hours. Findings were more severe in unwashed eyes including iridial inflammation and findings persisted to Day 8/9.

Table B.6.2.5-1 Mean scores for ocular irritation- non-rinsed group

	Iris						Conjunctiva																	
	Irritation						Redness						Chemosis						Discharge					
Time/Rabbit	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
1 hr	1	0	0	1	0	0	1	2	2	2	2	2	0	1	1	1	1	0	2	2	2	1	2	2
24 hrs	0	1	0	0	0	1	2	2	2	1	1	2	0	1	0	0	0	1	0	1	0	0	0	1
48 hrs	0	0	0	0	0	1	2	2	2	1	1	2	0	0	0	0	0	0	0	0	1	0	0	0
72 hrs	0	0	0	0	0	1	2	1	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
means scores 24-72 hrs	0.22						1.56						0.11						0.17					
Day 8/9	0	0	0	0	0	0	1	0	2	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Day 15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Additional criteria in Directive 93/21/EEC Point 3.2.6.2 fulfilled: Yes
Note corneal opacity and area were measured, and in all animals at all time points a score of 0.0 was returned

Table B.6.2.5-2 Mean scores for ocular irritation- rinsed group

	Iris						Conjunctiva																	
	Irritation						Redness						Chemosis						Discharge					
Time/Rabbit	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
1 hr	0	0	0	0	0	0	2	2	2	2	2	2	1	1	2	1	1	1	2	2	2	2	2	2
24 hrs	0	0	0	0	0	0	1	1	2	1	2	1	0	1	0	0	0	0	0	0	0	0	0	0
48 hrs	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
72 hrs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
means scores 24-72 hrs	0.0						0.78						0.06						0.0					

Additional criteria in Directive 93/21/EEC Point 3.2.6.2 fulfilled: Yes
Note corneal opacity and area were measured, and in all animals at all time points a score of 0.0 was returned

The test material was found to be a moderate eye irritant (in unwashed eyes) under the conditions of this study. For the purposes of classification, the guidance on CLP has been consulted and in studies where 6 animals have been used, the criteria for Category 2 eye irritants (Table 3.3.3 Regulation (EC) 1272/2008) must be observed in 4 of 6 animals. Only two animals have conjunctival redness above the threshold of 2 over 24-72 hours which is not sufficient to trigger classification.

[redacted] 1987b

B.6.2.6 Skin sensitisation (IIA 5.2.6)

This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report. The tables have been updated to aid classification to Regulation (EC) 1272/2008 and subsequently classification to this regulation has been completed.

Study	FR 1398/1: Delayed contact hypersensitivity study in Guinea-pigs
Reference	[redacted] 1987c
Date performed	27 November 1987 - 10 January 1988
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	OECD 406 (1981)
Deviations from the guideline	No positive control group or reference to positive control study
GLP	Yes
Test material	Asulam-sodium Batch NPD W002; 88% purity
Study acceptable	Yes: the positive control information is considered unnecessary given the clear positive response in this study

The potential of the test material to induce delayed contact hypersensitivity was investigated in a Maximisation study using Dunkin-Hartley guinea pigs. Concentrations used for induction and challenge exposures were based on the results of a preliminary study. A positive control group was not included in the study design and no reference is made to a separate positive control study.

Intradermal induction was performed on test animals (10/sex) using paired injections of test material (5% in distilled water), FCA or FCA/test material. Control animals (10/sex) were similarly treated during this phase, with vehicle in place of the test material. Six days following intradermal induction, irritation was induced at the application site on all animals by topical application of 10% sodium lauryl sulphate in petrolatum. The following day, topical induction was performed using a 48-hour occlusive application of 0.6 ml test material (50% in distilled water); control animals were similarly treated during this phase, with vehicle alone. Two weeks following topical induction, all animals were challenged using a 24-hour occlusive application of 0.03 ml test material (10% and 50% in distilled water). Dermal reactions were assessed at 24 and 48 hours following dressing removal, using the Draize scale.

Dermal reactions (slight erythema) were seen in test and control animals following intradermal induction; no reactions were seen following topical induction. Dermal reactions following challenge were seen in test animals only and are detailed below.

Table B.6.2.6-1 Skin sensitisation study: summary of reactions following challenge

		Males										Females									
Phase/animal		1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control Group																					
10%	24h	0[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	48h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50%	24h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	48h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Test Group																					
10%	24h	0	0	0	0	±	0	0	0	0	±	0	±	0	0	±	1	0	0	0	0
	48h	0	0	0	0	±	0	0	0	0	±	0	0	0	0	±	±	0	0	0	0
50%	24h	0	0	2	±	±	±	0	1	2	1	1	±	0	1	1	2	2	1	2	1
	48h	0	0	2	±	±	±	0	1	2	1	±	1	0	±	±	1	2	±	2	1

[a] 0 = no response; ± = barely perceptible erythema; 1 = slight erythema; 2 = moderate erythema

Clear evidence of skin sensitisation was seen under the conditions of this study; 12/20 at 24 hours and 9/20 at 48 hours post challenge with 50% test substance. The lack of a positive control group or reference to a separate positive control study (reliability check) does not raise any concerns given the clear positive result in this study.

The test material should be classified as "Category 1", with the signal word "Warning" and the hazard statement "H317: May cause an allergic skin reaction" according to Regulation (EC) 1272/2008. Sub-categorisation as skin senistisation 1A or 1B is not possible due to insufficient data at lower intradermal induction concentrations.

B.6.2.7 Summary of acute toxicity, irritancy and sensitisation studies

Asulam was found to be of low toxicity in the rat by the oral, dermal and inhalation routes and was also of low acute dermal toxicity in the rabbit. Signs of toxicity in these studies were transient and non-specific. Slight skin and moderate eye irritation was seen in studies in the rabbit; the reactions are not of sufficient severity to warrant classification. Clear evidence of sensitisation was seen in a Maximisation test. Asulam is therefore classified as "Category 1", with the signal word "Warning" and the hazard statement "H317: May cause an allergic skin reaction" according to Regulation (EC) 1272/2008.

Table B.6.2.7-1 Summary of acute toxicity studies

Test	Species	Result	Classification	Reference
Acute oral	Rat	LD_{50} >5000 mg/kg bw	-	[redacted] 1987a
	Rabbit	LD_{50} >5000 mg/kg bw	-	[redacted] 1988
Acute dermal	Rat	LD_{50} >2000 mg/kg bw	-	[redacted] 1987b
	Rabbit	LD_{50} >4000 mg/kg bw	-	[redacted] 1988
Acute inhalation	Rat	LC_{50} >5.46 mg/l (whole body)	-	[redacted] 1988
Skin irritation	Rabbit	Slight irritant	-	[redacted] 1987a
Eye irritation	Rabbit	Moderate irritant	-	[redacted] 1987b
Skin sensitisation	Guinea pig	Sensitiser (Maximisation test)	H317	[redacted] 1987c

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

A series of studies via the oral route have been conducted in the rat, mouse and dog with Asulam. In the rodent species, there are only 90 day exposures to the test substance whilst in the dog, 6 and 12 month studies are available. All dietary studies have been evaluated previously and the assessments are consistent with those in the EFSA conclusions from 2010.

In addition, a 21 dermal toxicity study in the rabbit has been evaluated. This study was not assessed previously despite being available but has been presented in this submission.

B.6.3.1 Oral short-term toxicity in the rat (IIA 5.3.1, 5.3.2)

This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report. The RMS has included more detail in the conclusion but the NOAEL has not been altered.

Study	90-Day Toxicity Study in the Rat by Dietary Administration
Reference	[redacted] 2000
Date performed	25th August – 25 November 1999
Test facility	[redacted]

Report reference	[redacted]
Guideline(s)	92/69/EEC B.26, OECD 408 (1981), (EPA) OPPTS 870.3100 (1998), JMAF 59 NohSan no.4200 (1985)
Deviations from the guideline	None
GLP	Yes
Test material	Asulam sodium, batch PN-24049; 896g/kg purity
Study acceptable	Yes

Wistar rats (10/sex/group) were administered test material in the diet at concentrations of 0, 2000, 6000 or 20000 ppm for at least 90 days (equivalent to 128.5, 387.0, and 1327.3mg/kg/ bw/d for males and 157.9, 479.4 and 1651.5mg/kg bw/d in females). Animals were observed daily for clinical signs; more detailed physical examinations were conducted pre-test and weekly throughout the study. Sensory reactivity (grasping, righting, corneal, papillary, auditory startle and head shaking reflexes) was assessed pre-test in all animals and during Week 12 in control and high dose group animals. Bodyweights and food intake were measured weekly. Ophthalmoscopy was performed pre-test (all animals) and during Week 12 (control and high dose group animals).

A week prior to necropsy, blood samples were collected from all animals for haematological and clinical chemistry investigations. Urine samples were collected overnight prior to necropsy. All animals were subjected to gross necropsy; weights of the adrenals, brain, epididymis, heart, kidneys, liver, ovaries, pituitary gland, prostate, spleen, testes, thymus, thyroid and uterus were recorded. Histopathology was performed on a comprehensive selection of tissues taken from all animals in the control and high dose groups and from decedents. Investigations in intermediate groups were limited to the liver, lungs, thyroids and kidneys.

Results

Dietary analyses revealed the achieved concentration, stability and homogeneity to be within acceptable limits.

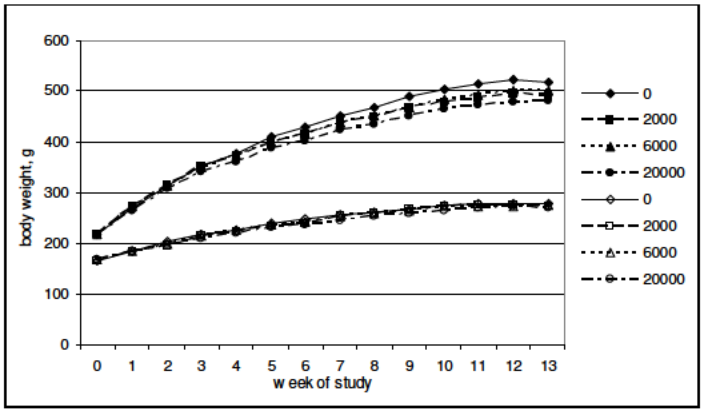
Mortality and clinical signs

One 6000 ppm female died during blood sampling on Day 87. No treatment-related deaths occurred and no signs of toxicity were observed during the study period. No treatment-related findings were noted during the neurotoxicity assessment or ophthalmoscopy.

Bodyweights and food consumption

Mean bodyweights of top dose males were significantly lower than controls from Week 5-13; overall weight gain by this group was also significantly reduced. Food consumption by top dose males was significantly reduced during the first week of the study. No effects on bodyweight or food consumption were noted in males at lower doses, or in females.

Figure B.6.3.1-1 Rat 90-day study: mean bodyweights



Haematology and clinical chemistry

Haematology revealed statistically significant effects on red blood cell parameters consistent with mild macrocytic anaemia in both sexes at the top dose level; findings were more marked in males. Clinical chemistry revealed apparently treatment-related reductions in the levels of plasma proteins in males at ≥6000 ppm and in females at ≥2000 ppm. Plasma phosphate concentration was significantly elevated in top dose females only. Increases in serum AST and ALT activities were noted in males at 6000 ppm; this effect was not statistically significant, and resulted from a single outlier (which also had increased total bilirubin concentration and alkaline phosphatase activity). Urinalysis did not reveal any treatment-related findings.

Organ weights and pathology

Mean absolute and relative spleen weights were slightly (but significantly) higher in top dose males; findings are consistent with the haematological findings in this group. Mean thyroid weights were slightly (but significantly) higher in top dose females. Prostate weight was slightly lower in top dose males, however values do not attain statistical significance. Mean relative testes weight was significantly higher in top dose males; this value is largely (but not entirely) attributable to one male with an unusually high absolute testes weight (5.65 g).

Gross necropsy did not reveal any treatment-related findings; one observation of 'large testes' was made in top dose males, corresponding to the individual with high absolute testes weight. Microscopically, moderate unilateral seminiferous tubular dilatation was observed in this animal. The incidence and severity of thyroid follicular hypertrophy was increased in high dose animals of both sexes; the affected follicles were also reported to be generally smaller. The severity of splenic extramedullary haematopoiesis was increased in males at ≥6000ppm; there was also an increase in the severity of haemosiderin deposition in the spleen in both sexes at the top dose level and in 6000 ppm males. An increase in the incidence and severity of mineral deposits located free in the renal pelvis or papilla was seen in both sexes at the top dose level and in 6000 ppm females. A slight or minimal hyperplasia of the adjacent transitional epithelium occurred occasionally as a result of the mineral deposition, causing a greater incidence of focal urothelial hyperplasia in both sexes at the top dose level and

in 6000 ppm males. In addition to these changes, an increase in the severity and incidence of hydronephrosis was observed in females at ≥6000 ppm.

Table B.6.3.1-2 90-day rat study: summary of findings

Parameter		Dose level							
		Males				Females			
		0	2000	6000	20000	0	2000	6000	20000
Intake (mg/kg bw/d)		-	129	387	1327	-	158	479	1652
Food (g/d)		26.5	26.3	26.1	26.0	19.1	18.8	19.2	19.3
Wt Gain (g)		301.3‡	276.6	283.7	263.8**	114.2	109.3	109.1	101.3
Body weight (g)		493.8‡	467.3	476.8	458.5**	260.4	255.2	255.2	249.6
Haematology									
RBC (10^{12}/L)		9.55‡‡‡	9.40	9.25	8.68***	9.01‡‡	8.90	8.86	8.27**
HGB (g/dl)		16.20 ‡‡	15.80	15.66	15.02**	15.72‡‡	15.68	15.71	14.49**
HCT (l/l)		0.485‡	0.473	0.469	0.458**	0.460‡‡	0.462	0.464	0.428*
MCV (fl)		50.7‡‡	50.3	50.7	53.0**	51.2	52.1	52.4	51.4
MCH (pg)		17.00	16.83	16.94	17.32	17.44	17.62	17.73	17.54
MCHC (g/dl)		33.44‡‡	33.47	33.37	32.78**	34.15	33.89	33.86	33.83
Clinical chemistry									
PTT (s)		13.48‡‡‡	13.79	13.26	15.77**	12.60	12.55	13.10	13.00
Protein (g/l)		70.1‡‡	67.9	66.2	64.0**	66.7	64.9	65.1	61.4
Albumin (g/l)		35.8	35.6	36	35.7	36.1	37.0	37.4	36.1
Globulin (g/l)		34.3‡‡‡	32.3	30.2**	28.3***	30.6‡‡	27.9	27.7	25.3**
A:G Ratio		1.05‡‡‡	1.11	1.19***	1.27***	1.19‡‡‡	1.33*	1.37**	1.43***
P (mM)		1.856‡	1.820	1.703	1.664*	1.624	1.642	1.431	1.495
Organ weights									
Spleen	(g)	1.057‡	1.025	1.172	1.208*	0.772	0.771	0.803	0.788
	(%)	0.215‡‡	0.219	0.246	0.264	0.297	0.303	0.316	0.315
	rel	50.81‡‡	49.49	57.13	59.01*	39.92	39.94	40.75	41.18
Thyroid	(g)	0.024	0.021	0.021	0.023	0.017‡	0.017	0.017	0.020*
	(%)	0.0048	0.0044	0.0045	0.0049	0.0066‡	0.0066	0.0068	0.0081*
	rel	1.129	1.006	1.039	1.097	0.877‡	0.865	0.880	1.056*
Prostate	(g)	0.753	0.749	0.698	0.657				
	(%)	0.152	0.159	0.147	0.144				
	rel	36.24	36.06	34.25	32.07				
Testes	(g)	3.748	3.760	3.776	4.049				
	(%)	0.760‡‡	0.805	0.792	0.882**				
	rel	180.2	181.3	184.1	197.5				
Testes[a]	(g)	3.748	3.760	3.776	3.871				
	(%)	0.760‡‡	0.805	0.792	0.851				
	rel	180.2	181.3	184.1	190.1				
Histopathology									
Thyroid hypertrophy		2 (2,0,0)	1 (1,0,0)	2 (2,0,0)	10 (10,0,0)	1 (1,0,0)	-	-	8 (2,6,0)
Extramedullary haematopoiesis		10 (6,4,0)	10 (5,4,1)	10 (1,6,3)	10 (0,5,5)	10 (0,4,6)	10 (1,4,5)	9 (0,3,6)	10 (2,3,5)
Haemosiderin deposition		10 (4,5,1)	10 (4,5,1)	10 (1,9,0)	10 (0,0,10)	10 (0,7,3)	10 (0,7,3)	9 (0,6,3)	10 (0,3,7)
Kidney mineralisation		1 (1,0,0)	1 (1,0,0)	2 (2,0,0)	7 (4,2,1)	5 (4,1,0)	6 (4,2,0)	7 (1,3,3)	9 (4,3,2)
Kidney hyperplasia		2 (2,0,0)	1 (1,0,0)	5 (4,1,0)	6 (5,1,0)	5 (5,0,0)	4 (2,2,0)	5 (4,1,0)	8 (5,3,0)
Hydronephrosis		5 (1,2,2)	4 (3,1,0)	5 (2,2,1)	2 (1,1,0)	2 (1,1,0)	2 (1,1,0)	6 (3,2,1)	7 (1,4,2)

‡*significantly different to controls ($p<0.05$);* ‡‡*$p<0.01$;* ‡‡‡*$p<0.001$ [ANOVA test for overall effect]*

**significantly different to controls (p<0.05); **p<0.01; ***p<0.001 [Dunnett's test]*
rel: organ weight relative to brain weight
[a]*excluding outlier*

Conclusion

At the highest dose tested (20000ppm), there were statistically significant changes in body weight, body weight gain, haematology, clinical chemistry and histopathology of the kidney and thyroid in both sexes of the rat. Organ weight changes were confined to males at the top dose and were reported in the spleen and testes only. The clinical chemistry and increased incidence of histopathology changes in the kidney were also reported in both sexes at 6000ppm and this dose level is determined to be the LOAEL.

Whilst there was a statistical difference in the albumin/globulin ratio in females treated with 2000ppm, this is not considered to be of significance in the absence of any significant effect on albumin or globulin concentration and the effect not being replicated in males at this dose level. Therefore, a NOAEL of 2000 ppm (equivalent to 129 and 158 mg/kg bw/d) can be determined from this study, based on clinical chemistry in males and histopathology observations in both sexes at 6000 ppm.

[redacted] 2000

B.6.3.2 Oral short-term toxicity in the mouse

This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report.

Study	Eight-Week Range-Finding Dietary Toxicity Study in Mice
Reference	[redacted] 1989a
Date performed	29 August – 25 October 1988
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	-
Deviations from the guideline	No histopathology
GLP	Yes
Test material	Asulam sodium; batch XN-36111, 88% purity
Study acceptable	Yes (range-finding study)

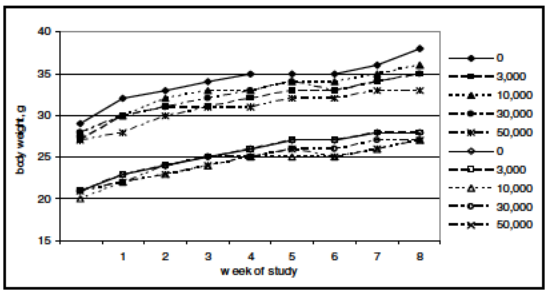
CD-1 mice (10/sex/group) were administered the test material in the diet at concentrations of 0, 3000, 10000, 30000 or 50000 ppm for 8 weeks. Animals were observed daily for clinical signs; bodyweights and food consumption were measured weekly. Terminal blood samples were taken for the assessment of plasma and erythrocyte cholinesterase activity. Gross necropsy was performed on all animals; weights of the brain, heart, kidneys, liver, testes, ovaries and adrenals were recorded. No histopathology was performed in this study. Brain cholinesterase activity was measured at termination.

Results

Dietary analyses revealed the achieved concentration, stability and homogeneity of the test material to be within acceptable limits.

No deaths occurred and no signs of toxicity were observed in any group. Mean bodyweights of all treated groups of males were lower than controls throughout the study period. A consistent, clearly dose-related and statistically significant effect is only apparent at the top dose level, however the terminal bodyweight of 30000 ppm males is also significantly lower than controls. Bodyweights were broadly comparable in all groups of females, however transient weight loss was observed at the top dose level.

Figure B.6.3.2-1 8-week mouse study: mean bodyweights



Absolute food consumption was unaffected by treatment; food consumption relative to bodyweight was significantly higher in treated groups of males, most markedly and consistently at the top dose level. Findings may be attributable to the high level of the test material in the food (5%). Plasma, erythrocyte and cholinesterase activities were comparable in all groups. Gross necropsy did not reveal any treatment-related findings. Organ weights were unaffected by treatment.

Table B.6.3.2-1 8-week mouse study: summary of findings

Parameter/ Week		Dose level (ppm)									
		Males					Females				
		0	3000	10000	30000	50000	0	3000	10000	30000	50000
Intake (mg/kg bw/d)		-	512	1673	5103	9022	-	675	2263	6835	10828
Body weight (g)	0	29	27*	28	28	27*	21	21	20	21	21
	1	32	30	30	30	28**	23	23	22	22	22
	2	33	31	32	31	30**	24	24	23	24	23
	3	34	31	33	32	31*	25	25	24**	25	24
	4	35	32*	33	33	31**	26	26	25	25	25
	5	35	33	34	34	32	27	27	25**	26	26*
	6	35	33	34	33	32	27	27	25	26	25
	7	36	34	35	34	33*	28	28	26	27	26
	8	38	35*	36	35*	33**	28	28	27	27	27
Gain (g)		9	8	8	7	6	7	7	7	6	6

**significantly different from controls (p<0.05); **p<0.0l [Dunnett's test]*

Conclusion

NOAELs of 10000 ppm (equivalent to 1673 mg/kg bw/d) and 30000 ppm (equivalent to 6835 mg/kg bw/d) can be determined for males and females respectively in this

study, based on minor bodyweight effects at higher dose levels. Whilst it is appreciated the objective of the study was not to derive NOAELs, it can be agreed that 1000mg/kg bw/d is well tolerated in the mouse and justifies the use of this concentration in long term toxicity studies with the mouse.

[redacted] 1989a

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

A.

This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report. The RMS has included more detail in the conclusion but the NOAEL has not been altered.

Study	Asulam: Six Month Oral Toxicity Study in Beagles
Reference	[redacted] 1980
Date performed	August 1978 - February 1979
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	-
Deviations from the guideline	-
GLP	No: pre-GLP
Test material	Asulam, batches JN 120 and JN 165 (99% purity after drying)
Study acceptable	Yes

Beagle dogs (6/sex/group) were gavaged with the test material (suspended in 0.2% aqueous tragacanth) at dose levels of 0, 60, 300 or 1500 mg/kg bw/d for 26 weeks. Animals were observed for clinical signs daily during the working week; at weekends and on bank holidays for faecal consistency and vomiting only. Food consumption was measured daily (excluding weekends/bank holidays); bodyweights were recorded twice weekly. Water consumption was measured over two day periods pre-test and during the first, third and sixth months of treatment. Ophthalmoscopy was performed pre-test and prior to necropsy. Blood samples were taken pre-test and during Weeks 4, 8, 12, 17, 21 and 26 for the measurement of haematological and clinical chemistry parameters; additional samples were taken pre-test and during Weeks 9, 17/18 and 25/26 for the determination of whole blood and plasma cholinesterase activity. Urine samples (obtained by housing animals individually in metabolism cages overnight) were taken pre-test and during Weeks 7, 16 and 25. Liver function (bromosulfophthalein retention) was assessed pre-test and during Weeks 4, 8, 12, 17, 22 and 26. Bone marrow samples were obtained under general anaesthesia pre-test and at termination and smears examined qualitatively. All animals were subjected to gross necropsy; weights of the adrenals, heart, liver, pituitary, brain, kidneys, lungs, prostate, thyroids, testes and ovaries were recorded. A comprehensive list of tissues from all animals was examined microscopically; brain samples were taken for the assessment of cholinesterase activity.

Results

Mortality and clinical signs

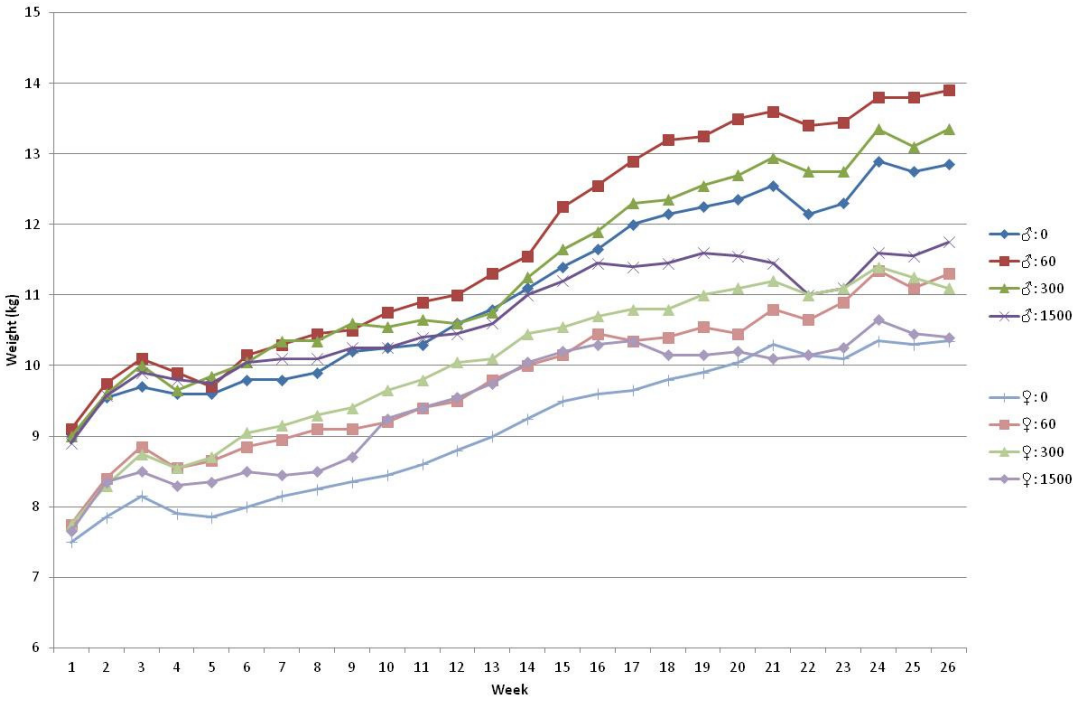
One 60 mg/kg bw/d male died during Week 14 as a result of injuries caused by fighting. One top dose male died during Week 19; this animal had exhibited laboured

respiration approximately 4 hours after dosing and was found dead the following morning. One top dose female also died during Week 9; this animal appeared lethargic at the end of Week 8, had lost weight and was in poor condition. Loose and sometimes liquid faeces were observed in control and treated groups; the incidence of findings was similar in all groups. A single top dose male was also observed to pass faeces containing blood during Week 22. Vomiting immediately following dosing was observed with increased incidence in both sexes at the top dose level and in 300 mg/kg bw/d females. Ophthalmoscopy did not reveal any treatment-related findings.

Bodyweights, food and water consumption

Terminal bodyweights and overall weight gains were lower in both sexes at the top dose level; findings were more marked in males. A reduction in mean weekly food consumption was noted at the top dose level from Week 4 in males and Week 5 in females, resulting in a decrease in total food intake in these groups. Food consumption by 300 mg/kg bw/d females was also slightly lower from Week 20. Water consumption was increased in females at the top dose level.

Figure B.6.3.3-1 6-month dog study: bodyweights



Haematology and clinical chemistry

Red blood cell parameters (erythrocyte count, haemoglobin concentration and haematocrit) were depressed in top dose males at Week 26; however similar findings were not apparent at earlier time points. Mean cell volume was significantly higher in top dose females at the majority of time points; similar findings were not seen in males. The total white blood cell count was significantly lower in treated groups at the majority of time points, however values are variable; a dose-response relationship is not always apparent and no treatment-related effect was seen at termination.

Differential counts were unaffected by treatment and histopathological examination of terminal bone marrow smears did not reveal any effect of treatment.

In males, a dose-related increase in glucose concentration was apparent from Week 8; values attained statistical significance from Week 8-26 at the top dose level, from Week 12-26 at 300 mg/kg bw/d and at Week 26 only at 60 mg/kg bw/d. Less consistent increases were observed in females, although values at 1500 mg/kg bw/d were higher than those in control animals at all time points. A tendency to increased cholesterol levels was seen in treated groups of females from Week 4, although statistical significance was attained only at 1500 mg/kg bw/d at Week 17. Reduced serum LDH activity was seen in top dose females at a number of time points, however this finding is not considered to be of toxicological significance. A significantly increased chloride concentration in treated groups is not considered to be of toxicological significance in the absence of similar findings at any other time point. Whole blood, plasma and brain cholinesterase activities were unaffected by treatment.

Urinalysis revealed increased volume in both sexes at the top dose level; values in intermediate dose groups are also higher than controls, however there is no consistent relationship to dose. Specific gravity was also reduced (and is stated to be below the lower limit of normality) in individual animals at the top dose level at some time points.

Organ weights and pathology

Absolute and relative lung weights were lower in top dose males; absolute weights attained statistical significance. Relative kidney weight was significantly higher in top dose males. Absolute and relative thyroid weights were increased in both sexes at the top dose level; absolute thyroid weight was also significantly increased in females at 300 mg/kg bw/d. Mean absolute testes weight was significantly lower in top dose males. Necropsy did not reveal any gross or microscopic findings attributable to treatment.

Table B.6.3.3-2 6-month dog study: summary of findings

Parameter	Time point	Dose level (mg/kg bw/d)							
		Males				Females			
		0	60	300	1500	0	60	300	1500
Terminal weight (kg)		13.0	14.2	13.5	11.6	10.6	11.4	11.0	10.3
Weight gain (kg)		4.0	4.6	4.2	3.1	2.8	3.7	3.2	2.5
Vomiting incidences		-	1	-	11	-	-	4	18
Food intake	W 1-12	295	299	296	270	251	277	275	240
	W 1-26	342	350	337	286	281	298	287	266
Urine volume (ml)	W 7	196	244	217	225	185	232	235	348
	W16	191	258	202	238	214	257	245	310
	W 25	86	233	230	304	145	213	204	277
Haematology									
RBC (10^{12}/l)	W 26	6.89	6.80	6.91	6.18*	6.52	7.04	6.75	6.55
Hb (g/dl)	W 26	15.7	15.4	15.2	14.2*	14.7	16.0*	15.6	15.6
PCV (%)	W 26	46.5	46.7	45.8	42.1*	43.9	47.9	46.5	46.3
MCV (fl)	Pre-test	72.3	72.1	71.3	72.0	71.1	72.4	72.3	72.5
	W 4	68.9	69.5	66.8	67.2	67.7	68.7	67.9	69.7

	W 8	72.5	70.0*	70.0*	73.0	70.9	70.8	71.5	75.1*
	W 12	73.9	72.4	71.7	75.5	72.4	72.4	73.5	77.0*
	W 17	69.4	68.9	68.3	72.6*	68.9	69.9	69.7	72.9*
	W 21	70.8	69.1	67.6*	71.9	67.7	70.2	68.9	72.3*
	W 26	68.2	69.2	67.2	68.8	67.7	68.7	69.6	71.5**
WBC (10^9/l)	Pre-test	16.5	17.7	16.6	15.2	14.8	16.3	16.1	17.2*
	W 4	14.1	14.1	13.3	13.0	16.1	14.8	14.0	12.1**
	W 8	16.9	14.0*	14.5	13.4*	18.5	14.6*	12.7*	10.4**
	W 12	13.5	14.0	14.4	13.1	16.3	12.3**	13.0*	10.6*
	W 17	17.2	14.3*	14.1*	13.6*	15.0	13.9	13.0	11.1
	W 21	19.3	15.9	13.6*	10.9**	13.5	13.8	13.3	9.9*
	W 26	15.1	16.3	13.5	13.8	15.3	15.8	16.1	12.6
Clinical chemistry									
Glucose (mM)	Pre-test	6.4	6.6	6.3	6.6	6.6	6.6	6.9	6.6
	W 4	7.0	7.0	7.1	7.3	6.4	6.5	6.8	6.6
	W 8	7.0	7.3	7.3	7.7*	6.0	6.3	6.9**	7.1**
	W 12	6.3	6.9	7.4**	7.8**	7.0	6.9	7.2	7.5
	W 17	6.5	6.9	7.2*	7.0*	6.5	6.6	6.5	7.1*
	W 21	6.6	7.7	8.0*	8.4**	7.5	7.1	7.5	7.1
	W 26	6.5	7.7**	7.9**	8.0**	6.7	7.2	7.1	7.8**
Cholesterol (mM)	Pre-test	3.07	2.63	2.90	2.95	2.93	2.63	2.68	2.99
	W 4	4.34	3.86	3.86	3.97	4.06	3.93	3.78	4.24
	W 8	3.55	3.28	3.20	3.79	3.13	3.47	3.24	3.61
	W 12	3.19	2.54	3.06	3.27	3.31	3.04	3.13	3.71
	W 17	3.44	2.82*	3.14	3.44	3.48	3.30	3.21	4.34*
	W 21	4.07	4.08	4.35	4.94	4.25	4.07	4.36	5.30
	W 26	4.13	4.11	4.15	3.96	4.28	4.18	4.21	5.17
Cl- (mM)	W 26	118	122	124**	128**	122	126	124	128**
Organ weights									
Testes	(g)	19.7	19.6	20.4	14.8*				
	(%)	0.153	0.139	0.15	0.129				
Lungs	(g)	127.3	124.8	113.8	98.6*	99.5	99.7	103.7	93.4
	(%)	0.985	0.872	0.851	0.856	0.960	0.886	0.945	0.912
Kidney	(g)	59.5	72.6	59.2	62.6	50.0	50.2	49.7	50.6
	(%)	0.45	0.509	0.439	0.543**	0.468	0.452	0.448	0.497
Thyroid	(g)	0.87	0.88	0.91	2.00**	0.72	0.73	1.12	1.79**
	(%)	0.66	0.60	0.68	1.75**	0.69	0.65	1.06	1.78*

**significantly different to control (p<0.05); **p<0.01; ***p<0.001*

Conclusions

At the top dose, vomiting, lower terminal body weight and body weight gain, reduced food consumption, increased urine volume and increased thyroid weights were reported in both sexes. Sex specific changes at 1500mg/kg bw/d included increased water consumption, increased cholesterol and increased mean cell volume in females whilst males exhibited a depression in red blood cell parameters, an increase in glucose concentration, decreased liver and lung weight and an increase in kidney weight. The elevated plasma glucose concentrations seen in this study with male rats are of unclear toxicological significance in the absence of similar findings in the 12-month dog study ([redacted] 2004a).

At the mid-dose, there were effects reported on white blood counts but similar effects on white blood cell counts were not seen in the 12-month dog study and the effects in the study above were largely reversible by termination (the study report states that treatment with asulam resulted in a reversible leucopoenia). A high incidence of

roundworm infestation was noted, particularly in the control and 60 mg/kg bw/d groups; this may also have influenced the white blood cell count.

A NOAEL of 60 mg/kg bw/d can be determined for this study, based on clinical signs (females), consistent effects on white blood cell count (both sexes), consistent effects on plasma glucose concentration (males) and increased thyroid weight (females) at 300 mg/kg bw/d.

[redacted] 1980

B.

This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report. The RMS has included more detail in the conclusion but the NOAEL has not been altered.

Study	52-week toxicity study by oral route (gavage) in beagle dogs (Asulam)
Reference	[redacted] 2004a
Date performed	2 May 2001 – 26 July 2002
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	OECD 409 (1981), EPA OPPTS 870.4100 (1998), 96/54/EEC B.32, JMAFF 59 NohSan 4200 (1985)
Deviations from the guideline	None
GLP	Yes
Test material	Asulam, batch OP210109; 82.2% purity
Study acceptable	Yes

Beagle dogs (5/sex/group/dose) were administered daily doses of the test material (suspended in aqueous methylcellulose) by gavage at dose levels of 0, 100, 300 or 600 mg/kg bw/d for 52 weeks. Animals were observed at least daily for clinical signs; more detailed physical examinations were performed pre-test and weekly throughout the study period. Bodyweights and food consumption were recorded weekly throughout the study. Ophthalmoscopy was performed pre-test and during Weeks 12, 25 and 52. Blood samples were taken pre-test and during Weeks 12/13, 25 and 51 for the measurement of haematological and clinical chemistry parameters; urinalysis was also performed at these time points. Gross necropsy was performed on all animals; weights of the adrenals, brain, epididymides, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes, thymus, thyroids and uterus were recorded. A comprehensive list of tissues from all animals was investigated microscopically.

Results

Mortality and clinical signs

One 300 mg/kg bw/d female died on Day 47 following an accident (no further details are reported and this animal was replaced); the replacement animal was dosed for 52 weeks. No treatment-related deaths occurred during the study period. Signs of toxicity (salivation and vomiting) were seen in treated animals; the number of affected animals and the frequency and severity of these findings increase with the dose level. Haematemesis was additionally noted in top dose males on a small number of

occasions. The study report states that these clinical signs are treatment-related but are not considered to be 'indicative of toxicity of the test material'. Although salivation and emesis are known to be caused by gavage dosing, the clear dose-related increase in incidence, severity and frequency indicate an effect of the test material. One 300 mg/kg bw/d female exhibited emaciation from Day 167 to the end of the study; this finding is not considered to be treatment-related as similar findings were not present at 600 mg/kg bw/d. Ophthalmoscopy did not reveal any treatment-related findings.

Bodyweights and food consumption

Weight loss and reduced weight gain was observed in the 300 mg/kg bw/d female exhibiting emaciation; the terminal bodyweight in this animal was 6.8 kg. This animal received extra food; the reported figures mean food consumption and bodyweight therefore exclude this individual. Mean bodyweights, weight gains and food consumption were unaffected by treatment.

Haematology and clinical chemistry

Haematological parameters were unaffected by treatment. Clinical chemistry revealed elevated ALP activities in treated groups of females, however the magnitude of change was not great and there was no apparent relationship to treatment. AST activity was slightly elevated in top dose females; values attained statistical significance at some time points. ALT activity was also consistently slightly higher in top dose females, however values did not attain statistical significance. Mean CK activity was elevated in top dose females at Week 25; Week 52 values were elevated at ≥300 mg/kg bw/d, however a dose-response relationship is not apparent for this finding and values do not attain statistical significance. Clinical chemistry findings are therefore considered to represent a marginal effect in top dose females. Urinalysis did not reveal any treatment-related findings.

Organ weights and pathology

Mean absolute adrenal weights were slightly higher at the top dose level, significantly in females. Absolute and relative heart weights were slightly lower in both sexes at the top dose level, however values do not attain statistical significance. Spleen, prostate and testes weights were slightly (but not significantly) lower in top dose males. Mean absolute and relative thyroid weights are higher in all treated groups in a dose-related fashion; values attained statistical significance in males at ≥300 mg/kg bw/d and in females at the top dose level.

Gross necropsy revealed increased incidences of 'small' spleen in males and enlarged thyroid in both sexes at the top dose level, corresponding to the organ weight findings in these groups. Microscopically, a marginally increased incidence and/or severity of splenic haemosiderosis/sclerosiderosis was seen in males at ≥300 mg/kg bw/d. Findings are not considered to be of clear toxicological significance in the absence of any treatment-related effects on red blood cell parameters. Thyroid follicular cell hypertrophy was seen in both sexes at ≥300 mg/kg bw/d; the incidence and severity of this finding was increased at the top dose level.

Table B.6.3.3-3 52-week dog study: summary of findings

Parameters		Dose level (mg/kg bw/d)							
		Males				Females			
		0	100	300	600	0	100	300	600
Clinical signs									
Salivation	Incidence	-	2 (6)	5 (286)	5 (466)	-	-	5 (87)	5 (728)
	Severity	-	Slight	Marked	Marked	-	-	Marked	Marked
	Duration	-	5-8h	8-24h	8-24h	-	-	8-24h	8-24h
Vomiting	Incidence	-	-	2 (2)	5 (20)	-	-	2 (3)	0.5h
	Duration	-	-	0.5h	0.5h	-	-	5 (68)	0.5h
Body weights									
Weight (kg)	Week 53	11.6	11.1	11.7	11.7	9.8	9.6	9.3	10.4
Weight gain (kg)	Week 1-53	2.3	1.7	2.2	2.3	2.1	1.9	1.4	2.5
Clinical chemistry									
ALP (IU/l)	Pre-test	204	199	235	202	181	229	211	217
	Week 13	114	113	140	112	108	119	146	148
	Week 25	105	19	101	102	98	96	139	140
	Week 51	81	84	87	92	95	106	130	145
AST (IU/l)	Pre-test	22	19	21	21	17	19	20	21
	Week 13	19	19	19	22	18	18	19	22*
	Week 25	22	22	23	27	20	18	24	26
	Week 51	21	24	22	25	19	18	19	27*
ALAT (IU/l)	Pre-test	20	19	20	21	19	18	21	24
	Week 13	23	29	24	25	18	20	35	37
	Week 25	22	28	25	26	25	12	26	29
	Week 51	26	26	21	20	20	13	23	28
CK (IU/l)	Pre-test	82	92	101	104	98	91	71	97
	Week 13	73	95	69	70	117	91	85	83
	Week 25	53	69**	63*	63	73	76	73	91
	Week 51	68	84	64	67	61	77	114	101
Organ Weights									
Adrenals	(g)	1.34	1.37	1.30	1.44	1.28	1.38	1.26	1.62*
	(% x 10^2)	1.15	1.24	1.14	1.23	1.32	1.48	1.39	1.61
Heart	(g)	93.25	89.48	93.32	86.94	69.35	71.29	70.65	66.76
	(% x10)	8.15	8.17	8.12	7.54	7.23	7.64	7.87	6.68
Prostate	(g)	10.97	8.51	10.52	8.56				
	(% x10^2)	9.31	7.75	9.28	7.34				
Spleen	(g)	33.88	31.72	34.01	28.53	26.32	26.58	26.65	28.18
	(% x10)	2.95	2.88	2.99	2.45	2.74	2.82	2.88	2.82
Testes	(g)	13.60	13.68	13.75	11.80				
	(% x10)	1.19	1.24	1.20	1.02				
Thyroid	(g)	0.730	0.815	1.08#	1.22##	0.693	0.761	0.919	1.19**
	(% x10^3)	6.29	7.39	9.42*	10.34**	7.11	7.97	10.19	12.07*
Pathology									
Spleen reduced		-	-	-	2	-	-	1	-
Thyroids enlarged		-	-	-	2	-	-	-	1
Spleen haemosiderosis	Total	5	4	4	5	5	5	5	5
	Severity	2,2,1	-,3,1,-	1,-,3,-	-,1,4,-	1,2,2,-	-,2,3,-	2,1,1,1	-,2,3,-
Spleen sclerosiderosis	Total	-	1	2	3	1	-	-	2
	Severity	-	1,-,-,-	1,1,-,-	1,-,2,-	1,-,-,-	-	-	1,1,-,-
Thyroid hypertrophy	Total	-	-	2	5	-	-	3	5
	Severity	-	-	2,-,-,-	2,3,-,-	-	-	3,-,-,-	4,1,-,-
	Mean severity	-	-	1.0	1.6	-	-	1.0	1.2

#significantly different to controls (5% level); ## (1% level) [Dunn's test]
**significantly different to controls (5% level); ** (1% level) [Dunnett's test]*
severity: laboratory scale Grade 1-4

Conclusions

At the highest dose tested, an increased incidence of salivation, increased thyroid weight and accompanying histopathology were reported in both sexes of the dog. At the top dose, there were changes in AST levels in females but these were sporadic, not replicated in males and there were no accompanying changes e.g. ALT levels.

At 300mg/kg bw/d, salivation and vomiting were marked in both sexes. The increase in thyroid weight was statistically significant in males only but changes in thyroid histopathology were reported in both sexes, particularly, thyroid hypertrophy.

At 100mg/kg bw/d, there were no adverse effects reported in either sex.

Overall, the NOAEL for this study is 100 mg/kg bw/d based on the clinical signs, increased thyroid weights (males) and thyroid histopathology (both sexes) at 300 mg/kg bw/d.

[redacted], 2004

B.6.3.4 Short-term dermal toxicity in the rabbit (IIA 5.3.3)

This study was available but not submitted previously. As this study has not been evaluated, an assessment is presented here for consideration.

Study	21-day repeated dose dermal toxicity study in rabbit
Reference	[redacted] (1989b)
Date performed	2nd- 23rd November 1988
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	US FIFRA 82-2
Deviations from the guideline	None
GLP	Yes
Test material	Asulam-sodium 88% purity Batch 264-NC-01
Study acceptable	Yes

Asulam-sodium was administered to male and female New Zealand White rabbits (10 animals/sex/group) *via* dermal (occluded) application. The test material was formulated in 0.2% or 0.5% (aq) CMC for the first 9 days and remaining 12 days respectively. The increase in vehicle concentration was to increase viscosity of the test substance and minimise run off from the application site. The test substance was applied every day for 3 weeks to the shaved intact dorsal skin of each rabbit. Each exposure lasted 6 hours. A single dose level of 1000 mg/kg bw/d was used. A concurrent vehicle control group received the vehicle on a comparable regimen. Signs of toxicity and dermal scoring were performed daily, whilst body weight and food consumption were determined weekly. Haematology and blood biochemistry were performed on all rabbits immediately following the final administration period. Opthalmoscopic examination was performed prior to study initiation and in week 3. Organ weights, macroscopic and microscopic findings were reported at necropsy.

Results

There were no treatment-related clinical signs of toxicity observed during the study. There was no evidence of dermal irritation in treated males. Three high dose females exhibited very slight erythema on day 11 and was seen intermittently throughout the study until necropsy in these animals. One of these animals also showed evidence of slight oedema on day 11, but by day 12 this had resolved. There were no treatment related mortalities and no differences in opthalmoscopic examinations were seen in control versus treated animals.

No treatment related effects on body weight or body weight gain were observed and there was no treatment related effect on food consumption

Statistical significance was attained for some haematology and clinical chemistry parameters (see table 6.3.4-1). These were not considered a toxicological concern because the effects were not replicated across both sexes and in relative terms, the difference between pre-test and termination in controls and test animals was similar. There were no significant differences in plasma and red cell cholinesterase activity.

Table 6.3.4-1 Haematology and blood clinical chemistry in the rabbit after 21 days dermal exposure to Asulam-sodium

Parameter/ time		Dose level (mg/kg bw/d)			
		Males		Females	
		0	1000	0	1000
Haematology					
Leukocytes ($x10^3$/cm)	Pre-test	8.4	7.9	9.0	7.7
	Terminal	7.4	7.0	7.9	6.6*
Erythrocytes ($x10^6$/cm)	Pre-test	5.03	5.12	4.8	5.09*
	Terminal	5.9	5.84	5.34	5.57*
Lymphocytes ($x10^3$/cm)	Pre-test	5.7	5.4	5.8	4.7
	Terminal	4.4	4.2	4.7	3.7*
Clinical chemistry					
Urea nitrogen (mg/dl)	Pre-test	15.4	14.8	17.3	19.4
	Terminal	23.4	20.9*	26.7	28.4

There were no treatment related changes in absolute or relative organ weight and no gross macroscopic changes were reported. No treatment related histopathological changes were observed in treated skin or any other tissues examined.

Conclusions:

Based on the results from this study, the NOAEL for both local and systemic toxic effects of asulam-sodium when administered dermally to male and female rabbits for 21 consecutive days was 1000 mg/kg bw/d (the limit dose).

B.6.3.5 Short-term inhalation toxicity in the rat (IIA 5.3.3)

Inhalation studies were not conducted for 28 or 90 days as they were not required given the vapour pressure of asulam-sodium is less than 10^{-2} Pa (actual 5x 10^{-7} Pa at 45°C).

B.6.3.6 Summary of short-term toxicity studies

Asulam was found to be of low toxicity via the dermal route in the rabbit and the oral route in short term studies with the rat, mouse and dog.

The main targets identified in the 90-day rat study were the erythrocyte and thyroid; results show mild macrocytic anaemia (and histopathology consistent with increased erythrocyte turnover) and thyroid follicular hypertrophy at the top dose level (20000ppm). Additional effects in both sexes at the top dose included an increased incidence of histopathology changes in the kidney and clinical chemistry changes plus an increase in spleen and testes weight of males. The clinical chemistry and increased incidence of histopathology changes in the kidney were also reported in both sexes at 6000ppm and this dose level is determined to be the LOAEL. Whilst there was a statistical difference in the albumin/globulin ratio in females treated with 2000ppm, this is not considered to be of significance in the absence of any significant effect on albumin or globulin concentration and the effect not being replicated in males at this dose level. Therefore, a NOAEL of 2000 ppm (equivalent to 129 and 158 mg/kg bw/d) can be determined from this study, based on clinical chemistry in males and histopathology observations in both sexes at 6000 ppm.

Treatment-related findings in the mouse were limited to minor bodyweight effects at high dose levels (>10000ppm) but this study was intended as a range finder and not for determination of NOAELs or reference doses.

In the dog, histopathological investigations also identified the thyroid as a target of toxicity in both studies (6 month and 1 year). Thyroid changes were noted at 300mg/kg bw/d in the 1 year study and 1500mg/kg bw/d in the 6 month study. Significantly reduced white blood cell counts and significantly elevated plasma glucose concentration seen in treated groups in the 6-month dog study were not apparent in the 1-year study. The toxicological significance of this finding is therefore unclear. Based on the effects in the dog the NOAELs were 60mg/kg bw/d and 100mg/kg bw/d in the 6 month and 1 year study respectively.

The consistent finding across all species was the effect on the thyroid and published literature was consulted in the previous review (see section 6.8) to decipher a mechanism of action. Sulphonamide antibiotics such as sulphamethazine and sulphanilamide (a metabolite of asulam) have been shown to reversibly inhibit the activity of thyroid peroxidase, the enzyme responsible for iodination of thyroglobulins in the thyroid ([redacted], 1994a). The inhibition of thyroid peroxidase results in the reduced synthesis of thyroid hormones.

In commonly used rodent species, perturbations of thyroid hormone synthesis can rapidly lead to decreases in circulating thyroid hormone concentration which in turn triggers the increased production of thyroid stimulating hormone; the increased thyroid

activity can result in follicular hyperplasia. In humans, however, a large proportion of circulating thyroid hormone is bound to carrier proteins such as thyroxine binding globulin (TBG, the major protein); transthyretin, and albumin. Bound thyroid hormone acts as a buffer against alterations in thyroid hormone concentrations, reducing the feedback signal resulting in release of thyroid stimulating hormone (TSH) and reducing the stimulatory effect on the thyroid. Rodents have markedly lower concentrations of TBG and most circulating thyroid hormone in the rat is free, resulting in little reserve capacity. The thyroid is therefore much more sensitive to perturbations of thyroid hormone homeostasis. Other evidence suggests that the rodent thyroid is much more sensitive than the human thyroid, as serum TSH concentration is much lower in rats, and thyroxine half life is shorter ([redacted], 1999a).

In rats, mice and dogs, administration of sulphonamides has been shown to produce thyroid gland hyperplasia related to increased TSH concentration ([redacted], 1998a), whereas administration of therapeutic levels to humans has no effect on thyroid function. Monkey thyroid peroxidase has been shown to be much less sensitive than rat thyroid peroxidase to inhibition in vitro by administration of sulphamonomethoxine ([redacted], 1995) and administration of two sulphonamides to monkeys at doses of up to 300 mg/kg bw/day for up to 1 year had no effect on thyroid function or morphology.

It is therefore concluded that the thyroid effects of asulam-sodium seen in rats, mice, and dogs (increased organ weight and follicular hyperplasia) are related to the sulphonamide moiety of the asulam molecule. The effects are due to sensitivity of the test species and are of limited relevance to the risk assessment.

Table B.6.3.6-1 Summary of short-term toxicity studies

Study	NOAEL	LOAEL	Effects at LOAEL	Reference
	[ppm] mg/kg bw/d			
Rat 90-day	M: [2000 ppm] 129 F: [2000 ppm] 158	M:[6000 ppm] 387 F: [6000 ppm] 479	Histopathology, clinical chemistry Histopathology	[redacted] 2000
Mouse 8-week	M: [10000 ppm] 1673 F: [30000 ppm] 6835	M: [30000 ppm] 5103 F: [50000 ppm] 10828	Marginally reduced bodyweight and weight gain Marginally reduced bodyweight	[redacted] 1989a
Dog 6-month	M: 60 F: 60	M: 300 F: 300	Haematology, clinical chemistry Clinical signs, clinical chemistry, ↑thyroid weight	[redacted] [redacted] 1980
Dog 52-week	M: 100 F: 100	M: 300 F: 300	Clinical signs, ↑thyroid weight, thyroid histopathology Clinical signs, thyroid histopathology	[redacted], 2004a
Rabbit 21 day dermal	M:1000 F: 1000	M: >1000 F:>1000	No treatment related effects were observed at the highest dose tested	[redacted] 1989b

B.6.4 Genotoxicity studies (IIA 5.4)

B.6.4.1 Genotoxicity *in vitro* (IIA 5.4.1)

Two negative bacterial reverse mutation studies were evaluated in the original submission of Asulam-sodium but were deemed supplementary only (McGregor & Crichton 1977; Hoom 1983). These Ames studies have not been presented or re-submitted here in light of 2 more recent studies; one was evaluated as part of the additional report to the DAR and considered acceptable (Van den Wijngaard 2008). The second has been completed to address the new proposed source of asulam (Tendulkar 2013).

A. This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report.

Study	Bacterial reverse mutation test with asulam
Reference	Van den Wijngaard, MJM (2008)
Date performed	12 – 31 March 2008
Test facility	TNO, Zeist, Netherlands
Report reference	V7905/05
Guideline(s)	OECD 471
Deviations from the guideline	None
GLP	Yes
Test material	Asulam sodium, 90.2% (equivalent to Asulam 82.3%) Batch ENAS000019 (Bayer source)
Study acceptable	Yes

Asulam sodium was dissolved in phosphate buffered saline and tested for its potential to induce reverse mutations in *Salmonella typhimurium* strains TA98, TA 100, TA1535 & TA 1537 and *E. Coli* WP2 *uvrA*. The assay was performed in the presence and absence of metabolic activation (S9) derived from Aroclor 1254 induced rat livers. Concentrations tested were 62, 185, 556, 1667 & 5000 ug/plate. Cytotoxicity was evident at 1667 ug/plate and marked at 5000 ug/plate. Positive control compounds produced significant increases in mutation rates. Asulam sodium did not produce any indications of positive responses either with or without S9.

Asulam sodium was not mutagenic in this bacterial reverse mutation assay.

Wijngaard, 2008

B. Since the EFSA conclusion report, confirmation of a new manufacturing source, and transfer of production to a new manufacturing plant has occurred. The production process is unchanged and the technical specification can be seen in volume 4 Table C.1.1 for the original process. To confirm that the new source of technical material is comparable to previous batches regards genotoxicity, a new *in vitro* genotoxicity bacterial assay for gene mutation has been undertaken. Therefore as this study has not previously been reviewed at EU level a summary of the study has been provided.

Study	Bacterial reverse mutation test of asulam sodium (50%w/v) aqueous concentrate using *Salmonella typhimurium*
Reference	Tendulkar, 2013
Date performed	3rd – 17th June 2013
Test facility	Jai Research Foundation, Valvada-396 108, Dist Valsad, Gujarat, India
Report reference	Study number 481-1-06-6691
Guideline(s)	OECD 471
Deviations from the guideline	None
GLP	Yes and QA statement provided
Test material	Asulam-sodium aqueous concentrate, Batch AJ09ASM-15, purity 44.2% (w/w)
Study acceptable	Yes

Method

In a reverse gene mutation assay in bacteria, *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 were exposed to asulam sodium (50% w/v) formulated in distilled water. The assay was performed using the plate incorporation method.

Following a preliminary toxicity-mutation assay with TA100, dose levels of 6.89, 20.58, 61.73, 185.19, 555.56 and 1666.67 µg/plate in the presence and absence of S9 activation were assessed in two independent tests. Trial I was perfomed with 5% S9 whilst Trial II was completed with 10% S9.

Positive controls in the presence and absence of S9 were consistent with those recommended in OECD 471. Assay acceptance criteria was well-defined including confirmation of tester strain integrity, culture density and a minimum of 5 concentrations not suffering from signs of cytotoxicity. Data sets were judged positive if the increase in mean revertant at the peak of the dose response were equal to or greater than 2-3 times (depending on strain) the negative control value.

Results

Ten doses of the test material ranging from 0.25 to 5000 µg/plate were evaluated in the cytotoxicity range-finder assay using TA100 in the absence and presence of S9. No precipitation was observed up to the limit dose, 5000 µg/plate. Pronounced cytotoxicity was noted from a concentration of 1666.67 µg/plate and greater. Hence, 1666.67 µg/plate was chosen as the maximum dose for the mutation studies based on cytotoxicity.

The mean number of his^+ revertant colonies of the negative controls were within the acceptable range. The positive controls gave the expected increase in the mean number of revertant colonies.

Five concentrations ranging from 6.89 to 1666.67 µg/plate were employed in both experiments in the absence and presence of S9.

In experiments 1 (+S9 5%) and 2 (+S9 10%) pronounced cytotoxicity as evidenced by complete inhibition of the background lawn with reduction in the revertant numbers was observed at 1666.67 µg/plate in all strains in both the absence and presence of S9 mix. No mutagenic effect was observed in the strains tested.

Of note, some researchers have suggested that 2-aminoanthracene should not be used as the only positive control to evaluate S9-mix activity as it has been shown that the

chemical may be activated by enzymes other than the microsomal cytochrome P450 family. The study report contains the S9-production certificate (MOLTOX®) which confirms that the batch of S9 used in this study was sensitive to another known mutagen that requires metabolic activation, benzo[a]pyrene.

Conclusions

It was concluded that asulam showed no evidence of mutagenic activity following testing in five *S. typhimurium* (TA98, TA100, TA102, TA1535 and TA1537) strains when tested up to 1666.67 µg/plate (a concentration that induced cytotoxicity in all strains) in both the absence and presence of metabolic activation (S9 mix present at 5% or 10%) using the plate incorporation methodology.

Tendulkar, 2013

C. This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report. The report was available previously to the RMS and archived but was not re-submitted for this approval by the notifier.

Study	An Assessment of the Mutagenic Potential of Asulam Using in vitro mammalian cell test system
Reference	Richold M, Grantham C, Ransome S & Basham K; 1982a
Date performed	March-August 1982
Test facility	Huntingdon, Cambridgeshire, UK
Report reference	R001258; M&B 148/82816
Guideline(s)	-
Deviations from the guideline	-
GLP	-
Test material	Asulam, Batch JN 120 (purity 99%)
Study acceptable	Yes

Duplicate cultures of L5178Y mouse lymphoma cells (TK locus) were exposed for three hours to the test material (dissolved in DMSO) at four concentrations between 4000-5250 µg/ml in the absence and five concentrations between 4400-5200 µg/ml in the presence of an exogenous metabolic activation system (Aroclor 1254-induced Sprague-Dawley rat liver S9). Cultures were incubated for a further 48 hours (expression time) prior to sub-culture and incubation in the presence of trifluorothymidine for 12 days. The concentrations of test material were based on the results of a preliminary study.

In the main assay, mild cytotoxicity was observed at the highest concentration of 5250 µg/plate; no evidence of an increase in mutation frequency was seen at any concentration in the absence of S9. In the presence of S9, more marked cytotoxicity was observed at the highest concentration of 5200 µg/plate. A concentration-related increase in mutation frequency was observed at ≥4800 µg/plate, with the mutation frequency reaching statistical significance at 5200 µg/plate. Appropriate positive control compounds (ethylmethanesulphonate and 20-methylcholanthrene) confirmed the sensitivity of the assay.

Table B.6.4.1-1 Mammalian cell mutation assay: summary of findings

Concentration (µg/plate)	Survival (%)		Mutation (colonies/plate)				Mutation frequency (/10^6 survivors)	
			1	2	3	Total		Average
-S9								
0	100	100	24	26	23	73	46	46
			40	37	29	106	46	
4500	134	131	27	21	21	69	35	38.5
	128		33	29	25	87	42	
4750	157	152.5	28	24	21	73	31	38.5
	148		41	50	29	120	46	
5000	143	149	26	32	25	83	35	36.5
	155		32	30	24	86	38	
5250	37	66	30	34	30	94	55	50
	95		26	24	44	94	45	
EMS	60	55	290	286	300	876	628	667
	50		295	323	325	943	706	
+S9								
0	100	100	17	23	9	49	42	45
			24	17	-	41	63	
			17	24	27	68	32	
			39	35	29	103	42	
4400	106	119.5	19	20	17	56	31	38
	133		33	43	23	99	44	
4600	103	106	25	22	17	64	34	31
	109		18	18	14	50	27	
4800	95	77.5	40	39	29	108	55	56
	60		27	39	29	92	56	
5000	95	81.5	36	44	36	116	61	68
	68		30	43	49	122	74	
5200	75	44	67	88	94	249	128	131**
	13		108	119	115	342	134	
20-MC	73	73.5	83	92	86	261	165	180
	74		107	103	125	335	195	

[a]*Plate discarded due to contamination; figure in brackets (1.5x colony count, is used in all calculations)*
****significantly different to controls (p<0.001)*

Evidence of mutagenicity was observed in the presence of metabolic activation under the conditions of this study. The study report states that the results provide evidence of weak mutagenic potential. The Notifier considers that the results are not indicative of a mutagenic effect in the absence of a dose-related response and due to an association with 'very low' cell survival. This argument is not accepted as the mutation frequency was increased at this concentration in both duplicates, whereas marked cytotoxicity was only apparent in one duplicate.

Richold, 1982

D. This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report.

Study	Gene mutation test at the TK-locus of L5178Y cells with asulam.
Reference	Steenwinkel M-J ST (2009)
Date performed	10 March to 1 April 2008

Test facility	TNO, Zeist, Netherlands
Report reference	V7903/02
Guideline(s)	OECD 476
Deviations from the guideline	None
GLP	Yes
Test material	Asulam sodium, 90.2% pure (equivalent to Asulam 82.3%), Batch ENAS000019 (Bayer source)
Study acceptable	Yes

Asulam sodium was tested for its potential to induce reverse mutations at the TK-locus of mouse lymphoma, L5178Y cells. Assays were performed in the presence and absence of metabolic activation (S9) derived from Aroclor 1254 induced rat livers (male Wistars). A range of concentrations was tested (0.03 – 10 mMolar; 0.6 – 2300 µg/ml), with a minimum of 8 concentrations scored for mutation frequencies. Exposure times were 4 hours in the presences of S9 and 24 hours in the absence of S9. Expression times were 44 – 48 hours, followed by a 10 to 14 day culture period. Cytotoxicity was not seen at any concentration of asulam sodium.. Positive control compounds produced significant increases in mutations. Asulam sodium did not produce any indications of positive responses either with or without S9.

Asulam sodium was not mutagenic in this mammalian cell gene mutation assay. Although this contrasts with study by Richold (1982), the study has been completed to a modern standard and to guidelines applicable at the time. Overall, it is considered the conclusion of this study overrides the previous one by Richold.

Steenwinkel, 2009.

E. This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report. The report was available previously to the RMS and archived but was not re-submitted for this approval by the notifier.

Study	Asulam: Test to determine the ability of asulam to induce unscheduled DNA synthesis in HeLa cells
Reference	Martin CN;1982
Date performed	February – March, 1982
Test facility	Microtest Research, UK
Report reference	C030509; MB1/HE/JC3
Guideline(s)	-
Deviations from the guideline	-
GLP	-
Test material	Asulam, batch JN 120; 99% purity after drying
Study acceptable	Supporting information only

Triplicate cultures of HeLa S3 cells were exposed for two hours to the test material (dissolved in DMSO) at eight concentrations between 0.125-250 µg/ml in the presence and absence of an exogenous metabolic activation system (Aroclor 1254-induced male Wistar rat S9 fraction). The culture medium also included hydroxyurea to reduce normal DNA replication. Unscheduled DNA synthesis (UDS) was investigated using liquid scintillation counting.

No increase in UDS was observed in cells exposed to asulam. The positive control compound 4-nitroquinoline-1-oxide confirmed the sensitivity of the assay in the

absence of S9, however the performance of positive control used in the presence of S9 (3,3-dichlorobenzidine) is considered to be unsatisfactory. This compound is not one of the positive controls recommended by the current (1997) or previous (1986) OECD guideline 482.

Table B.6.4.1-2 UDS *in vitro*: summary of findings

Concentration (µg/ml)	DNA repair activity (dpm/µg DNA)	
	-S9	+S9
0	84.2 ±6.1	107.0 ±9.7
0.125	76.7 ±11.1	99.7 ±5.9
0.25	80.1 ±4.0	88.2 ±4.0
1.25	79.8 ±1.1	86.1 ±5.6
2.5	84.1 ±0.4	90.8 ±9.2
12.5	76.8 ±6.7	81.9 ±4.7
25	71.0 ±4.9	85.2 ±1.6
125	70.0 ±1.8	82.3 ±6.6
250	75.6 ±15.7	72.8 ±10.8
NQO (1 x10^{-5}M)	1661.1** ±288.0	
DCB (5 x10^{-5}M)		142.0** ±6.3

No evidence of UDS was seen under the conditions of this study, however the sensitivity of the study in the presence of metabolic activation is questionable due to the poor performance of the positive control compound. The use of liquid scintillation counting to determine UDS is also less sensitive than autoradiography due to problems in distinguishing between unscheduled and replicative DNA synthesis, although hydroxyurea was used in this study to reduce replicative synthesis. The study does not comply with the current or previous versions of OECD guideline 482, which recommend a minimum of six replicates per concentration for LSC determinations.

Martin, 1982

F. This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report. The study was deemed supplementary by the RMS due to study design not complying with the contemporary guidelines at performance (1983) or modern standards at the first review (1997).

Study	Mutagenicity evaluation of Asulam Technical (dried) Batch: PN 85, in an *in vitro* Cytogenetics Assay Measuring Chromosome Aberration Frequencies in Human Lymphocytes
Reference	Galloway SM & Myrh BC, 1984
Date performed	October-December, 1983
Test facility	Litton Bionetics, USA
Report reference	R001262; Report No. 20090
Guideline(s)	-
Deviations from the guideline	-
GLP	-
Test material	Asulam technical, PN 85 (purity 97.5-98.2% after drying)
Study acceptable	Supporting information only

Duplicate cultures of PHA-stimulated human lymphocytes were exposed to the test material (dissolved in DMSO) for 49 hours (-S9) or 1 hour (+S9) at five concentrations between 0.125-2.5 mg/ml in the presence and absence of an exogenous metabolic activation system (Aroclor 1254-induced male rat liver S9 fraction). Cultures were incubated with colcemid for one hour; 100 metaphases from each concentration were assessed for chromosomal aberrations following fixation at 49 hours after the start of exposure. The concentrations used were based on the results of a preliminary study in which cells were exposed to asulam at concentrations of up to 3.3 mg/ml. The highest concentration was cytotoxic, causing cell lysis. Cells were obtained at up to 1.1 mg/ml although a delay in cell cycle progression was observed at this concentration.

In the main study, total cytotoxicity was evident at 1.0 and 2.5 mg/ml in the absence of S9; a slightly increased proportion of cells with chromosomal aberrations was seen at 1.0 mg/ml, however this value is reported to be within the historical control range of the laboratory. No evidence of cytotoxicity was seen in the presence of S9; exposure to the test material did not result in an increased proportion of cells exhibiting chromosomal aberrations. Appropriate positive control compounds (ethylmethanesulphonate and cyclophosphamide) confirmed the sensitivity of the test system.

Table B.6.4.1-3 Clastogenicity *in vitro*: summary of findings

Concentration (mg/ml)	-S9		+S9	
	Cells scored (#)	Cells with aberrations (%)	Cells scored (#)	Cells with aberrations (%)
0	100	3.0	100	3.0
0.125	100	3.0		
0.25	100	3.0		
0.5	100	3.0		
1.0	34	5.8	100	1.0
1.5			100	2.0
2.0			100	0.0
2.5			100	0.0
+control	23	39.1**	50	12.0**

Some evidence of clastogenicity was found under the conditions of this study at the highest concentration in the absence of S9, however this finding is reported to be within the laboratory's historical control range and is associated with cytotoxicity. The study does not comply with the current (1997) OECD guideline 473 due to the scoring of 100 cells per concentration (at least 200 are specified). The study also does not comply with the current or contemporary (1983) version of the guideline with regard to exposure times and the inclusion of a repeated assay.

Galloway, 1984

B.6.4.2 Genotoxicity *in vivo* (IIA 5.4.2)

The original evaluation at EU level for the *in vivo* micronucleus test ([redacted] 2004) concluded that the results were equivocal. A re-evaluation has been conducted by the applicant for this submission which took into consideration corrected historical control data. The RMS did not agree, however, with the re-calculation as the applicant had

considered all vehicles from the historical control data not just water which is used in this study with Asulam. Subsequently, the RMS has presented the original evaluation which was reviewed during the EU process.

Study	AE F10278900 SG80 A102: Micronucleus Test on the Male Mouse
Reference	[redacted] 2004
Date performed	June – August 2004
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	OECD 474, USEPA OPPTS 870.5395
Deviations from the guideline	None
GLP	Yes
Test material	Asulam sodium, batch WN 42016; 89.3% purity
Study acceptable	Yes

Male NMRI mice (5/group) were administered the test material (dissolved in deionised water) by intraperitoneal injection at dose levels of 0, 1000, 2000 or 4000 mg/kg bw/d on two consecutive days. A positive control group was administered a single intraperitoneal dose of cyclophosphamide. Animals were sacrificed and femoral bone marrow harvested at 24 hours following the last administration. 2000 polychromatic erythrocytes (PCEs) per animal were scored for the presence of micronuclei. The ratio of PCEs:NCEs was also calculated. A single sex was used in this study because of the absence of any sex differences in toxicity seen in a preliminary study.

No mortality occurred. Signs of toxicity including apathy, digging and grooming movements, loss of weight, spasm, ptosis and difficulty in breathing were observed in animals administered asulam. The PCE:NCE ratio was slightly increased in animals administered 4000 mg/kg bw/d asulam and exceeds the laboratory's historical control range of 2.0-5.6 micronucleated PCEs (27 studies performed in 2002-2003). The proportion of micronucleated PCEs was slightly higher at 2000 mg/kg bw/d, however a similar finding was not seen at the top dose level. Administration of cyclophosphamide resulted in a significant increase in the number of micronucleated PCEs.

Table B.6.4.2-1 Mouse micronucleus study: summary of findings

Dose group	**NCEs** (/2000 PCEs)	**Micronuclei** (/2000 PCEs)	**Micronuclei** (/2000 NCEs)
- control	2036 ±690	3.8 ±0.4	1.9 ±1.6
1000 mg/kg bw/d	2348 ±845	3.6 ±2.3	1.7 ±1.7
2000 mg/kg bw/d	1493 ±402	6.0 ±2.8	1.5 ±1.1
4000 mg/kg bw/d	2978 ±2015	2.8 ±2.2	1.6 ±1.0
+ control	1564 ±516	17.8* ±8.7	2.2 ±2.7

The results of this study are considered to be equivocal, and also appear to meet the laboratory's criteria for an equivocal study ('..an increase of micronucleated polychromatic erythrocytes above the range of historical negative controls, provided the increase was not significant and the result of the negative control was not closely

related to the data of the respective treatment group. In this case, normally a second test will be performed'), however it is recognised that the increase was seen only at an intermediate dose level but not the highest dose level.

[redacted], 2004

B.6.4.3 Summary of genotoxicity studies

The genotoxicity of asulam was investigated in a number of studies *in vitro* and *in vivo*. No evidence of mutagenicity was seen in two recent Ames tests (Wijngaard 2008 and Tendulkar, 2013). A significantly increased mutation frequency was seen in a mammalian cell mutation (mouse lymphoma) assay at the highest (cytotoxic) concentration in the presence of metabolic activation (Richold, 1982) but a subsequent study by Steenwinkel (2009) showed that Asulam was negative in the mouse lymphoma assay. An equivocal result (increased proportion of cells with chromosomal aberrations at the highest (cytotoxic) concentration in the absence of metabolic activation) was seen in a study of clastogenicity *in vitro* (Galloway and Myhr, 1984). There are deficiencies with this study as it did not comply with the guidelines available at the time and subsequently, the RMS considers it supplementary. No evidence of unscheduled DNA synthesis was seen in a study *in vitro*, however there are concerns over the design and performance of this study (Martin, 1982). Therefore, the UDS test is seen as supplementary information by the RMS. An equivocal result was also seen in the only *in vivo* study, where the proportion of micronucleated PCEs was elevated (and exceeded the historical control range, 1.2-5.8) at an intermediate concentration.

This suite of studies could be enhanced by additional *in vitro* data on clastogenicity to clarify the equivocal outcomes from the *in vitro* clastogenicity assay and *in vivo* micronucleus but in the absence of further data, the RMS agrees with the previous EFSA conclusions (2010) that Asulam does not have genotoxic potential. The RMS concurs with the conclusion based on the studies completed to modern regulatory standards and the fact that positive/equivocal results only arise at high dose levels of asulam, in the absence of a dose response relationship or without statistical significance compared to negative controls.

The classification of Asulam according to Regulation (EC 1272/2008) has been proposed by the UK CA in an accompanying C&L dossier for submission to ECHA and justification for non-classification provided in more detail in volume 1 of the active substance RAR.

Table B.6.4.3-1 Summary of genotoxicity studies

Study	Test system	Result	Reference
In vitro			
Bacterial reverse mutation assay	*S. typhimurium* TA1535, TA100, TA1537, TA98 and E.coli WP2 uvrA	Negative	Van den Wijngaard, 2008
	S. typhimurium TA1535, TA100, TA1537, TA102 & TA98	Negative	Tendulkar, 2013
Mammalian cell mutation	L5178Y (TK locus)	Positive	Richold, 1982
		Negative	Steenwinkel,

			2009
Clastogenicity	Human lymphocytes	Equivocal	Galloway and Myrh 1984
UDS	HeLa cells	Negative	Martin, 1982
In vivo			
Micronucleus	Mouse bone marrow	Equivocal	[redacted], 2004

B.6.5 Chronic toxicity and carcinogenicity studies (IIA 5.5)

Two studies were submitted in rodents and assessed in the previous review of Asulam-sodium. They were previously considered acceptable and have been presented here in accordance with the EFSA report from 2010. The studies remain acceptable for the purposes of pesticide active substance data requirements (admissibility), deriving NOAELs and subsequently risk assessment. In generating a C&L report, however, the studies were deemed limited for classification of Asulam (see volume 1 and accompanying C&L report by the UK CA for further details).

B.6.5.1 Chronic toxicity and carcinogenicity in the rat

This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report. The conclusion has been updated to accommodate additional information provided by the applicant but no change was made to the NOAELs.

Study	Asulam - Toxicity and tumorigenicity in prolonged dietary administration to rats [and study addendum]
Reference	[redacted] 1981a
Date performed	January 1978 - February 1980
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	None stated
Deviations from the guideline	-
GLP	No: pre-GLP, but QA audited
Test material	Asulam batches HN 9 and JN 266; purity not reported
Study acceptable	Yes

(Historical control data reference- [redacted] (1987). Background data related to H.R.C. report number [redacted]. Asulam - toxicity and tumorigenicity in prolonged dietary administration to rats. Unpublished report No.: [redacted] Company report No.: [redacted]

CD rats of Sprague Dawley origin (50/sex) were administered the test material in the diet at concentrations of 0, 1000, 5000 or 25000 ppm for up to 108 weeks; all groups of males or females were sacrificed when survival in any group of that sex reached 30%. Additional groups of 15 rats/sex were sacrificed following treatment for 78 weeks. Animals were observed daily for clinical signs during the first four weeks of the study and weekly thereafter. Palpation was performed weekly throughout the study. Bodyweights and food consumption were measured weekly. Water consumption was monitored visually throughout the study period and was measured (in control and top dose level animals) during weeks 6, 12 and 24. Ophthalmoscopy was performed on all control and top dose level animals pre-test and during weeks 6, 13, 26, 52, 78 and 101. Blood samples were taken pre-test and during weeks 12, 24,

39, 50, 64 and 76 from satellite group animals and during weeks 90 and 102 from the main study groups (15 rats/sex). Clinical chemistry parameters were investigated pre-test and during weeks 5, 12, 24, 50 and 76 from satellite group animals and from main group rats (15/sex) during Week 102. Urine samples were collected from control and top dose level animals (5/sex) during weeks 4, 11, 23, 49, 75 and 101; additional samples (5/sex/group) were collected during weeks 5 and 77. Gross necropsy was performed on all animals; weights of the adrenals, brain, heart, kidneys, lungs, liver, ovaries, pituitary, prostate, spleen, testes, thymus and uterus were recorded. A comprehensive list of tissues from all main group animals was investigated microscopically; investigations in satellite group animals were limited to macroscopically abnormal tissues.

Diets were prepared every two weeks; dietary analyses are stated to have shown 'good agreement' with the nominal dose levels, however further details are not reported.

Results

Dietary intake of Asulam

The doses of Asulam administered were 0, 1000, 5000 and 25000ppm which equated to 0, 36, 180 and 953mg/kg bw/d in males and 0, 47, 243 and 1280mg/kg bw/d in females.

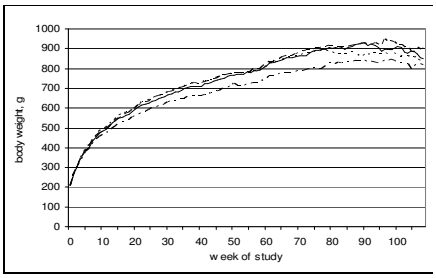
Mortality and clinical signs

Survival to study termination at week 107 (F) or 108 (M) was slightly greater at the top dose level and remained above 25% in all groups except males dosed with 5000ppm Asulam. In accordance with current OECD guidelines, the mortality data is sufficient to prevent the termination of the study as the number of animals did not decrease below 25% in the control or low dose group. To attain acceptability criteria, at least 50% should be present at study termination which is not the case but the RMS is satisfied that the study remains valid as 50% or more of each sex were alive at week 93 for males and week 101 for females (~90% or more of the study completed), there was >45% alive at the top dose for both sexes at week 104 and all tumours for decedents were recorded to give a full tumour profile for Asulam. No signs of toxicity were observed during the study period. Ophthalmoscopy did not reveal any treatment-related findings.

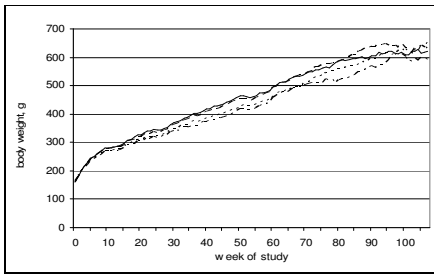
Bodyweights, food and water consumption

Weight gain by animals of both sexes was slightly (but significantly) reduced at the top dose level, resulting in mean bodyweights lower than controls from approximately Week 10; bodyweight values did not attain statistical significance. Weight gains and mean bodyweights of 5000 ppm females were also slightly lower than controls. Food and water consumption were unaffected by treatment; food conversion efficiency was slightly impaired in both sexes at the top dose level.

Figure B.6.5.1-1 Chronic rat study: bodyweights



Males



Females

Table B.6.5.1-1 Chronic rat study: survival and bodyweights

Parameter	Week	Dose level (ppm)							
		Males				Females			
		0	1000	5000	25000	0	1000	5000	25000
Intake (mg/kg bw/d)		-	36	180	953	-	47	243	1280
Survival	78	73%	80%	80%	80%	67%	80%	80%	67%
	52	94%	96%	96%	98%	96%	94%	100%	96%
	80	84%	86%	80%	80%	84%	78%	84%	74%
	104	38%	42%	26%	52%	38%	40%	44%	46%
	107/8	32%	32%	22%	48%	30%	26%	34%	40%
Body weight (g)	26	640	655	655	607	344	336	325	319
	52	769	778	775	712	460	454	427	415
	78	897	909	890	812	572	578	553	523
	104	885	885	856	796	627	626	638	590
	107/8	852	901	847	816	622	653	628	591
Weight gain	0-6	193	198	203	184	89	87	86	80***
	6-52	363	371	364	316**	208	206	181*	171**
	0-26	429	447	445	395	182	175	165	157
	0-52	558	570	565	500	298	293	267	253
Food consumption	1-6	5.4	5.2	5.2	5.7	9.5	9.7	9.7	10.8
	7-26	14.0	13.5	13.8	15.4	26.2	26.6	29.2	30.1

**significantly different to controls (p<0.05); **p<0.01; ***p<0.001*
[a]*satellite group*
[b]*expressed as group mean food consumption (g)/bodyweight gain (g)*

Haematology

Haematology revealed treatment-related findings on red blood cell parameters consistent with mild macrocytic anaemia in all treated groups of both sexes; findings were apparent largely during the first year of the study. Although the effects on some parameters attained statistical significance at 1000 ppm, findings are inconsistent, of small magnitude and/or do not form part of a dose-response relationship. Neutrophil counts were consistently lower in top dose females; a similar effect was not apparent in males.

Table B.6.5.1-2 Chronic rat study: haematology

Parameter/ Week		Dose level (ppm)							
		Males				Females			
		0	1000	5000	25000	0	1000	5000	25000
RBC (10^6/µl)	**12**	7.3	7.8**	7.8***	7.5	7.2	6.8**	6.5***	6.6***
	24	7.2	7.1	7.3	6.7*	6.0	6.1	6.1	5.9
	39	7.8	7.5	7.2***	7.1***	6.8	6.5*	6.4**	6.3***
	50	6.5	6.0**	6.2	6.4	5.9	6.1	5.8	5.6**
	64	7.7	7.5	8.0	7.6	7.1	7.3	6.9	6.7*
	76	7.4	7.2	7.5	7.5	6.8	6.9	6.8	6.8
	90	7.3	7.0	6.5	7.2	6.9	6.9	6.6	6.4*
	102	6.9	6.8	6.4	6.9	6.3	6.3	6.5	6.8
PCV (%)	**12**	53	54	53	53	53	53	53	50***
	24	56	54	55	56	53	54	55	53
	39	54	54	52**	53	52	52	52	50**
	50	48	45*	47	47	44	46	44	44
	64	50	49	50	49	47	49*	47	46
	76	51	51	50	50	48	49	48	45
	90	46	45	42	46	45	44	45	44
	102	45	44	44	45	44	45	46	47
Hb (g/dl)	**12**	16.5	16.6	16.0*	16.1*	16.2	16.2	16.0	15.6*
	24	15.2	14.4*	14.4*	14.2**	13.7	14.4***	14.6***	14.3**
	39	17.5	16.9*	16.1***	16.5***	16.6	16.9	17.2*	16.6
	50	15.4	14.8	14.9	15.6	15.5	14.8**	14.2***	13.9***
	64	14.8	14.7	14.8	14.6	14.7	15.5**	14.8	14.3
	76	14.6	14.3	14.2	14.9	14.3	14.6	14.7	13.9
	90	13.3	13.4	12.5	14.1	14.1	14.4	14.1	13.7
	102	13.5	13.1	12.5	13.6	13.5	13.3	13.7	14.3
MCHC (%)	**12**	31	31	30***	30**	31	30	30	31
	24	27	27**	26***	26***	26	27**	27**	27***
	39	32	31***	31***	31***	32	33*	33***	33**
	50	32	33	32	33	35	32***	32***	32***
	64	30	30	30	30	32	32	32	31
	76	28	28	28	30***	30	30	30	31
	90	29	29	29	30*	32	32	31	31
	102	30	30	28**	30	31	29	30	30
MCV (µl)	**12**	73	70*	68***	71	73	79***	81***	76
	24	77	77	76	83***	89	88	90	91
	39	70	72*	73**	75***	77	79	81**	81**
	50	74	76	76	74	75	76	76	78**
	64	65	65*	62	64	66	66	68	68
	76	69	71	67	68	70	71	71	67*
	90	63	65	66	65	65	64	69*	69*
	102	65	65	70**	65	70	72	71	70
Neutrophil (10^6/µl)	**12**	2.6	1.3	1.6	1.4	1.1	1.0	0.6	0.7
	24	2.3	2.4	2.1	2.3	1.4	1.0*	1.0*	0.8**
	39	1.3	1.1	1.1	0.9	0.9	1.1	0.7	0.5
	50	0.7	0.6	0.8	0.7	0.9	0.8	0.6	0.4*
	64	2.2	1.7	1.7	2.2	2.2	1.5	1.0**	1.3
	76	2.6	1.7	2.5	1.6	2.1	1.4*	1.3*	1.2**
	90	4.8	2.7	5.1	2.5	2.3	1.8	2.3	1.5
	102	4.3	4.8	3.4	2.5	2.8	5.2	3.8	1.6

**significantly different to controls (p<0.05); **p<0.01; ***p<0.001*

Clinical chemistry

Significantly increased clotting times were noted in top dose males at Weeks 12, 14 and 24. As a result of these observations, the diet was supplemented with Vitamin K and similar effects were not apparent at later time points. No clearly treatment-related effects were apparent on other clinical chemistry parameters, including erythrocyte and plasma cholinesterase activity. Urinalysis revealed a slightly reduced urinary pH in both sexes at the top dose level.

Table B.6.5.1-3 Chronic rat study: clotting parameters

Parameter / Week		**Dose level (ppm)**							
		Males				**Females**			
		0	**1000**	**5000**	**25000**	**0**	**1000**	**5000**	**25000**
Thrombotest (s)	**12**	32	29	34	41***	23	21**	20**	21*
	14	39	39	50*	56**	21	21	21	19*
	24	39			62***				
	33	26			26				
	39	28	29	28	29	25	25	24	25
	50	28	26	26**	26*	25	24	24	25
	64	23	23	23	25**	22	21	21	21
	76	24	24	25	24	22	21	23	20
	90	25	24	24	23**	21	21	21	21
	102	20	21	22*	24***	21	22	22	21
APPT (s)	**14**	40.3	32.2***	35.2**	42.1				
	24	29.0	29.4	34.2	38.4**				
Prothrombin (s)	**14**	13.1	10.9***	12.0**	14.3*				
	24	12.7	12.5	14.6	17.1***				

**significantly different to controls (p<0.05); **p<0.01; ***p<0.001*

Organ weights and pathology

No effects were seen on organ weights at the interim sacrifice. Gross necropsy did not reveal any treatment-related findings. At the terminal sacrifice, mean absolute and relative liver and kidney weights were elevated in top dose females only. Absolute and relative ovary weights were slightly lower in this group, however ovary weights are noted to be variable in all groups.

Gross necropsy of main group animals revealed increased incidences of enlarged thyroid in both sexes at the top dose level and a slightly increased incidence of uterine masses in top dose females. Microscopically, thyroid effects were characterised by diffuse or focal follicular hyperplasia in both sexes and an increased incidence of epithelial whorls in males at the top dose level. Increased incidences of unilateral or bilateral adrenal medullary hyperplasia were noted in males at ≥5000 ppm; similar findings were not apparent in females. Other potentially treatment-related findings were seen at the top dose level, including a slightly higher incidence of bile duct hyperplasia in both sexes, an increased incidence of prominent splenic siderocytes (consistent with the haematological findings) and pituitary hyperplasia in males.

Tumorigenicity

Neoplastic findings were limited to an increased incidence of phaeochromocytoma in top dose males with no associated dose response; the histopathology report did not differentiate between benign and malignant tumours, however the summary sections of the study report state that 'benign phaeochromocytomas' were increased, with 'no evidence of malignancy'. The increased incidence of benign phaeochromocytoma is considered to be related to the adrenal medullary hyperplasia seen in this group; the study report states that clear differentiation between hyperplastic and neoplastic change is difficult and therefore the combined incidence for these findings in males is also reported. The incidence of phaeochromocytoma in top dose males (20%) exceeds the laboratory's historical control range for benign tumours (six studies commenced in 1978) of 2-16% (mean 8.6%). Data published by the animal supplier (11 studies performed during 1977-85) report control incidences for benign tumours of 0-18.0% (6.0%); and malignant tumours of 0-2.2% (0.3%).

A marginal increased incidence of liver carcinoma in top dose males is not considered to be related to treatment in the absence of a dose response and was within the published historical control range from the supplier (0-11.1%). Increased incidences of thyroid parafollicular tumours in treated groups of males are not considered to be related to treatment in the absence of a dose-response relationship. An increased incidence of interstitial cell tumours in all groups of treated males is noted.

Table B.6.5.1-4 Chronic rat study: organ weights and pathology

Finding		Dose level (ppm)							
		Males				Females			
		0	1000	5000	25000	0	1000	5000	25000
Organ weights									
Liver	(g)	27.3	28.7	27.3	23.3	17.4	18.8	17.9	19.2
	relative	3.25	3.26	3.14	3.10	3.16	3.18	3.24	3.37
Kidney	(g)	5.3	6.5	6.2	5.2	3.5	3.8	3.7	4.1
	relative	6.31	7.39	7.13	6.91	6.35	6.42	6.69	7.19
Ovaries	(mg)					94	88	109	76
	relative					1.71	1.49	1.97	1.33
Gross pathology									
Thyroid enlarged		1	6	5	11	4	3	7	11
Uterus: mass						2	-	2	5
Histopathology									
Bile duct: hyperplasia		10	9	14	17	6	4	9	13
Spleen: prominent siderocytes		1	1	1	7	2	4	6	6
Thyroid: hyperplasia		-	-	4	11	-	1	1	3
Thyroid: epithelial whorls		1	2	1	5	2	2	6	4
Pituitary: hyperplasia		6	6	8	13	5	9	4	4
Adrenals: medullary hyperplasia		8	8	14	17	5	5	5	5
Tumours									
Phaeochromo-cytoma	Intercurrent	1	5	2	6	1	-	1	-
	Terminal	2	0	2	4	-	-	1	1
	Total no.	3	5	4	10	1	-	2	1
	Total %	*6%*	*10%*	*8%*	*20%*	*2%*	-	*4%*	*2%*
Adrenals: proliferative change	Intercurrent	*6*	*10*	*10*	*10*				
	Terminal	*4*	*1*	*6*	*13*				
	Total	*10*	*11*	*16*	*23*				
Testes:interstitial cell tumour		-	1	1	1				
Thyroids:parafollicular adenoma		-	4	2	-	1	-	-	-
Thyroids: parafollicular carcinoma		-	5	5	2	1	-	2	1
Liver carcinoma		-	1	-	2	1	-	1	1

[a]*enlarged/one lobe enlarged*
n=50

Conclusion

Treatment related effects at 25000ppm in both sexes of the rat were confined to reduced body weight gain at the 6-52 week interval, bile duct hyperplasia, prominent siderocytes, enlarged thyroid and haematological changes. The haematological changes persisted in both sexes at 5000ppm and were consistent with microcytic anaemia. Where there were statistical changes at 1000ppm for haematology, these were not related to exposure to asulam as the findings were in the absence of a dose response and were not replicated across both sexes.

Sex specific differences were reported; in females, this was reduced body weight gain and prominent siderocytes in the mid dose group. Males had epithelial whorls and hyperplasia of the thyroid, pituitary and adrenal medulla at the top dose; the tissue hyperplasia was also reported at the mid dose.

Based on all the findings, the non-neoplastic NOAEL is 1000ppm for both sexes of the rat (equivalent to 36 and 47mg/kg bw/d in males and females respectively).

There was evidence of carcinogenicity in male rats as the incidence of phaeochromocytomas was elevated at the top dose. The phaeochromocytomas were accompanied by histopathology observations (proliferative changes in the adrenals) and were marginally outside of historical control data for the laboratory and published data for this strain of rat during the study period (1977-1985). Published data from other studies with Sprague Dawley rats are supportive of the spontaneous nature of phaeochromocytomas in aging animals and the incidence is variable from 4-33% ([redacted] 1979, [redacted] 1992 and [redacted] 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%.

Phaeochromocytomas are tumors originating from chromaffin cells of the adrenal medulla. Such tumours produce mainly adrenaline and noradrenaline and have relatively low rates of malignancy in humans (<5%). Unlike in humans, spontaneous phaeochromocytomas can occur relatively frequently in rats depending on strain, with a greater degree of incidence in males than females. Changes in the adrenal medulla occur in the form of hyperplasia as well as benign and malignant phaeochromocytomas. Increased incidences of hyperplasia in the adrenal medulla are indicators for the proliferation of chromaffin cells; whether it is a physiological adaptation reaction or a direct effect of the chemical tested remains unclear. In most cases, no correlation between the chemical under study and increased incidencies of phaeochromocytomas has been determined ([redacted], 2009).

The conditions leading to phaeochromocytomas in animal studies include hypoxia, uncoupling of oxidative phosphorylation, disturbance in calcium homeostasis and disturbance of the hypothalamic endocrine axis ([redacted] 2009). Taking into consideration all available data, there is no evidence that Asulam directly generates the required conditions for phaeochromocytoma formation i.e. there is no pulmonary toxicity leading to low oxygen levels, calcium concentrations have not been reported to be affected by treatment, kidney function is not altered and there is no evidence that asulam uncouples mitochondrial respiration as no increase in brown adipose has been reported.

In long term studies, treatment related phaeochromocytomas occur together with other tumors or toxic effects in other organs. Typically these tumors cause nephrotoxic effects, neoplastic liver changes or endocrine disturbances, with tumours in different endocrine glands such as the thyroid, pancreas, preputial gland, zymbal gland or Harderian gland previously reported ([redacted] 2009, [redacted] 1979). With the exception of the thyroid effects reported, no liver, kidney or endocrine disturbances were evident. The thyroid effects reported were consistent, however, with the laboratory historical control range and therefore not considered treatment related.

Overall, there is insufficient evidence that asulam-sodium causes phaeochromocytomas in male rats when the study data are considered in the context of the historical control incidence and the plethora of public domain data.

[redacted] 1980

B.6.5.2 Chronic toxicity and carcinogenicity in the mouse

This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report and expert consultations. The study has been presented here to include the original assessment and changes made as part of the additional report and addendum 2.

Study	2-Year Dietary Oncogenicity Study in Mice
Reference	[redacted] 1992a
Date performed	1989-1991
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	EPA 83-2
Deviations from the guideline	None at time of guideline
GLP	Yes
Test material	Asulam sodium, batch XN36111; 88% purity
Study acceptable	Yes

CD-1 mice (75/sex/group) were administered test material in the diet at concentrations of 0, 500, 5000 or 50000 ppm for up to 2 years; 10 mice/group/sex were sacrificed following treatment for 12 months and 15 mice/sex/group were used for clinical investigations only. Animals were observed daily for clinical signs. Bodyweights were measured weekly up to Week 16 and 4-weekly thereafter. Blood samples were taken from 15 mice/sex/group following treatment for 6, 12 and 24 months and at the interim sacrifice (all animals) for the assessment of haematological parameters. Gross necropsy was performed on all animals; weights of the brain, kidneys, liver, spleen and testes were recorded. A comprehensive list of tissues from all animals was examined histopathologically.

Dietary analyses showed that the achieved concentration, stability and homogeneity of the test material were largely within acceptable limits. Minor deviations are not considered to affect study integrity.

Results

Dietary intake of Asulam

The doses of Asulam-sodium administered were 0, 500, 5000 and 50000ppm which equated to 0, 74, 730 and 8040mg/kg bw/d in males and 0, 95, 938 and 10353mg/kg bw/d in females.

Mortality and clinical signs

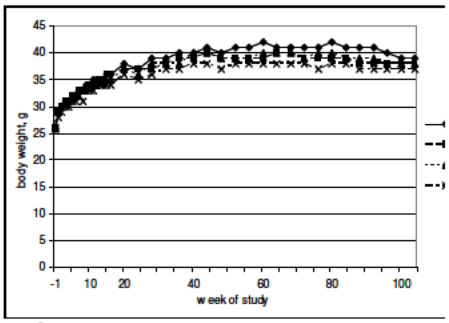
Increased mortality was seen in top dose males during the early part of the study; findings are attributed by the study report to compaction of the diet due to the high proportion of test material, which resulted in reduced availability of the diet to the

animals in this group. Incidences of discoloured skin were higher in both sexes at ≥5000 ppm, however findings are not consistent, a dose-response relationship is not apparent and the toxicological significance of this finding is unclear.

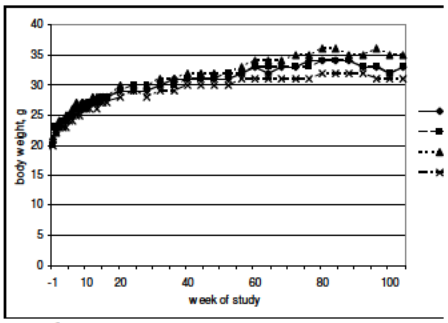
Bodyweights and food consumption

Mean bodyweights of top dose males were slightly (but significantly) lower than controls throughout the study period; no treatment-related effects were seen in other groups of males. Mean bodyweights of top dose females were also consistently lower than controls; values attained statistical significance at some time points. Food consumption was consistently higher in both sexes at the top dose level; values consistently attained statistical significance in males and were occasionally statistically significant in females. Findings are likely to be due to the inclusion of the test material at a high level (5%) in the diet.

Figure B.6.5.2-1 Chronic mouse study: bodyweights



males



females

Haematology

Treatment-related effects on haematological parameters indicative of mild anaemia were noted in both sexes at ≥5000 ppm. Findings were more marked in males; values in 5000 ppm females were less consistently affected but occasionally attained statistical significance.

Table B.6.5.2-1 Chronic mouse study: haematology

Parameter/ Month		Dose level (ppm)							
		Males				Females			
		0	500	5000	50000	0	500	5000	50000
RBC (10^6/µm)	**6**	8.30	8.39	8.01	7.41**	8.31	7.65	8.06	7.24**
	12	7.89	8.11	7.49	6.72**	7.83	7.64	7.41	6.84**
	12[a]	7.90	7.71	7.80	7.00**	7.59	7.83	7.27	7.06*
	18	7.68	7.95	7.26	7.07	8.10	7.79	7.15*	6.94**
	24	7.73	7.87	7.46	6.88	7.32	6.99	7.04	6.20
Hb (g/dl)	**6**	16.4	16.3	16.4	17.3	16.8	15.7	16.5	16.6
	12	15.5	15.8	16.1	15.9	16.2	15.7	15.6	16.3
	12[a]	15.6	15.4	15.8	15.8	15.5	15.4	15.3	15.4
	18	14.9	14.8	14.7	16.5	15.8	15.0	14.2	15.7
	24	14.9	14.8	15.2	15.8	14.2	13.5	14.3	14.0
Hct (%)	**6**	49.4	49.4	47.3	44.1**	50.4	46.6	48.8	45.0**
	12	46.2	46.6	43.8	39.3**	47.0	45.4	44.3	41.8**
	12[a]	44.4	44.3	42.7	38.9**	44.5	44.2	41.3*	40.6**
	18	45.5	45.8	41.5	41.9	48.0	45.9	42.1*	41.7**
	24	41.8	40.9	39.7	38.2	39.4	37.7	38.9	36.3
MCV (μm^3)	**6**	60	59	59	60	61	61	61	62
	12	59	58	59	59	60	59	60	61
	12[a]	56	58	55	55	59	57	57	58
	18	60	58	58	60	59	59	59	60
	24	54	52	53	56	54	54	55	59
MCH (µg)	**6**	19.8	19.5	20.5	23.4**	20.3	20.6	20.5	23.0**
	12	19.7	19.5	21.5*	23.7**	20.8	20.6	21.1	23.9**
	12[a]	19.7	20.0	20.2	22.6**	20.4	19.7	21.0	22.0*
	18	19.5	18.7	20.4	23.4**	19.5	19.2	19.9	22.6**
	24	19.3	18.8	20.5	23.0**	19.3	19.3	20.3	22.7**
MCHC (g/dl)	**6**	33.3	33.2	34.7**	39.2**	33.4	33.7	33.9	37.0**
	12	33.6	33.9	36.8**	40.6**	34.5	34.7	35.2	39.2**
	12[a]	35.0	34.8	37.0**	40.7**	34.7	34.9	37.1**	38.0**
	18	32.8	32.4	35.3*	39.4**	32.8	32.6	33.7	37.7**
	24	35.6	36.1	38.4**	41.4**	36.1	35.7	36.7	38.5*

**significantly different to controls (p<0.05); **p<0.01*
[a]*interim sacrifice*

Organ weights and pathology

Mean absolute and relative spleen weights were significantly increased in both sexes at the top dose level at the interim sacrifice; terminal sacrifice values were also elevated in this group but did not attain statistical significance. Findings are consistent with the haematological effects seen in these groups. Absolute brain weight was slightly (but significantly) lower in top dose females at the terminal sacrifice; mean relative brain weight was unaffected by treatment and this finding is considered to be secondary to effects on bodyweight in this group. Gross necropsy revealed an increased incidence of lung nodules in males at the interim sacrifice; similar findings were not seen in females or in either sex at the terminal sacrifice. A treatment-related increase in renal cystic change was seen in males at the terminal sacrifice; a similar finding was not apparent in females. Microscopic treatment-related findings were limited to the accumulation of a brown granular pigment in the spleen (in both sexes at the top dose level and in 5000 ppm males), renal proximal tubule epithelium (in top dose females)

and hepatic Kupffer cells (in both sexes at the top dose level). Findings are consistent with the haematological effects and indicate the increased breakdown of erythrocytes.

Table B.6.5.2-2 Histopathological findings in the mouse

ppm in diet	0		500		5000		50000	
	M	F	M	F	M	F	M	F
Interim kill (n==10)								
Liver inflammation	1	4	5	2	4	1	2	4
Hepatocyte hypertrophy	0	0	2	0	1	2	0	0
Alveolar haemorrhage	1	10	5	4	2	2	1	2
Stomach hyperplasia	3	0	0	7	0	6	3	6
Spleen brown pigment	0	0	0	10	1	9	5	8
2 years (all animals)								
Stomach adenoma	0	2	3	3	2	1	1	0
Stomach adenocarcinoma	1	0	2	0	0	0	0	0
Hepatocellular adenoma	8	0	16	4	4	2	6	0
Hepatocellular carcinoma	3	1	10	4	9	1	2	0
Alveolar adenoma	17	11	8	16	8	12	11	8
Pituitary adenoma	0	1	0	5	0	5	0	0
Stomach epithelial hyperplasia	10	25	8	33	9	32	10	30
Testes mineralisation (1 / 2)	16 /7	X	14/7	X	15 / 10	X	18 / 12	X
Uterine corpus cystic endometrial hyperplasia	X	23	X	30	X	40	X	36
Spleen brown pigment	4	39	1	32	9	33	6	40

With regard to tumour incidence, historical control data was submitted from the performing laboratory and the period covering two years prior to and 2 years after the Asulam study were deemed appropriate for comparison. No survival information was submitted for the studies forming the historical control data set.

The historical data for liver tumours (mean and range) are presented in Table 6.2, together with the equivalent data for the low dose group from the asulam study. The incidences in the low dose asulam groups are greater than the upper end of the historic control range for both hepatocellular adenomas and carcinomas and in both sexes. However, the relevance of this finding is unclear as there is no increase in incidence with increasing dose over a 100 fold range, if anything the doe response is an inverse one. The incidences of hepatocellular carcinomas in mid-dose animals are also outside the historical control range indicating there might be a relatively high spontaneous incidence, but this is not supported by the relatively low incidence at the highest dose level. There were no obvious differences in survival in the asulam study that might explain the observed tumour profile. Overall, it is considered that the hepatocellular

tumours seen at the low dose are unlikely to be a direct result of asulam administration due to the absence of a dose response relationship.

Table B.6.5.2-3 Liver tumour data for CD-1 mice (%)

	Historic data (initiated 1986 – 1996)		Historic data (initiated 1987 – 1991)		Asulam 500 ppm
	Mean	Range	Mean	Range	
Males					
Hepatocellular adenoma	14.1	7 - 22	13.5	7 - 22	32
Hepatocellular carcinoma	4.4	0 - 10	4.9	0 - 10	20
Females					
Hepatocellular adenoma	2.9	0 – 8	4.0	0 – 8	8
Hepatocellular carcinoma	0.4	0 - 2	0.3	0 - 2	8

No treatment-related effects were seen on the incidence of tumours in any other tissue.

Table B.6.5.2-4 Mouse carcinogenicity study: summary of findings

Parameter/ time point			**Dose levels (ppm)**							
			Males				**Females**			
			0	**500**	**5000**	**50000**	**0**	**500**	**5000**	**50000**
Intake (mg/kg bw/d)			-	74	730	8040	-	95	938	10353
Survival (%)		10	100	100	99	93	99	99	100	72
		26	100	99	96	92	96	95	100	93
		52	97	97	92	91	96	95	96	89
		78	88	80	82	56	80	88	85	72
		105	43	51	38	43	46	58	45	34
Bodyweight (g)		1	29	29	29	28**	22	23*	22	22
		13	35	35	35	34*	27	27	28	26
		24	37	37	36	35*	29	30	30	29
		52	41	39	39	38*	31	32	32	30
		80	42	39*	40	38**	34	34	36	32*
		104	39	39	38	37	33	33	35	31
Organ weights										
Spleen	**12**	(g)	0.14	0.15	0.13	0.20*	0.14	0.16	0.15	0.30
		Rel[a]	3.61	4.01	3.66	5.55**	4.39	4.84	5.18	9.54**
		Rel[b]	2.69	2.94	2.60	4.03*	2.62	2.94	2.92	5.70
	24	(g)	0.13	0.15	0.22	0.24	0.21	0.19	0.30	0.20
		Rel[a]	3.31	3.88	5.90	6.35	5.95	6.07	8.18	6.37
		Rel[b]	2.44	2.80	4.31	4.51	3.76	3.62	5.56	3.94
Gross pathology										
Lung nodule		12	-	1	-	3	-	-	-	-
		24	15	10	10	10	9	18	15	8
Kidney cyst		12	-	-	-	-	-	-	-	-
		24	6	7	10	15	3	3	2	2
Spleen: brown granular pigment deposition										
[Total]			4	1	11	13	29	44	44	51
Found dead (0-12m)			0/2	0/1	1/5	1/6	0/3	0/4	2/3	3/6
Moribund (0-12m)			0/0	0/1	0/1	1/1	0/1	0/1	0/0	0/1
Interim sacrifice			0/10	0/10	1/10	5/10	0/10	10/10	9/10	8/10

Found dead (12-24m)	1/24	1/22	4/22	3/20	8/22	2/11	9/18	17/25
Moribund (12-24m)	3/9	0/8	5/11	3/8	3/9	6/10	8/15	7/11
Terminal sacrifice	0/28	0/33	0/25	0/28	18/30	24/38	16/29	16/21
Liver: brown granular pigment deposition								
[Total]	0	9	10	13	1	1	2	12
Found dead (0-12m)	0/2	0/1	0/5	0/6	0/3	0/4	0/3	0/7
Moribund (0-12m)	0/0	0/1	0/1	0/1	0/1	0/1	0/0	1/1
Interim sacrifice	0/10	0/10	0/10	8/10	0/10	0/10	0/10	2/10
Found dead (12-24m)	0/24	0/22	0/23	2/21	0/22	0/11	1/18	3/25
Moribund (12-24m)	0/9	0/8	2/11	1/9	1/9	1/10	0/15	3/11
Terminal sacrifice	0/28	9/33	8/25	2/28	0/30	0/38	1/29	3/21
Kidneys: brown granular pigment deposition								
[Total]	1	1	2	5	9	8	7	16
Found dead (0-12m)	0/2	0/1	0/5	0/6	0/3	0/4	0/3	0/7
Moribund (0-12m)	0/0	0/1	0/1	0/1	0/1	0/1	0/0	0/1
Interim sacrifice	0/10	0/10	0/10	3/10	0/10	1/10	1/10	4/10
Found dead (12-24m)	0/25	0/22	2/23	2/21	1/22	0/12	1/18	5/25
Moribund (12-24m)	0/9	0/8	0/11	0/9	3/9	1/10	2/15	1/11
Terminal sacrifice	1/28	1/33	0/25	0/28	5/30	6/38	3/29	6/21

**significantly different to controls (p<0.05); **p<0.01*
rel[a]: organ weight relative to bodyweight rel[b]; organ weight relative to brain weight

Significant toxicological findings in both sexes of the mouse were confined to haematological effects at ≥ 5000ppm; reduced red blood cell count, haematocrit and MCHC and increased mean cell volume. At terminal sacrifice there was an increase in spleen weight at the top dose in both sexes and microscopic changes in the spleen, liver and kidney but these are a result of the haematological changes. An increased incidence of brown pigment in Kupffer cells was also reported at the lowest dose tested in males but in the absence of haematological correlates this is not an adverse effect. Overall, a non-neoplastic NOAEL can be determined at 500ppm for both sexes of the mouse.

There were no treatment related tumours in either sex of the mouse and the NOAEL for neoplastic findings is > 50000ppm.

[redacted] 1992

B.6.5.3 Summary of chronic toxicity and carcinogenicity studies

The most significant and consistent effects across both species were alterations to haematology parameters. The haematological effects were indicative of mild anaemia and the histopathology data on the spleen, kidney and liver are in support of an increased breakdown of red blood cells. Minor effects on bodyweight were also seen at high dose levels but these were attributable to high compaction of the diet, particularly in the mouse study.

In the rat chronic study, increased thyroid weight and hyperplastic changes were reported which are consistent with the data from the 90 day rat study and dog study. The effects on the thyroid are of limited relevance to risk assessment based on the published literature regarding the species differences in inhibition of thyroid peroxidase (see section 6.3.6). The adrenal was also identified as a target organ in the male rat, with increased incidences of medullary hyperplasia at high dose levels.

Evidence of carcinogenicity was limited to the male rat, with an increased incidence of phaeochromocytoma at the top dose level. The phaeochromocytomas were accompanied by histopathology observations (proliferative changes in the adrenals) and were marginally outside of the laboratory's historical control data and published data for this strain of rat during the study period (1977-1985). According to the study report, CD rats of Sprague Dawley origin were purchased from Charles Rivers Laboratories. There is evidence that the incidence of phaeochromocytomas with Asulam is within the variability seen in Sprague Dawley rats in published studies (4-33%). The most relevent paper being the paper by [redacted] 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%. There is also no evidence in the current database of studies with Asulam that suggest the mechanism of the substance fulfils the conditions to cause phaeochromocytomas e.g. perturbation of calcium homeostasis, uncoupling of mitochondrial respiration and hypoxia ([redacted], 2009).

In the original assessment of Asulam, no evidence of carcinogenicity was seen in the mouse. The incidence of hepatocellular adenoma and carcinoma are however increased at the lowest dose of Asulam (74 mg/kg bw/d) in both sexes and are outside the historical control data. In the absence of a dose response, general toxicity in higher doses to account for the decrease in tumour incidence, accompanying histopathology or liver weight changes, these tumours are insufficient for classification purposes (see Volume 1 and C&L report by the UK CA).

The conclusion of the previous EU review was that asulam had no carcinogenic potential relevant to humans.

Table B.6.5.3-1 Summary of chronic toxicity and carcinogenicity studies

Study	NOAEL (ppm and mg/kg bw/d)	LOAEL (ppm and mg/kg bw/d)	Effects at LOAEL	Reference
2 year rat carcinogenicity	Systemic M&F: 1000ppm (36 and 47mg/kg bw/d)	Systemic M & F: 5000ppm (180 and 243 mg/kg bw/d)	Haematological effects and proliferation of the adrenal medulla	[redacted], 1980
	Neoplastic M & F:> 25000ppm (953 mg/kg bw/d)	Neoplastic M & F: Not determined	M: Phaeochromocytoma but not considered to be treatment related therefore no effects at LOAEL. F: Not determined	
2 year mouse carcinogenicity	Systemic M & F: 500ppm (74 and 95 mg/kg bw/d)	Systemic M & F: 5000ppm (730 and 938 mg/kg bw/d)	Systemic:Haematological effects including decreased Hct and increased MCV	[redacted] 1992
	Neoplastic M & F: >50000ppm (8040 and	Neoplastic M & F: Not determined	Neoplastic: Not determined	

	10353 mg/kg bw/d)			

B.6.6 Reproductive and developmental toxicity studies (IIA 5.6)

A two generation reproduction study in the rat and two developmental studies in the rat and rabbit have been submitted. These were all evaluated previously by the RMS and the most recent versions of the studies have been presented. The studies are acceptable for satisfying the data requirements for a pesticide active substance, deriving NOAELs and use in risk assessment but have been deemed as compromised for the purposes of classification (see Volume 1 and accompanying C&L report by the UK CA).

B.6.6.1 Multi-generation study in the rat

This study was not fully compliant with modern guidelines at its previous assessment in a number of areas, however the study was sufficient to identify the critical effect of the test material on reproduction. The study design was also non-standard in some areas (specifically the use of 1:2 mating, the lack of litter standardisation and the untreated period prior to necropsy of F_2 pups), however these aspects were not considered to significantly compromise the study.

The notifier has commented on the deficiencies with the study and the justification for not completing additional vertebrate studies to fulfil any data gaps in the current submission. These are presented below:

1. Litters were not standardised on lactation day 4, F_2 pups were switched to controlled diets 30 days prior to necropsy (thereby making any organ weight findings were considered unlikely to be treatment related) and mating involved the use of 1 male to 2 females. These aspects however are not considered to comprise the study.

2. Sperm parameters not measured. Mating performance and fertility were comparable in all groups from both the P_0 and P_1 parents. The number of live pups/litter was decreased in the F_1 generation (5000 and 25000 ppm from day 0 onward) with a slight non dose related decrease in the F_2 litters. No explanation was provided to clarify these findings in the absence of maternal toxicity.

3. Whilst it is recognised that spermatozoa parameters were not investigated, further vertebrate testing was considered unjustified based on the following: i) the sub-chronic (mouse, rat, dog), chronic (dog) and carcinogenicity toxicity (mouse, rat) and 2-generation studies showed no evidence of adverse effects on the testes (+epididymides)/seminal vesicles; ii) this was not identified as a critical area during the review conducted in 2010. The RMS agreed to adjust the LoEP to indicate that “reduced litter size” was observed; iii) although no explanation has been provided for the effects observed, this is not expected to affect the derivation of the ADI (for which a lower NOAEL in the 2yr rat study is proposed). This is a

view that was also shared during the review; iii) a clear NOAEL was established for reproductive effects (1000 ppm [46 mg/kg bw/d] vs. the LOAEL of 5000 ppm [224 mg/kg bw/d]).

Despite the deficiencies, the results and conclusions of the study are acceptable for derivation of NOAELs and risk assessment as required. The RMS has updated the conclusion but no alterations were made to the NOAELs.

Study	Asulam: two generation reproduction study in the rat
Reference	[redacted], 1981a
Date performed	August 1978 - April 1980
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	-
Deviations from the guideline	-
GLP	No: pre-GLP, no reference to QA audit
Test material	Asulam, batches JN120 and JN165; 99% purity after drying
Study acceptable	Yes: deviation from modern guidelines is not considered to significantly reduce the quality of the study. The relevant reproductive effects have been identified.

Groups of 12 male and 24 female 35 day-old CD rats (F_0 generation) were administered test material in the diet at concentrations of 0, 1000, 5000 or 25000 ppm for 100 days prior to mating (1:2) and throughout mating, gestation and lactation of the resulting (F_1) litters. The F_1 litters were not standardised on Day 4 *post partum*, but were allowed to grow through to weaning at Day 30 *post partum*, when F_0 animals were sacrificed. Selected F_1 weanlings (16 males and 32 females/group) were mated at 120 days following weaning to produce the F_2 generation. F_2 litters were not standardised on Day 4 *post partum*, but were allowed to grow through to weaning at Day 30 *post partum*. Following weaning, all F_2 pups were fed control diet for 30 days. Mating was confirmed by vaginal smear; females failing to mate after 10 days were housed with another male from the same group. Gross necropsy was performed on F_0 parents; the testes of all males were weighed and investigated histopathologically. Gross necropsy was also performed on all non-selected F_1 pups at weaning; weights of the pituitary, adrenals, ovaries, testes, heart, lungs, kidneys, spleen, brain and prostate were recorded (5/sex/group). Gross necropsy was performed on all non-selected F_1 parents at 30 days following weaning of the F_2 litters; weights of the pituitary, adrenals, ovaries, testes, heart, lungs, kidneys, spleen, brain and prostate were recorded (10 males and 25 females/group). Gross necropsy was performed on all F_2 pups also at Day 60 *post partum*; weights of the pituitary, adrenals, ovaries, testes, heart, lungs, kidneys, spleen, brain and prostate were recorded (5/sex/group).

<u>F_0 parents</u>

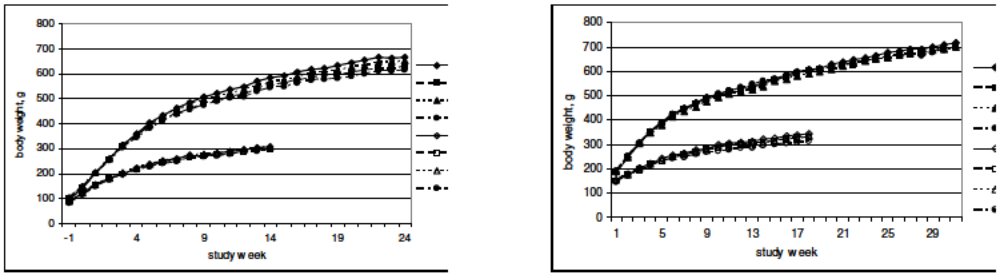
No deaths occurred and no signs of toxicity were observed. Mean bodyweights of top dose males were slightly lower than controls throughout the study period; no treatment-related effects on bodyweight were noted in females during the pre-mating, gestation or lactation periods. Food consumption during the pre-mating and gestation periods was unaffected by treatment. Mating performance and fertility were

comparable in all groups. Gross necropsy did not reveal any treatment-related findings; testes weights were unaffected by treatment.

F1 parents

No treatment-related deaths occurred and no signs of toxicity were observed. Mean bodyweights of all groups of treated males were lower than controls from mating, however the absence of a dose-response does not indicate a relationship to treatment. Mean bodyweights of top dose females at mating were slightly (~10%, but significantly) lower than controls as a result of reduced weight gain during the pre-mating period. Bodyweights of top dose females were lower throughout gestation, although weight gain during gestation was comparable in all groups. Weight loss in all groups during lactation was comparable. Food consumption during the pre-mating period was unaffected by treatment; food consumption by top dose females during gestation was slightly (but consistently) lower than controls. Mating performance and fertility were comparable in all groups. Necropsy did not reveal any gross or microscopic findings related to treatment. 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.

Figure B.6.6.1-1 Mean pre-mating bodyweights in adult F0 and F1 rats



F0 rats *F1 rats*

Table B.6.6.1-1 Multi-generation study: summary of findings in adults

Parameter			Dose level (ppm)							
			Males				Females			
			0	1000	5000	25000	0	1000	5000	25000
Pre-mate intake (mg/kg bw/d)		F0	-	46-	224-	1162-	-	58-	278-	1531-
			-	124	568	3070	-	119	612	3409
		F1	-	47-	223-	1136-	-	58-	274-	1456-
			-	132	680	3274	-	130	674	3358
F0	Weight (g)	Pre-mate	585	555	566	545	308	296	309	297
		Gestation d22					430	415	417	406
		Lactation d30					319	318	325	311
		Terminal	668	629	652	615				
	Gain (g)	Pre-mate	487	455	463	445	221	209	219	215
		Gestation					124	118	111	112
		Lactation					-22	-13	-25	-25
		Terminal	570	529	549	515				
F1	Weight (g)	Pre-mate	608	601	589	596	342	331	333	310**
		Gestation					451	426	450	426

		d22								
		Lactation d30					326	299*	335	312
		Terminal	717	697	699	696				
	Gain (g)	**Pre-mate**	425	415	401	407	193	182	181	165
		Gestation					120	115	118	118
		Lactation					-40	-42	-51	-33
		Terminal	534	511	511	507				
Organ weights										
F1	**Liver**	(g)	26.3	26.6	25.6	25.4	13.5	12.0*	13.3	11.4**
		rel	3.78	3.77	3.68	3.55	3.57	3.45	3.54	3.27**
	Thyroid	(mg)	31.6	35.3	34.2	39.4**	29.4	26.9	29.1	29.0
		rel	4.6	5.2	5.0	5.5*	7.8	7.7	7.7	8.2

**significantly different to controls (p<0.05); **p<0.01*

F_1 pups

Mean litter size at birth (and subsequently at all time points up to weaning) was reduced in a dose-related fashion in all treated groups; values attained statistical significance at ≥5000 ppm. The number of pups dead at birth was comparable in all groups. The viability index was comparable in all groups; other survival indices (including the lactation index) were slightly greater at ≥5000 ppm, probably reflecting the smaller litter size in these groups. Mean pup weights were not adversely affected by treatment; a tendency to higher pup weight at ≥5000 ppm may also reflect the smaller litter size in these groups. Necropsy did not reveal any gross or microscopic findings related to treatment. Mean absolute and relative liver weights were lower in treated groups, however the absence of a dose-response does not suggest a relationship to treatment. Absolute and relative ovary weights were lower in top dose females.

F_2 pups

Mean litter size at birth was lower in all treated groups, however a dose-response relationship is not apparent and values only attain statistical significance at 5000 ppm. The number of pups dead at birth was comparable in all groups. Viability index was lower at 25000 ppm in terms of percentage but not in absolute numbers of pups dying. Post Day 4 survival indices were also lower at ≥5000 ppm (but without a dose-response relationship) and lactation index was lower at 5000 ppm only. Mean pup weights at 5000 ppm were significantly higher than controls from Day 4 *post partum*; findings reflect the smaller litter size in this group. Bodyweights of F_2 rats following weaning and up to termination at Day 60 *post partum* were slightly lower in both sexes at the top dose level (significantly in males) due to reduced weight gain. Mean absolute and relative pituitary and adrenal weights were significantly higher in females at the top dose level; liver weights were slightly lower in top dose males; relative kidney weights were significantly higher in males at ≥5000 ppm; thyroid weight was also elevated in this group. In contrast to F_1 pups, ovary weight was unaffected by treatment (but the later sampling time in F_2 pups is noted). Necropsy did not reveal any gross or microscopic findings related to treatment.

Table B.6.6.1-2 Multi-generation study: summary of findings in pups

Parameter / Time point			Dose level (ppm)							
			F1				F2			
			0	1000	5000	25000	0	1000	5000	25000
Litter size (#)		Day 0	12.4	11.1	9.6**	9.3**	11.0	10.1	8.0*	9.4
		Day 4	10.9	10.6	9.4	8.8*	9.3	9.9	7.1	7.9
		Day 11	11.3	10.6	9.3*	8.7**	9.3	9.9	7.2	7.9
		Day 18	11.3	10.6	9.2*	8.7**	9.3	9.8	7.2	7.9
		Day 25	11.3	10.6	9.1*	8.7**	9.3	9.8	7.2	7.9
		Day 30	11.2	10.5	9.1*	8.7*	9.3	9.8	7.2	7.8
Survival (%)		Day 4	93	95	98	94	88	92	88	82
		Day 11	92	92	97	94	87	91	82	82
		Day 18	92	92	96	94	87	91	82	82
		Day 25	92	91	95	94	87	91	82	82
		Day 30	92	91	95	94	87	91	82	82
Viability index (%)			93	95	98	94	88	92	88	82
Lactation index (%)			93	91	97	99	98	99	93	100
Pup weight (g)		Day 0	6.1	6.1	6.6*	6.2	6.0	6.3	6.2	6.2
		Day 4	9.1	8.8	10.3	9.2	8.6	9.3	10.1*	9.3
		Day 11	22.2	21.2	24.1	21.7	20.8	22.2	26.7**	22.4
		Day 18	36.0	35.7	39.7	37.4	33.9	36.1	43.1**	35.7
		Day 25	64.3	63.8	70.4	68.8	64.0	66.6	75.1**	66.1
		Day 30	92.2	91.2	100.9*	93.4	90.1	85.9	103.7*	91.8
		F2	Males				Females			
		Day 37	**152**	147	162	154	120	126	140**	126
		Day 44	**212**	206	223	207	159	156	167	155
		Day 51	**265**	262	281	260	182	181	192	174
		Day 58	**316**	309	324	303*	200	200	214**	195
Organ weights			Males				Females			
			0	1000	5000	25000	0	1000	5000	25000
F1	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
	Adrenal	(mg)	25.7	25.8	34.7	29.7	26.8	30.5	35.3*	24.4
		(rel)	25.5	25.8	31.0	29.4	28.8	30.7	34.4	29.2
	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*
	Kidney	(g)	1.24	1.27	1.38	1.24	1.14	1.16	1.33	1.01
		(rel)	1.22	1.23	1.22	1.23	1.23	1.17	1.29	1.19
	Ovary	(mg)					55.5	47.9	54.7	38.3*
		(rel)					58.6	48.1	54.3	47.0
	Pituitary	(mg)	10.3	9.8	11.2	10.9	10.3	10.2	11.7	13.2*

F2		(rel)	3.1	3.1	3.4	3.3	4.9	5.0	5.5	6.3*
	Adrenal	(mg)	45.7	42.0	51.3	49.3	60.3	57.9	60.4	66.5
		(rel)	13.8	13.5	15.7	15.1	28.2	28.0	28.5	31.9
	Liver	(g)	18.2	14.9	17.9	16.5	10.0	9.7	9.6	9.2
		(rel)	5.45	4.67*	5.47	4.96	4.64	4.67	4.43	4.40
	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*
	Ovary	(mg)					112	121	108	117
		(rel)					53.4	59.4	50.9	56.3

**significantly different to controls (p<0.05); **p<0.01*

Conclusion

Parental toxicity manifested as a decrease in body weight and a variation in organ weight at 25000ppm. In the F_0 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. Based on the effects at the top dose, 5000ppm (224 and 278 mg/kg bw/d) is proposed as the parental NOAEL.

A statistically significant reduced litter size at ≥ 5000ppm was reported in the F1 generation and in the F2 generation but without statistical significance being attained or a dose-response relationship. As this is the only reproductive effect in the study, the NOAEL for reproduction is 1000ppm (46 and 58 mg/kg bw/d) based on the results in the F1 generation.

There were no significant adverse effects reported in the offspring of either generation at the highest dose tested. The slightly (but significantly) increased relative kidney weight in F_2 males at 5000ppm is not considered to be clearly treatment-related in the absence of any microscopic correlates and the absence of renal effects in other studies. It is also noted that these animals had not been exposed to the test material for 30 days prior to necropsy; any organ weight findings are therefore considered unlikely to be related to treatment. Therefore, the NOAEL for offspring toxicity is 25000ppm (ca 1136mg/kg bw/d).

[redacted] 1981

B.6.6.2 Developmental toxicity in the rat (IIA 5.6.1)

This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report.

Study	Asulam: Teratology study in the rat
Reference	[redacted] 1982
Date performed	December 1981 – January 1982
Test facility	[redacted]
Report reference	[redacted]

Guideline(s)	None specified
Deviations from the guideline	-
GLP	Yes
Test material	Asulam, batches MN991 (98.4% purity) & MN1201 (98.5% purity)
Study acceptable	Yes

Mated female CD rats (20/group) were gavaged with the test material (suspended in aqueous tragacanth) 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.

Results

No clinical signs were observed. Group mean bodyweights and food consumption were unaffected by treatment. Litter parameters were comparable in all groups. Foetal examination did not reveal any external or visceral abnormalities attributable to treatment. A number of minor skeletal findings indicative of reduced or delayed ossification were increased in incidence at the top dose level. Increases were slight and were mainly within the laboratory's historical control range, however the incidences of some findings exceeded the historical control range or had not previously been reported in control animals and are therefore considered to be treatment-related. The incidence of extra rib(s) was increased in all treated groups, however all values lie within the historical control range and the incidences of these findings in the concurrent control group are low compared to the historical control range.

Table B.6.6.2-1 Group litter data and historical control data (based on a maximum of 10954 foetuses from 74 studies)

Parameter		Dose level (mg/kg bw/d)				Historical control mean (range)
		0	500	1000	2000	
Pregnant		20	20	20	20	
Corpora lutea		16.9 ±2.8	16.7 ±3.3	17.6 ±3.8	17.6 ±2.6	15.9 (14.0-18.3)
Implantations		14.7 ±1.5	14.3 ±1.9	14.6 ±2.2	15.2 ±1.4	14.2 (11.6-16.5)
Viable foetuses	**Males**	6.2 ±2.1	6.4 ±2.2	7.1 ±2.2	7.6 ±1.8	6.8 (5.0-8.0)
	Females	7.4 ±2.1	7.2 ±2.1	6.8 ±1.7	7.2 ±2.0	6.7 (5.5-8.4)
	Total	13.5 ±2.9	13.6 ±2.2	13.9 ±2.3	14.7 ±1.7	13.5 (10.9-15.9)
Resorptions	**Early**	0.95 ±0.97	0.60 ±0.77	0.50 ±0.71	0.30 ±0.55	0.55 (0.08-1.53)
	Late	0.20 ±0.45	0.10 ±0.32	0.25 ±0.50	0.15 ±0.39	0.13 (0.00-1.45)
	Total	1.15 ±1.07	0.70 ±0.84	0.75 ±0.87	0.45 ±0.67	0.68 (0.07-1.91)
Pre-implantation loss		13.3%	14.7%	17.0%	13.9%	11.0 (2.6-20.9)
Post-implantation loss		7.8%	4.9%	5.1%	3.0%	4.8 (0.5-14.0)
Foetal weight (g)		3.32 ±0.07	3.39 ±0.06	3.38 ±0.07	3.31 ±0.07	3.69 (3.5-4.04)
Placental weight (g)		0.46 ±0.02	0.47 ±0.02	0.48 ±0.02	0.47 ±0.01	0.50 (0.45-4.04)
Foetal findings (% foetal incidence, % litter incidence)						
↓ossification; cranium		-	-	-	0.5 (5)	-
Ribs 13/13		99.4 (100)	98.3 (100)	95.6 (100)	91.9 (100)	90.8 (63.1-100)

Ribs 13/14	-	1.7 (10)	3.3 (20)	4.6 (35)	5.4 (0.6-20.5)
Ribs 14/14	-	-	1.1 (5)	3.0 (25)	3.82 (0.0-17.9)
13th rib(s) short/absent	1.1 (10)	-	-	4.1 (15)	0.07 (0.0-1.7)
↓ossification; 5 sternebrae	-	-	0.6 (5)	1.0 (10)	1.18 (0.0-6.1)
↓ossification; 6 sternebrae	-	-	-	0.5 (5)	0.59 (0.0-4.4)
Fused cervical arches	-	-	-	0.5 (5)	-
↓ossification; lumbar centrae	-	-	-	0.5 (5)	1.11 (0-4.6)
↓ossification; caudal vertebrae	1.1 (5)	1.7 (3)	1.1 (2)	2.5 (5)	1.01 (0.6-1.2)
↓ossification; pubis	0.6 (5)	1.7 (15)	2.2 (5)	2.5 (20)	0.27 (0-3.1)
↓ossification; ischium	-	-	-	0.5 (5)	0.03 (0-0.6)

Conclusion

The dams did not exhibit any clinical signs, significant changes in body weight or food consumption at any of the tested doses. A maternal NOAEL of 2000 mg/kg bw/d can be determined, in the absence of any treatment-related findings.

There were 4 effects noted in the foetuses at the top dose which are outside the historical control range; decreased ossification of the cranium, short/absent 13th rib, fused cervical arches and decreased ossification of caudal vertebrae. None of these effects were seen at lower doses. Whilst the findings do occur in the absence of maternal toxicity they are only present at doses exceeding the limit dose for developmental studies. Subsequently, a developmental NOAEL of 1000 mg/kg bw/d can be determined, based on a pattern of minor foetal skeletal effects at the top dose level.

[redacted] 1982

B.6.6.3 Developmental toxicity in the rabbit (IIA 5.6.1)

This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report.

Study	Asulam: Teratogenicity study by the oral route in the rabbit
Reference	[redacted] 1981a
Date performed	26 April 1980 - 14 October 1980
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	None specified
Deviations from the guideline	-
GLP	No: pre-GLP but QA inspected
Test material	Asulam batch JN 165; 98.0% purity
Study acceptable	Yes; there are some concerns over study conduct, however the study is considered adequate to address developmental toxicity

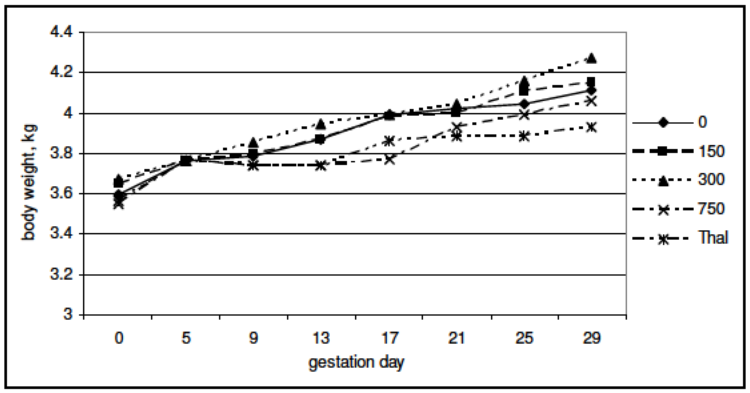
Mated female New Zealand White rabbits (15/group) were gavaged with the test material (suspended in aqueous gum tragacanth) on Days 5-20 of gestation at dose

levels of 0, 150, 300, 750 or 1500 mg/kg bw/d. A positive control group consisting of 11 dams was administered thalidomide (150 mg/kg bw) over the same period. It is reported that the female rabbits were delivered to the laboratory in five batches over a period of approximately 11 weeks; details of the day of dosing are not recorded, however dose analysis results show that doses of asulam were prepared over a three month period. The study was terminated on Day 29 of gestation and the uterine contents examined. Foetuses were investigated for external abnormalities and for visceral abnormalities by dissection. The brains of all foetuses were investigated by freehand serial sectioning and skeletal findings investigated following staining with Alizarin Red S.

Results

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 bodyweights of 750 mg/kg bw/d animals were lower than controls; more marked effects on bodyweight 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.

Figure B.6.6.3-1 Rabbit developmental toxicity study: bodyweights



Litter size and foetal weight were unaffected by treatment with asulam.

Table B.6.6.3-1 Rabbit developmental toxicity study: maternal and litter findings

Parameter		Dose level (mg/kg bw/d)				
		0	150	300	750	+ control
	Mated	30	28	23	23	14
	Not pregnant	4	1	2	4	2
	Total deaths	10	9	5	5	1
Reasons for death	Mis-dosed	2	7	3	4	-
	Suspected mis-dosed	4	-	1	-	-
	Respiratory distress/infection	2	-	-	-	-

	Broken back	1	-	1	1	-
	Found dead	1	-	-	-	-
	Abortion	-	2	-	-	1
Total resorption		-	-	-	-	2
Litters		16	18	16	14	8
Bodyweight (kg)	**Day 5**	3.70	3.58	3.60	3.76	3.54
	Day 9	3.72	3.61	3.69	3.74	3.52
	Day 13	3.81	3.69	3.78	3.74	3.52
	Day 17	3.93	3.80	3.83	3.77	3.64
	Day 21	3.96	3.82	3.88	3.93	3.66
	Day 29	4.05	3.97	4.11	4.06	3.71
Weight gain (kg)	**Day 0-29**	0.52	0.50	0.60	0.51	0.37
	Day 0-17	0.40	0.33	0.42	0.22	0.30
	Day 5-17	0.23	0.22	0.23	0.01	0.10
	Day 5-21	0.26	0.24	0.28	0.17	0.12
Food consumption (g/d)	**Day 5-9**	167	156	163	158	130
	Day 9-13	151	151	148	143	124
	Day 13-17	145	144	143	121	150
	Day 17-21	145	146	161	147	151
Corpora lutea (#)		10.4	10.3	11.4	10.9	10.8
Implantations (#)		9.3	8.7	10.6	9.1	8.7
Pre-implantation loss (%)		10.2	15.8	8.3	15.4	18.8
Post-implantation loss (%)		4.8	7.1	12.1	9.5	59.5
Foetal death (#)		6	11	22	12	52
Early (#)		4	6	11	1	18
Late (#)		2	5	11	11	34
Litter size (#)		8.9	8.1	9.2	8.2	3.5
Foetal weight (g)		35.5	38.7	36.1	36.6	35.1

The isolated incidence of foetal malformations was low in all groups and comparable to the control. There were 2 foetuses from 2 different litters at 300 and 750mg/kg which exhibited malformations (including cyclopia, distortion of skull bones, agenesis of lung lobe or kidney and hydrocephalus). The overall pattern of findings for malformations does not suggest a relationship to treatment with asulam.

Table B.6.6.3-2 Rabbit developmental toxicity study: foetal findings

Finding	**Dose group** (mg/kg bw/d)				
	0	**150**	**300**	**750**	**+ control**
Foetuses with minor malformations (#)	47 (38.5%)	27 (20.9%)	58 (38.7%)	33 (34.1%)	8 (23.1%)
Foetuses with major malformations (#)	-	-	2 (1.3%)	2 (1.9%)	19 (48.4%)
Incidence (foetal/litter)					
Sutural bone between frontal& parietal	1/1	1/1	-	1/1	
Sutural bone between parietals	-	-	1/1	-	
3 sutural bones between frontals & parietals	-	-	-	1/1	
Increased size of anterior fontanelle	-	5/3	4/3	-	
Palatine ridges appear inverted	-	-	-	1/1	
Body of hyoid, ossification absent	1/1	-	2/2	1/1	

5th sternebra, ossification absent	25/9	13/8	26/10	18/6	
6th sternebra, ossification absent	-	-	3/2	-	
5th and 6th sternebrae, ossification absent	-	2/1	1/1	1/1	
Ribs thickened	1/1	-	-	-	
7th and 8th ribs fused	-	1/1	-	-	
9th and 10th ribs fused	-	1/1	-	-	
2nd lumbar vertebra has no associated left vertebral arch, reduced central & right arch displaced to the right	-	-	-	1/1	
2nd and 3rd caudal vertebrae fused	-	-	-	1/1	
4th and 5th caudal vertebrae fused	-	-	-	1/1	
Incorrect articulation of both forelimbs	-	-	1/1	-	
Incorrect articulation of left forepaw	-	-	1/1	-	
Olecranon process/es, ossification absent	18/4	2/1	19/6	7/2	
Talus/tali, ossification absent	1/1	4/2	5/3	2/1	
Patella(e), ossification absent	18/4	5/3	24/5	12/3	
Malrotation of right hind limb about ankle	1/1	-	-	-	
Anury	-	-	1/1	1/1	
Blood-suffused iris, bilateral or unilateral	1/1	1/1	-	1/1	
Dextrocardia	1/1	-	-	-	
Agenesis of gall bladder	-	-	-	1/1	
Gall bladder and hepatic duct displaced	-	-	-	1/1	
Umbilical hernia	-	-	-	1/1	

Conclusion

Although the level of mortality in this study was high, the number of animals surviving to produce a litter exceeded the minimum (12) specified by the 1981 OECD guideline (414) and is comparable to the minimum (16) specified in the 2001 version. Despite concerns about the conduct of this study, it is considered that it is adequate to address the potential teratogenicity and developmental toxicity of the test material.

Reduced food consumption, body weight and body weight gain was recorded in dams at 750mg/kg bw/d. No effects were noted at lower doses and therefore the maternal NOAEL is 300 mg/kg bw/d.

No evidence of teratogenicity was seen in this study so a foetal NOAEL of 750 mg/kg bw/d can be determined in the absence of any treatment-related findings at the top dose level.

B.6.6.4 Summary of reproductive and developmental toxicity studies

Evidence of a reproductive effect was seen in the multi-generation study as litter size was significantly reduced at dose levels of ≥5000 ppm in the F1 generation. At 25000ppm (1136- 3409mg/kg bw/d), the reduction was in the presence of maternal toxicity including a reduction in body weight but there was no maternal toxicity at 5000 ppm to accompany the reduced litter size. The underlying reason for this effect was unclear as it was not due to an increase in the number of foetuses dead at birth. Furthermore, the effect was not statistically significant in the F2 generation. In addition, the rat developmental study did not report any effect on litter size nor effects on resorptions, abortions and implantation loss, even when tested at high doses of up to 2000 mg/kg bw/d. Thus, it appears that the reproductive findings in the F1 generation of the rat reproduction study are not repeatable in the current studies. There is also no evidence from the short and long term studies that asulam has any adverse effects on the reproductive organs which could contribute to a detrimental reproductive effect. Overall, the RMS concludes that asulam is not a reproductive toxicant.

The developmental toxicity of asulam has been investigated rats and rabbits. In rats, slight increases in supernumerary ribs and retarded ossification were observed at the top the dose of 2000 mg/kg/day only; twice the maximum recommended dose in the current OECD TG 414. As these changes were close to or within the relevant historical control range, they are regarded as slight increases within expected variation and can be discounted as not relevant for human health. No evidence of developmental toxicity was observed in rabbits at doses of up to 750 mg/kg/day, the highest tested. Overall, the RMS concludes that asulam is not a developmental toxicant.

Table B.6.6.4-1 Summary of reproductive and developmental toxicity studies

Study	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Effects at LOAEL	Reference
Multi-generation	Reproductive M: 46m/kg bw/d F: 58mg/kg bw/d	Reproductive M: 224m/kg bw/d F: 278mg/kg bw/d	Reduced litter size	[redacted] 1981
	Adults: M:224mg/kg bw/d F:278mg/kg bw/d	Adults: M:1136mg/kg bw/d F:1456mg/kg bw/d	Reduced body weight and organ weight effects	
	Offspring: 1136mg/kg bw/d	Not determined	No significant effects at the highest dose tested	
Rat developmental	Maternal: 2000mg/kg bw/d	Maternal: Not determined	No adverse effects at the highest dose tested	[redacted] 1981
	Developmental: 1000mg/kg bw/d	Developmental: 2000mg/kg bw/d	Minor skeletal effects	
Rabbit developmental	Maternal: 300mg/kg bw/d	Maternal: 750mg/kg bw/d	Reduced body weight during treatement and	[redacted] 1981

			reduced body weight gain at termination	
	Developmental: 750mg/kg bw/d	Not determined	No adverse effects at the highest dose tested	

B.6.7 Neurotoxicity studies (IIA 5.7)

As asulam contains a carbamate group, which is known to be associated with neurotoxicity, plasma, erythrocyte or brain cholinesterase activities were measured in the eight-week mouse study, 6-month dog study and the 21-day repeat dose dermal toxicity study in the rabbit. No significant inhibition was observed across the species or sexes in any of these studies.

As neither inhibition of cholinesterase has been reported nor neurotoxic signs in the short and long term studies, it is not necessary to conduct any other neurotoxicity studies.

B.6.8 Further toxicological studies (IIA 5.8)

B.6.8.1 Phototoxicity

No study has been submitted and this is agreed with as the dossier was submitted prior to 1st January 2014 and is being assessed to Regulation (EU) No 544/2011.

B.6.8.2 Published literature

A literature search is required for all new active substances under Regulation (EC) 1107/2009 Article 8(5) and the Notifiers for Asulam provided a report. The document addressed all specialisms and identified relevant publications accordingly.

Study	Asulam sodium literature review report: submission of scientific peer-reviewed open literature under regulation (EC) No 1107/2009
Reference	Tucker, K 2014
Date performed	-
Test facility	JSC International Limited
Report reference	UPL/10/02-KT 01
Guideline(s)	-
Deviations from the guideline	-
GLP	-
Test material	-
Study acceptable	Yes; there are some concerns over the search terms i.e whether Boolean term OR/AND was used for the metabolite search given the extensive number of returns (>10000) and most articles of a general/ broad range.

The report (Tucker, 2014) summarises the search for scientific peer-reviewed open literature on the active substance asulam (sodium) and its relevant metabolites(s). Health, the environment and non-target species were all included and the search was focused on the 10 years before the date of submission of the dossier.

In summary, an initial search was done to look for any references which included the active substance asulam and its synonyms, or its major metabolites, or its representative formulation. The returns for the metabolites were so extensive (>5000) that additional terms were included for specificity e.g. Tox, NOAEL.

The names searched for were:

- For asulam: asulam, asulam-sodium, asulox

- For the metabolites: sulphanilamide, suphanilic acid, acetyl-asulam, AP formamide, MCAPAP, malonyl-asulam, asulam-glc.

The additional key words combined with sulphanilamide and sulphanilic acid were designed to cover all endpoints, species, exposure routes/scenarios and risk for all specialisms. A total of 11 databases were searched and these covered a wide range of scientific areas from botany and agriculture to toxicology but with an emphasis on medical and biological sciences. A summary of the results is given in table 6.12.1-1.

Table 6.8.2-1 An overview of the results from the defined search terms and databases for asulam/ asulam-sodium/asulox

Data requirement(s) captured in the search	**Number**
Total number of *summary records* retrieved after *all** searches of peer-reviewed literature (excluding duplicates)	344
Number of *summary records* excluded from the search results after rapid assessment for relevance	333
Total number of *full-text* documents assessed in detail*	11
Number of *studies* excluded from further consideration after detailed assessment for relevance	10
Number of *studies* not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	1

*both from bibliographic databases and other sources of peer-reviewed literature

Table 6.8.2-2 An overview of the results from the defined search terms and databases for asulam sodium metabolites

Data requirement(s) captured in the search	**Number**
Total number of *summary records* retrieved after *all** searches of peer-reviewed literature (excluding duplicates)	10060
Number of *summary records* excluded from the search results after rapid assessment for relevance	10056
Total number of *full-text* documents assessed in detail*	4
Number of *studies* excluded from further consideration after detailed assessment for relevance	4
Number of *studies* not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	0

*both from bibliographic databases and other sources of peer-reviewed literature

The first tier relevance assessment was based on the study titles and abstracts alone and the second tier screen for relevance was completed following an assessment of the

full article. None of the publications were relevant to toxicology or non-dietary exposure and the RMS agrees.

Other papers have been used in the previous and current evaluation which are not covered by the scope of the literature review and presented below.

Doerge, D.R. & Decker, C.J. (1994). Inhibition of peroxidise-catalysed reactions by arylamines: Mechanism for the anti-thyroid action of sulfamethazine. *Chemical Research & Toxicology*, **7**, pp 164-169.

Capen, C.C. (1998). Correlation of mechanistic data and histopathology in the evaluation of selected toxic endpoints of the endocrine system. *Toxicology Letters*, **102-103**, pp 405-409.

McClain, R.M. (1995). Mechanistic considerations for the relevance of animal data on thyroid neoplasia to human risk assessment. *Mutation Research*, **333**, pp 131-142.

Poirier, L.A., Doerge, D.R., Gaylor, D.W., Miller, M.A., Lorentzen, R.J., Casciano, D.A., Kadlubar, F.F & Schwetz, B.A. (1999). An FDA review of sulfamethazine toxicity. *Regulatory Toxicology & Pharmacology*, **30**, pp 217-222.

These papers were assessed in the original DAR as a mechanism of action on the thyroid by Asulam-sodium and the conclusions have been presented in sections 6.3.6 and 6.10 (summary of mammalian toxicology and proposed ADI, AOEL, ARfD and MAC).

B.6.8.3 Relevant metabolites

In the final EFSA conclusion report, there was some indication that the metabolite, Sulphanilamide, was more toxic than asulam and residues were found in spinach. Consequently a data gap for consumers was identified. In order to address this data gap for consumers the following toxicological studies were undertaken by the notifier: acute oral toxicity, assessment of *in vitro* genotoxicity and sub-acute oral gavage study. As these studies have not previously been reviewed at EU level, an evaluation of these studies has been provided. In addition, published literature on Sulphanilamide has been referenced and discussed.

B.6.8.3.1 Acute toxicity in rats

Study	Sulphanilamide: Acute oral toxicity in the rat – fixed dose method.
Reference	[redacted] 2011
Date performed	1st - 20th September 2011
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	OECD 420, EU method B.1.bis Regulation (EC) No. 440/2008
Deviations from the guideline	None
GLP	GLP and QA statements provided
Test material	Sulphanilamide purity 99.7% Batch L011068299
Study acceptable	Yes

In a single-dose acute oral toxicity study one fasted, young adult female Wistar strain rat was given a single oral (gavage) dose of sulphanilamide in arachis oil at a dose of 2000 mg/kg bw. In the absence of mortality a further 4 animals were treated at the same dose level. A gross necropsy examination was performed on all animals at the time of death or scheduled euthanasia (study day 14).

No clinical signs of toxicity were observed and all animals gained weight over the study period. There were no mortalities reported and no gross abnormalities were observed at scheduled necropsy.

Based on the results of this study the oral LD_{50}- was determined to be >2000 mg/kg bw in female rats.

[redacted], 2011

B.6.8.3.2 Short term toxicity in the rat

Study	Sulphanilamide: Seven day repeated dose oral (gavage) range-finding study in the rat.
Reference	[redacted], 2011
Date performed	26th September- 3rd October 2011
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	None
Deviations from the guideline	N/A
GLP	GLP and QA statements provided
Test material	Sulphanilamide purity 99.7% Batch L011068299
Study acceptable	Yes as a range finding study

The objective of this study was to evaluate the toxicity of sulphanilamide when administered orally *via* gavage to rats for 7 days and assist in the dose selection for a 28-day study. Groups of rats (3/sex/gp) were administered sulphanilamide at doses of 0, 250, 500 or 1000 mg/kg bw/d. Animals were observed regularly for signs of toxicity and mortality. Bodyweight and food consumption were recorded periodically and at necropsy all animals were subject to macroscopic examination.

Results

There were no signs of toxicity and all animals survived to the scheduled necropsy. Males and females showed ~38% and 52% lower overall body weight gains when compared to controls, respectively. This finding was seen to a lesser degree at 500mg/kg bw/d where males and females showed ~20% and 42% lower overall body weight gains when compared to controls, respectively. No adverse effect on body weight or body weight gain was observed at 250 mg/kg bw/d in either sex.

In comparison to controls, overall dietary intake was reduced by approximately 41%, 43% or 47% for males dosed at 250, 500 or 1000 mg/kg bw/d, respectively. Dietary intake values for treated females were also reduced (between 8 and 15%) however a convincing dose-related response was not observed, although values were lower at the high dose level when compared to the control group.

In comparison to the concurrent control group, a dose related increase in water intake was observed in males dosed at 250, 500 and 1000 mg/kg bw/d, with an overall increase in water intake of 13%, 50% and 75%, respectively. For females, only the high dose group showed an increase in water intake, with an overall increase of 104%.

At necropsy, enlarged and dark spleens were evident for males treated with 1000 mg/kg bw/d. Dark spleens were also observed for two of the three females treated with 1000 mg/kg bw/d and all males treated with 500 mg/kg bw/d. No macroscopic abnormalities were evident for females treated with 500 mg/kg bw/d or for animals of either sex treated with 250 mg/kg bw/d.

The objective of this study was not to establish a NOAEL, but to provide a maximum dose for the 28-day toxicity study. Due to the substantial increase in water intake at doses of ≥500 mg/kg bw/d, 600 mg/kg bw/d was considered to be a maximum tolerated dose for the 28 day study.

[redacted], 2011

Study	Sulphanilamide: twenty-eight day repeated dose oral (gavage) toxicity study in the rat
Reference	[redacted] 2012
Date performed	21st October- 18th November 2011
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	OECD 407, Method B7 Regulation (EC) No. 440/2008
Deviations from the guideline	Potential for animals to be older than 9 weeks at the start of the study which exceeds the age recommended in the OECD 407 guideline but this has no impact on the outcome of the study.
GLP	GLP and QA statements provided
Test material	Sulphanilamide purity 99.7%, Batch L011068299
Study acceptable	Yes

In a 28 day study, groups of 5 rats/sex/dose were administered sulphanilamide suspended in arachis oil once daily *via* gavage at dose levels of 0, 30, 300 or 600 mg/kg bw/d. Animals were observed twice daily for morbidity and mortality and once daily for clinical signs of toxicity. Functional performance was assessed prior to study and during week 4 which included sensory reactivity, fore and hindlimb grip strength, pupil reflex and tail pinch. Body weight, food and water consumption were measured weekly. Blood samples for haematology and clinical chemistry were collected prior to necropsy. Gross pathological examination was performed and organ weights were recorded for all animals. All tissues were observed under light microscopy for control and high dose animals and the liver and spleen were examined in all dose groups. Where macroscopic findings were observed, tissues from the 300 and 30mg/kg bw/d groups were examined too.

Results

All animals dosed with 600mg/kg bw/d and males treated with 300mg/kg bw/d showed transient periods of post-dose increased salivation. Increased incidence of urination during the 2 week assessment was recorded in males at all dose levels. At the 3 week

assessment this effect was observed in all animals dosed with 600mg/kg bw/d and females treated with 300mg/kg bw/d when compared to controls. No treatment-related effects were witnessed in the functional performance or sensory reactivity tests.

Body weight changes deemed to be test article related were limited to males in the top dose group (600mg.kg bw/d), with a reduction in body weight gains when compared to controls during the first three weeks of treatment. This effect attained statistical significance ($p \leq 0.01$) during Week 2 and Week 3. No adverse effects were evident in females at any of the tested doses. Males treated at 600 mg/kg bw/d showed a slight reduction in dietary intake when compared to concurrent controls (~13% less).

Both sexes treated with 600 and 300 mg/kg bw/d showed increased water intake in comparison to controls. These effects were also observed in the female low dose group (30 mg/kg bw/d) where an approximate 28% increase in water consumption was observed. No overt differences in the low dose male group were observed.

Animals treated with 300 and 600mg/kg bw/d showed statistically significant reductions in haemoglobin, erythrocyte and haematocrit counts when compared to concurrent controls, with males showing a greater decrease. Furthermore, increases in MCH and MCV of animals of either sex were evident for animals treated with 300 and 600 mg/kg bw/d. Statistically significant reductions in MCHC were only achieved at the top dose.

Males treated with 600mg/kg bw/d showed statistically significant increases in A/G ratio with corresponding reductions in total protein and albumin compared to controls. Males treated with 600 and 300 mg/kg bw/day also showed higher blood urea values when compared to controls ($p<0.01$), with one high value observed at each treatment level. Males treated with 600 mg/kg bw/day showed an increase in alanine aminotransferase values when compared to controls ($p<0.05$). All individual values were within the normally expected ranges, and in the absence of any adaptive changes observed following histopathological examination of the liver, this increase was considered to have arisen incidentally. Furthermore, the statistically significant reduction in cholesterol values observed for males treated with 600 mg/kg bw/day was considered to be attributable to three higher than expected values observed in the control group. None of these effects on blood chemistry parameters were replicated in females at any dose level.

Table 6.8.3.2-1 Summary of haematological and clinical biochemical findings (% of control) in the 28-day oral gavage study with sulphanilamide

Parameter (% of control)	Males (mg/kg bw/d)				Females (mg/kg bw/d)			
	0	30	300	600	0	30	300	600
Haematology								
Hb (g/dl)	16.4	16.5 100	14.62 89**	12.38 76**	15.14	14.12 93	13.28 88**	13.68 90**
RBC (10^{12}/l)	8.618	8.574 99	7.264 84**	6.038 70**	8.020	7.606 94	6.918 86*	6.880 86*
Hct (%)	46.00	46.88 102	42.24 92*	36.12 79**	43.46	40.98 94	38.56 89*	39.8 91*
MCH (pg)	19.06	19.32 101	20.18 106*	20.48 108*	18.86	18.5 98	19.2 102	19.88 105
MCV (fl)	53.48	54.72 102	58.3 109**	59.94 112**	54.22	53.76 99	55.74 103	57.92 107**
MCHC (g/dl)	35.64	35.5 99	34.64 97	34.18 96*	34.8	34.44 99	34.42 99	34.40 100
Ret (%)	4.6	4.16 90	8.22 179**	9.4 204**	4.08	4.64 114	6.60 162**	8.22 201**
WBC (10^9/l)	8.26	8.14 99	7.62 92	5.84 71*	5.18	5.62 109	5.12 99	4.50 87
Lymp (10^9/l)	7.356	6.948 94	6.788 92	4.886 66*	4.634	5.00 108	4.360 94	3.594 76
Clinical chemistry								
Tot Prot (g/dl)	7.052	7.640 92	7.114 101	6.004 85**	7.604	7.664 101	7.260 105	7.346 97
Albumin (g/dl)	3.92	4.22 108	4.02 103	3.60 92*	4.54	4.54 100	4.38 96	4.50 99
A/G ratio	1.258	1.242 99	1.284 102	1.518 121**	1.504	1.456 97	1.538 102	1.600 106
Glucose (mg/dl)	165.0	150.4 91	152.0 92	177.4 108	144.2	133.4 93	144.8 100	159.4 111

Dark spleens were evident for animals of either sex treated with 600 mg/kg bw/day, and for one female and two males treated with 300 mg/kg bw/day. Males also showed enlarged spleens when compared to controls. To accompany the gross pathology findings, increased absolute and relative spleen weights were evident for animals of both sexes treated with mid and high doses of sulphanilamide compared to controls.

The increase in spleen weight was statistically significant in males but in females there was no clear dose response and statistical significance was not attained. A decrease in absolute and relative prostate weight was recorded in males treated with 600 mg/kg bw/d which attained statistical significance but this effect was not as marked at lower dose levels. Other statistically significant effects in the weight of the pituitary, heart and kidney are not toxicologically important as they do not exhibit a dose response, were not accompanied by histopathological changes or were attributable to control values outside the expected range.

Histopathology revealed an increased incidence of liver haematopoietic foci in both sexes at 600mg/kg bw/d and in males at 300mg/kg bw/d. There was increased incidence and severity of splenic congestion, haemosiderin deposits and extramedullary haematopoiesis in both sexes at mid and high dose of sulphanilamide. The bone marrow was affected by sulphanilamide and erythropoiesis was observed in the sternum and femur bone marrow of males and females treated with high dose of sulphonilamide. At 300mg/kg bw/d, increased incidence of erythropoiesis in the sternum and femur was reported in males but only in the sternum for females.

Table 6.8.3.2-2 Summary of organ weight and histopathological findings in the 28-day oral gavage study with sulphanilamide

Parameter		Males (mg/kg bw/d)				Females (mg/kg bw/d)			
		0	30	300	600	0	30	300	600
Organ weights									
Spleen	Abs (g)	0.67686	0.66620	0.96666 **	1.19254 **	0.45566	0.51308	0.55138	0.53142
	Rel (% of bodyweight)	0.196	0.215	0.299**	0.401**	0.236	0.251	0.273	0.271
Kidney	Abs (g)	2.11736	2.07684 **	2.13154 **	2.00176 **	1.28454	1.31092	1.37360	1.30144
	Rel (% of bodyweight)	0.609	0.666**	0.662**	0.663**	0.664	0.644	0.679	0.665
Prostate	Abs (g)	2.16800	2.04934	1.89914	1.65752 *				
	Rel (% of bodyweight)	0.614	0.664	0.595	0.552*				
Pituitary	Abs (g)	0.14272	0.1120	0.00936 *	0.00884 **	0.01054	0.01484	0.01208	0.01182
	Rel (% of bodyweight)	0.004	0.004	0.003*	0.003*	0.005	0.007	0.006	0.006
Heart	Abs (g)	1.00546	0.99218*	1.01802 *	0.96352 *	0.68934	0.73572	0.73838	0.69738
	Rel (% of bodyweight)	0.290	0.318*	0.316*	0.318*	0.357	0.363	0.365	0.358

Histopathology *Incidence [Mean severity grade]*								
Liver								
Haemopoietic cell focus/foci	0/5 [-]	0/5 [-]	2/5 [1.0]	4/5 [1.0]	0/5 [-]	0/5 [-]	0/5 [-]	3/5 [1.0]
Spleen								
Extramedullary haematopoiesis	5/5 [1.2]	4/5 [1.5]	5/5 [2.6]	5/5 [2.8]	4/4 [1.3]	4/4 [1.5]	5/5 [1.6]	5/5 [2.2]
Congestion	1/5 [1.0]	1/5 [1.0]	5/5 [1.8]	5/5 [1.8]	1/5 [1.0]	2/5 [1.0]	4/5 [1.0]	4/5 [1.0]
Haemosiderin deposits	0/5 [-]	0/5 [-]	5/5 [1.0]	5/5 [1.0]	2/5 [1.0]	3/5 [1.0]	5/5 [1.6]	5/5 [2.0]
Capsulitis	0/5 [-]	0/5 [-]	0/5 [-]	1/5 [2.0]	0/5 [-]	0/5 [-]	0/5 [-]	0/5 [-]
Bone marrow								
Sternum Increased erythropoiesis / erythroid series predominance	1/5 [1.0]	1/5 [1.0]	3/5 [1.0]	5/5 [1.0]	2/5 [1.0]	3/5 [1.0]	5/5 [1.2]	5/5 [1.6]
Femur Increased erythropoiesis / erythroid series predominance	1/5 [1.0]	1/5 [1.0]	4/5 [1.0]	4/5 [1.0]	2/5 [1.0]	2/5 [1.0]	3/5 [1.0]	4/5 [1.3]
Urinary bladder								
Hypertrophy, transitional epithelium	0/5	0/5	3/5 [1.0]	4/5 [1.3]	0/5	0/5	0/5	0/5
Mononuclear cell focus/foci, lamina propria	0/5	0/5	1/5 [1.0]	4/5 [1.0]	0/5	0/5	0/5	0/5

*p <0.05

**p <0.01

Conclusions

Increased water intake, organ weight changes and haematopoietic effects in the liver, spleen and bone marrow at 300mg/kg bw/d in both sexes are adverse and this is considered to be the LOAEL.

Treatment-related changes detected at 30 mg/kg bw/d were confined to an increase in water intake for females. Males treated with 30 mg/kg bw/d showed an increase in urination during Week 2 of the open-field arena observations. In view of similar effects observed at the higher dose levels, the relationship to treatment could not be disregarded. As such a NOEL could not be established, however, the effects at 30mg/kg bw/d are not adverse as they are not accompanied by any changes in the histopathology of the kidney or urinary bladder at this dose level. Therefore, a NOAEL was established at 30 mg/kg bw/d.

[There is no comparable 28d study with asulam. The NOAEL and LOAEL in the 90 day asulam study are 129/158 and 387/479 mg/kg bw/d respectively. These data indicate asulam is of similar toxicity to sulphanilamide.

[redacted], 2012

B.6.8.3.3 Genotoxicity

Three *in vitro* studies have been submitted with Sulphanilamide to determine mutagenicity and clastogenicity of the metabolite. All three studies have been evaluated and are presented in this submission for consideration.

A.

Study	Sulphanilamide: Bacterial reverse mutation test
Reference	May, 2011
Date performed	3rd August- 31st August 2011
Test facility	Huntingdon Life Sciences, Suffolk
Report reference	Report no. YRK0016
Guideline(s)	OECD 471, Method B.13/14 Regulation (EC) No 440/2008, OPPTS 870.5100, JMAFF 2-1-19-1
Deviations from the guideline	None
GLP	GLP and QA statements provided
Test material	Sulphanilamide purity 99.7%, Batch L011068299
Study acceptable	Yes

In a reverse gene mutation assay in bacteria, one *E.coli* (WP2*uvr*A (pKM101)) strain and four *S. typhimurium* (TA98, TA100, TA1535, and TA1537) strains were exposed to sulphanilamide formulated in purified water.

Since it was suspected that the test substance would be toxic towards the tester bacteria, a preliminary toxicity test was performed using the treat-and-plate procedure, in which the treated bacteria were washed free of the test substance prior to plating. Since no toxicity was evident upto 500µg/plate, an additional toxicity test was performed using the standard plate incorporation procedure. In the absence of toxicity, two independent mutation tests were then performed in the presence and absence of liver preparations (S9 mix) from rats treated with phenobarbital and 5,6-benzoflavone. The first test was a standard plate incorporation assay; the second included a pre-incubation stage. The doses for the initial test were 0, 5, 15, 50, 150, 500, 1500 and 5000µg/plate whilst the second test employed 0, 50, 150, 500, 1500 and 5000µg/plate. Appropriate positive and negative controls were included in each experiment in accordance with OECD 407.

Results

An initial preliminary experiment using the treat and wash procedure was undertaken to determine cytotoxicity but exposure to sulphanilamide upto 500µg/plate did not produce any toxicity. As no toxicity was evident, a further preliminary cytotoxicity test was undertaken using the plate incorporation method. Again no toxicity was evident up to 500µg/plate and subsequently two independent tests were performed with all tester strains.

The initial plate incorporation mutation did not show any increase in revertant colonies with any tester strains in presence or absence of S9. No evidence of cytotoxicity or precipitation were observed.

The pre-incubation test did not produce any increase in revertant colony number for any tester strain with or without S9. No precipitation of test material or cytotoxicity were reported.

Positive and negative controls were within the laboratory's ranges for revertants for both test methods and validate the study.

Conclusions

Based on the results from this study, it can be concluded that sulphanilamide showed no evidence of mutagenic activity following testing in one *E. coli* (WP2*uvr*A (pKM101)) strain and four *S. typhimurium* strains (TA98, TA100, TA1535, TA1537) when tested up to 5000 µg/plate (the maximum concentration as recommended by current regulatory guidelines) in the presence and absence of metabolic activation, under the test conditions employed.

May, 2011

B.

Study	Sulphanilamide: L5178Y TK$^{+/-}$ Mouse Lymphoma assay
Reference	Brown, R 2012
Date performed	14th November- 27th December 2011
Test facility	Harlan Laboratories Ltd, Shardlow, Derbyshire
Report reference	Report no. 41101395
Guideline(s)	OECD 476, Method B17 Regulation (EC) No. 440/2008, US EPA OPPTS 870.5300 and Japanese METI/MHLW
Deviations from the guideline	Minor- the positive control without S9 is not that stated in OECD 476 paragraph 19 but it is structurally similar and mutagenic based on the study results and historical control data. This has no impact on the outcome of the study.
GLP	GLP and QA statements provided
Test material	Sulphanilamide Purity 99.7% Batch L011068299
Study acceptable	Yes

In a mammalian cell gene mutation assay, mouse lymphoma L5178Y cells were exposed to sulphanilamide formulated in DMSO at concentration of 170.2 mg/mL. Forward mutation at the thymidine kinase ($tk^{+/-}$) gene locus was measured. A

preliminary cytotoxicity test was performed with the test substance up to 1720µg/ml and relative suspension growth (RSG) determined.

Dose concentrations of sulphanilamide were added to cultures at 1% (v/v), achieving a final maximum concentration of 1720 ug/mL in both the absence (4 and 24 h) and presence (4 h) of S9 mix. Appropriate positive controls (Cyclophosphamide, CP or ethylmethanesulphonate, EMS) were included in the assay in the presence and absence of S9. Following exposure, percentage relative suspension growth (%RSG), percentage viability (%V), cloning efficiency or relative total growth (%RTG) and mutant frequency (MF) were calculated. For the test material to be considered positive, the global evaluation factor was used, where a test agent is positive if the mutant frequency of any concentration exceeds the sum of the mean concurrent solvent control mutant frequency and the GEF ($126*10^{-6}$) and is accompanied by a positive linear trend.

Results

In the preliminary cytotoxicity assay, no precipitate (observed by eye at the end of treatment) was observed at any concentration following a 4 hour exposure. Exposure to sulphanilamide at concentrations from 6.72 to 1720 µg/mL in the absence and presence of S9 mix (4 hour exposure) resulted in RSG values from 119 to 80% and from 133 to 95% respectively.

Following a continuous exposure for 24 hours, no precipitation (assessed by eye at the end of treatment) was observed at any concentration. Exposure to concentrations from 6.72 to 1702 µg/mL resulted in RSG values from 101 to 72%

In the main test, cultures exposed to sulphanilamide at concentrations up to 1720 µg/mL in the absence and presence of S9 mix following a 4 hour exposure and in the absence of S9 mix following a 24 hour exposure returned RTG values of 89, 75 and 64%, respectively. The confirmatory 4 hour treatment in the presence of S9 returned an RTG value of 64% at the highest concentration tested. These results confirmed that sulphanilamide was not overtly toxic to L5178Y cells.

There were no increases in the mean mutant frequencies of any of the test concentrations assessed that exceeded the sum of the mean concurrent vehicle control mutant frequency and the GEF, within acceptable levels of toxicity in any of the treatment conditions (see table 6.8.3.3-1).

The positive controls induced an acceptable increase in mutation frequency and an acceptable increase in the number of small colony mutants, thereby demonstrating the sensitivity and specificity of the test system (see table 6.8.3.3-1).

Table 6.8.3.3-1 Summary of results in the TK mouse lymphoma assay following exposure to Sulphanilamide

Experiment 1							
Treatment (µg/ml)	4-Hour –S9			Treatment (µg/ml)	4-Hour +S9		
	SG	RTG	MF		SG	RTG	MF
0	9.88	1.00	117.27	0	12.71	1.00	110.68
107.5	9.10	1.04	104.20	107.5	12.17	0.95	120.96
215	9.35	1.14	118.70	215	11.92	0.93	110.76
430	10.33	1.25	86.28	430	11.76	0.90	124.04
860	8.89	1.00	106.21	860	11.07	0.75	159.30
1290	8.87	1.04	91.25	1290	10.95	0.82	117.41
1720	9.14	0.89	126.16	1720	10.18	0.80	99.08
	Linear trend- not significant				Linear trend- not significant		
EMS	6.14	0.46	814.11	CP	7.18	0.35	1231.34
Experiment 2							
Treatment (µg/ml)	24-Hour –S9			Treatment (µg/ml)	4-Hour +S9		
	SG	RTG	SG		SG	RTG	MF
0	70.44	1.00	151.75	0	10.90	1.00	134.07
107.5	60.80	0.96	114.54	107.5	12.53	1.18	124.19
215	70.74	1.04	181.85	215	12.87	0.95	139.75
430	63.93	0.95	148.01	430	12.53	1.09	139.94
860	57.15	0.83	162.05	860	12.56	0.95	150.24
1290	51.30	0.73	110.62	1290	11.77	0.94	108.70
1720	48.97	0.64	162.65	1720	13.10	0.86	121.07
	Linear trend- not significant				Linear trend- not significant		
EMS	42.16	0.39	1273.95	CP	9.22	0.39	1503.10

Conclusions

Based on the results from this study, it can be concluded that sulphanilamide did not induce mutation at the *tk* locus of L5178Y mouse lymphoma cells when tested under the conditions employed in this study. These conditions included treatments up to 1720 µg/mL (equivalent to 10 mM, the maximum recommended dose in accordance with current *in vitro* genotoxicity regulatory guidelines) in four independent experiments in the absence (4 and 24 hours) and presence (4 hours) of a rat liver metabolic (S9) activation system.

Brown, 2012

C.

Study	Sulphanilamide: chromosome aberration test in human lymphocytes *in vitro*
Reference	Morris and Lacey, 2012
Date performed	7th September- 12th December 2011
Test facility	Harlan Laboratories Ltd, Shardlow, Derbyshire
Report reference	Report no. 41101392
Guideline(s)	OECD 473, Method B10 Regulation (EC) No. 440/2008
Deviations from the guideline	None
GLP	GLP and QA statements provided
Test material	Sulphanilamide Purity 99.7% Batch L011068299
Study acceptable	Yes

In a mammalian chromosomal aberration assay, human lymphocytes, *in vitro* were exposed to sulphanilamide using DMSO as the solvent in either the presence of metabolic activation (+S9, 4 hours) or absence of metabolic activation (-S9, 4 and 24 hours).

A preliminary toxicity test was conducted first to ensure toxicity was not observed at doses up to 1720µg/ml for 4 hours with S9 and 24 hours without S9.

In the absence of toxicity, experiment 1 was conducted at concentrations of 107.5, 215, 430, 860, 1290 and 1720 µg/mL in the presence and absence of S9 for 4 hours but only metaphase analysis was performed on cells treated with 430, 860, 1290 and 1720 µg/mL.

In experiment 2, the same concentrations as used in experiment 1 were examined for the 24 hour –S9 exposures but for 4 hours with S9 the concentrations were as follows; 430, 860 and 1720 µg/mL.

Appropriate positive controls were included (MMC – S9, CP +S9) in both experiments. The mitotic index was also calculated in each experiment for the vehicle, test concentrations and positive controls as a measure of cytotoxicity.

Results

In the preliminary test, no test material precipitate was observed at any dose level in either of the treatment conditions used. Evidence of toxicity was observed at 1720 µg/mL in the 24 h –S9 treatment (33% of controls) but not in cells exposed 4 hours only.

In experiment 1, the absence or presence of S9 caused no overt reduction in MI when tested up to a maximum dose of 1720 µg/mL (equivalent to 10 mM) for 4 hours. No increase in the percentage of cells with aberrations (excluding gaps) was observed when tested up to a dose deemed to be the maximum recommended dose (10 mM). No biologically relevant increases in polyploidy were observed in either of the treatment conditions tested.

In experiment 2, cells treated with the top dose of sulphanilamide had a reduced MI (65% of controls) in the absence of S9 after a 24 hour exposure where as in the presence of S9 for 4 hours no marked toxicity was observed. Again, no increase in % cells with aberrations (excluding gaps) was observed when tested up to a dose deemed to be the maximum recommended dose under either test condition. No biologically relevant increases in polyploidy were observed in any of the treatment conditions tested.

Positive controls induced the appropriate response and validate the study.

Table 6.8.3.2-2 Summary of the results in the *in vitro* mammalian chromosomal aberration test with Sulphanilamide

Experiment 1									
4 Hours –S9					4 Hours +S9				
Treatment	% cells SA	Gaps	Mitotic index (% control)	% cells NA	Treatment	% cells SA	Gaps	Mitotic index (% control)	% cells NA
NC	0.5	0.0	100	0.0	NC	0.5	0.0	100	0.0
430	0.0	0.5	95	0.5	430	0.5	0.5	106	0.0
860	0.0	0.0	146	0.0	860	0.0	0.0	88	0.0
1290	1.0	1.0	109	0.0	1290	0.0	0.0	89	1.0
1720	0.0	0.0	114	0.0	1720	0.0	0.0	88	0.5
PC (MMC)	65.0	0.0	34	0.0	PC (CP)	28.0	8.0	26	0.0
Experiment 2									
24 Hours –S9					4 Hours +S9				
Treatment	% cells SA	Gaps	Mitotic index (% control)	% cells NA	Treatment	% cells SA	Gaps	Mitotic index (% control)	% cells NA
NC	0.0	0.5	100	0.0	NC	0.0	0.5	100	0.0
430	0.5	1.0	88.0	0.0	430	1.0	2.5	91	0.0
860	0.5	0.0	81.0	0.0	860	0.5	0.5	81.0	0.0
1290	0.0	0.0	81.0	0.0	1290	-	-	-	-

1720	0.0	1.0	65.0	0.5	1720	0.0	1.5	80.0	0.5
PC (MMC)	59.0	27.0	35.0	0.0	PC (CP)	37.0	18.0	21.0	1.0

NC= Negative control, PC= Positive control, SA= structural aberration, NA= numerical aberration, MMC= Mitomycin C, CP= Cyclophosphamide

Conclusions

Sulphanilamide did not show an increase in the incidence of chromosome aberrations in human lymphocytes. These conditions included four independent treatments tested up to the maximum recommended dose in accordance with current *in vitro* genotoxicity regulatory guidance [10 mM] in the absence (4[+20 hour recovery] and 24[no recovery]) and presence (4 [+20 hour recovery] hours at 1 and 2% (v/v) final concentration of S9 fraction) of a rat liver metabolic activation system.

Morris and Lacey, 2012

B.6.8.3.4 Report on the developmental toxicity study by Speert (1940)

Study	Sulphanilamide: toxicological relevance as a metabolite of the active substance Asulam
Reference	Bowen, D 2011
Date performed	N/A
Test facility	JSC International Ltd, Harrogate, UK
Report reference	UPL/10/01
Guideline(s)	N/A
Deviations from the guideline	N/A
GLP	N/A
Test material	N/A
Study acceptable	N/A

The purpose of this document was to provide a critical review of the study of the developmental toxicity study conducted by Speert.

The RMS is of the view that this study by Speert (1940) has significant deficiencies and that the LOAEL of 1000 mg/kg bw/day established from the study is not recommended for derivation of reference doses for Sulphanilamide. However, the effects reported in the treated group (1000 mg/kg bw/d) are not to be dismissed, despite the unreliability of the study, but they should be considered in conjunction with estimated exposure levels (see section B.7.16.2.3).

B.6.8.3.5 Additional information

The public perception of the toxicity of sulphanilamide is somewhat tainted due to the well-documented 'Elixir sulphanilamide' tragedy dating back to late 1937 when over 100 people taking sulphanilamide to treat streptococcal infections died following ingestion of the prescribed drug. Due to an increase in demand for this antibiotic in its liquid form, the solvent diethylene glycol was found to be an ideal solvent to increase

the demand of this antibiotic. As no toxicity testing had taken place on this new formulation (only assessment of flavour, appearance, fragrance which were all deemed satisfactory) the toxicological relevance of diethylene glycol was not realised. Thus the 'Elixir sulphanilamide' tragedy was not due to sulphanilamide *per se* but its preparation in a diethylene glycol based product.

The RMS has noted that there are references to sulphanilamide being a phototoxin/allergen. Original publications spanning 40 years (1939-1979) suggested that Sulphanilamide was phototoxic following intradermal (ID) and dermal administration. An example is the study by Kaidby and Kligman where intense erythema and edema was recorded after 4-6 h following ID administration of Sulphanilamide and UVB exposure. Whilst these original studies all consistently reported Sulphanilamide as phototoxic, it is recognised that there were differences across the UV spectrum required for activation and there was no consistent methodology. In 1995, the New York University Medical Centre included sulphanilamide in a photopatch test and concluded it had the potential to be a photoallergen (Casarett and Doull pp539-540). Sulphanilamide has also been referenced as a topical photoallergen in a publication into adverse effects in cosmetics and toiletries (Textbook of Contact Dermatitis, Rycroft R 2001 pp377 cites Nater and Groot 1985 Unwanted effects of cosmetics and drugs used in dermatology). Although it is recognised that the studies are limited, they are all consistent with their identification of Sulphanilamide as a phototoxin/allergen both systemically and topically. In the context of this submission, available data had suggested this could be an issue given radiolabelled Asulam and/or its metabolites were distributed to the skin in the ADME studies; thus there was a potential for Sulphanilamide to be activated by exposure to UV. After further deliberation, the RMS considers this to be a limited concern for the following reasons:

(1) The proportion of radiolabel present in the skin following exposure to Asulam is low compared to other tissues especially after 6 hours and at worst, only 1% is present as Sulphanilamide

(2) Acute and chronic exposure to sulphanilamide directly in consumed goods is low, (see section B.7, Table 7.20).

(3) Excretion of Sulphanilamide after exposure to Asulam is rapid (within 48 hours) and it could be expected that after direct exposure to Sulphanilamide via consumption of spinach, this will also be the case rather than distributing to the skin.

B.6.8.3.5 Summary of the toxicity of Sulphanilamide

Sulphanilamide was not acutely toxic by the oral route (LD_{50}>2000mg/kg) but in longer term studies adverse effects were reported. In a 7 day study dose range finder, reduced body weight gain, increased water consumption and alterations in the spleen were observed. These effects were also reported in the 28 day study. In addition, in this study, haematopoietic effects occurred from a dose of 300mg/kg bw/d, particularly in male rats. The NOAEL of the 28 day study is 30mg/kg bw/d and this is lower than the NOAEL (129mg/kg bw/d) for the parent compound over a 90-day exposure which suggests Sulphanilamide is more toxic than Asulam-sodium. However, the dose spacing in the sulphanilamide study is large and the LOAELs overlap (329mg/kg bw/d

for asulam, 300 mg/kg bw/d for sulphanilamide). Looking at the magnitude of effects at the LOAEL indicates the toxicity of sulphanilamide is similar to that of asulam.

Sulphanilamide was not genotoxic, in a battery of *in vitro* tests including the Ames, TK mouse lymphoma assay and chromosomal aberration test.

Table B.6.8.3.5-1 Summary of toxicity studies with Sulphanilamide, a metabolite of Asulam-sodium

Study	Results	Conclusion	Reference
Acute Oral-fixed dose method	No clinical signs of toxicity, mortality or changes at necropsy.	LD50> 2000 mg/kg bw No classification required under Regulation (EC) 1272/2008	[redacted] 2011
7-day range finding study in rats by oral gavage	No clinical signs of toxicity or mortality but dark spleen, ↓body weight gain, ↓food and ↑water consumption were reported.	Maximum tolerated dose is > 500 mg/kg bw/d. For future studies in the rat the maximum dose is 600 mg/kg bw/d.	[redacted], 2011
28-day oral gavage study	Effects observed at LOAEL (300mg/kg bw/d) include changes in organ weight, haematopoietic effects and increased water consumption	NOAEL= 30mg/kg bw/d	[redacted] 2012
Bacterial reverse mutation test	No cytoxicity or precipitation was observed. No increase in revertant colony number was reported in any tester strain with or without S9 up to 5000µg/plate Sulphanilamide.	Sulphanilamide is not mutagenic under the study conditions.	May, 2011
***In vitro* mammalian cell gene mutation test-TK Mouse lymphoma assay**	No increase in mutant frequency at any tested dose compared to concurrent controls. Positive controls showed an increase in mutant colony formation which was consistent with historical control data and this validates the study.	Sulphanilamide is not mutagenic in the mammalian cell mutagenicity test.	Brown, 2012
***In vitro* mammalian chromosomal**	Mitotic index as a % of the negative control decreased at the high	Sulphanilamide is not clastogenic under the conditions of the test.	Morris and Lacey, 2012

aberration test	dose only (1720µg/ml). No increase in % of structural or numerical aberrations with or without S9. Positive controls increased the % of structural aberrations and results were consistent with historical control data.		

Public domain toxicity data are available for sulphanilamide but these have been tainted by the 'Elixir' tragedy and the reports on this event should be interpreted with caution. Other studies are limited due to their age of publication and most are not conducted to any recognised regulatory guideline. Of these available studies the Speert (1940) development study which reported a LOEL value of 22 mg/kg bw/d is considered by the RMS to be unreliable and the data should not be used to determine reference doses for risk assessment of sulphanilamide. In addition, the RMS discovered references to Sulphanilamide being phototoxic but in the context of this submission, this has been concluded not to be a major toxicological concern based on the exposure assessments with residues on spinach (see section B.7.16.2.3).

B.6.8.3.6 **Reference doses for Sulphanilamide**

Based on the available data, an Acceptable Daily Intake value (ADI) can be derived for Sulphanilamide and used in exposure modelling. Only one repeat dose study is available which was performed in the rat over 28 days and the NOAEL was 30mg/kg bw/d. To convert the NOAEL to an ADI, a 100-fold safety factor is applied to account for inter and intra-species differences followed by a conversion factor of 6 to account for a short term study being used instead of a chronic/lifetime study.

Overall, the ADI for Sulphanilamide is **0.05mg/kg bw/d.**

An acute reference dose of 0.3mg/kg bw/d could be derived based on the NOAEL of the 28-day study with Sulphanilamide (30mg/kg bw/d) and a safety factor of 100.

The exposure to Sulphanilamide as a residue on spinach has been evaluated under B.7.16.2.3 and shows that the risk to consumers is within acceptable limits.

B.6.9 Medical data and information (IIA 5.9)

B.6.9.1 Medical surveillance of manufacturing plant personnel (AII 5.9.1)

Asulam-sodium has been produced since approximately 2008, with an average production of 1896 KL/year. Worker exposure is estimated at 132960 man hours since December 2008. Medical examinations have been conducted quarterly/half yearly since 2008, and consist of physical examination, blood tests (total count/ differential count) and urinalysis determinations. Medical surveillance of workers exposed to asulam sodium recorded no adverse findings. There are no known cases of ill health related to asulam sodium exposure among workers involved in its synthesis, during the production period from 2008 through to the present day.

B.6.9.2 Clinical cases and poisoning incidents (IIA 5.9.2)

There are no known cases of poisoning with asulam-sodium; nor are there any known clinical cases resulting from exposure to or application of asulam.

B.6.9.3 Exposure of the general population and epidemiological studies (IIA 5.9.3)

Since the market introduction if asulam-sodium, no adverse health effects have been reported from agricultural users or persons of the general population. Epidemiological studies are not available.

B.6.9.4 Clinical signs, symptoms of poisoning and details of clinical tests (IIA 5.9.4)

Compound-specific poisoning signs in man after oral ingestion are not known. Based on animal studies, there are few if any symptoms to be expected. The analytical demonstration of parent compound or metabolites in blood, urine or gastrointestinal contents is required for an exact diagnosis of poisoning.

B.6.9.5 First aid measures (IIA 5.9.5)

First aid measures have been summarised from the safety data sheet for asulam-sodium. No specific therapeutic regime is available for asulam-sodium. Symptomatic treatment is advised.

Skin:	Wash off immediately with plenty of soap and water. Removed contaminated clothing. Seek medical attention if irritation persists. May cause sensitisation by skin contact
Eye:	Rinse immediately with plenty of water for 15 minutes holding the eyelids open. Seek medical attention if irritation or symptoms persist.
Inhalation:	Move the exposed person to fresh air. Seek medical attention.
Ingestion:	Seek medical attention if irritation or symptoms persist. DO NOT INDUCE VOMITING.

Therapeutic regimes:

Expected effects of poisoning**:** No experiences in man are available. Severe cases of poisoning are to be expected only after accidental or intentional oral ingestion of high dosages. Although asulam is chemically a carbamate, it does not inhibit acetyl cholinesterase. Atropine therefore is not recommended in cases of poisoning.

B.6.9.6 Expected effects and duration of poisoning as a function of the type, level and duration of exposure or ingestion (IIA 5.9.6)

No poisoning cases have been reported so no data are available. As asulam-sodium is of low acute and repeat dose toxicity human poisoning is very unlikely.

B.6.9.7 Summary of medical data

Routine medical examinations of plant personnel have not indicated any specific adverse effects. No adverse health effects which could be directly linked to asulam-sodium have been reported from agricultural users or the general population. Epidemiological studies are not available. Compound-specific poisoning signs in man after oral ingestion are not known and are not expected as few if any specific clinical signs were observed in the animal studies conducted with asulam sodium. No specific antidote is available; symptomatic treatment is recommended. [NB: Although asulam is chemically a carbamate, it does not inhibit acetylcholinesterase. The use of atropine is therefore contraindicated].

B.6.10 Summary of mammalian toxicology and proposed ADI, AOEL, ARfD and MAC (drinking water limit) (IIA 5.10)

Toxicokinetics

Asulam sodium was found to be rapidly and extensively absorbed from the gastro-intestinal tract; the extent of oral absorption is estimated to be at least 85-90%. Asulam sodium was rapidly and widely distributed, with no evidence for bio-accumulation but some evidence for binding to blood cells. Excretion was rapid (largely within 24 hours) and largely (82.0-92.3%) in the urine following oral administration. Urinary excretion was slightly higher (and faecal excretion slightly lower) at the top dose level, indicating a saturation of metabolism or biliary excretion. Faecal excretion in intravenously-dosed animals (3.3-4.6% of the administered radioactivity) is consistent with limited biliary excretion of a conjugated metabolite. Asulam sodium was excreted largely unchanged in studies in the rat, with parent compound accounting for up to ~80% of the administered radioactivity. The major urinary and faecal metabolite was identified as acetyl-asulam, with sulphanilamide and acetyl-sulphanilamide also identified as minor urinary metabolites. A higher proportion of the administered radioactivity was excreted unchanged at the high dose level, suggesting the saturation of metabolism.

Toxicity

Asulam sodium is a carbamate herbicide. Its mode of action in plants is inhibition of 7,8-dihydropteroate synthase; there is no equivalent mammalian target. Although chemically a carbamate, no evidence of cholinesterase inhibition was seen in the 8-week mouse study ([redacted] 1989c), 21-day dermal study in rabbits and 6-month dog study ([redacted], 1980).

Acute toxicity

Asulam sodium is of low toxicity in the rat by the oral, dermal and inhalation routes and is also of low acute dermal toxicity in the rabbit; signs of toxicity in these studies were non-specific and transient. Slight skin and moderate eye irritation was seen in studies in the rabbit. Clear evidence of sensitisation was seen in a Guinea pig Maximisation test.

Short-term toxicity

Asulam/ asulam sodium was found to be of relatively low toxicity by the dermal route in rabbits and by the oral route in the rat, mouse and dog. The erythrocyte and thyroid were identified as targets of toxicity in the 90-day rat study; results show mild macrocytic anaemia (and histopathology consistent with increased erythrocyte turnover) and thyroid follicular hypertrophy at the top dose level. Treatment-related findings in the mouse were limited to minor bodyweight effects at high dose levels. In the dog, histopathology also identified the thyroid as a target of toxicity in both studies. Significantly reduced white blood cell counts and significantly elevated plasma glucose concentration seen in treated groups in the 6-month dog study were not apparent in the 1-year study.

The thyroid effects were a consistent finding across the test species but this was deemed not to be of relevance to risk assessment due to differences in sensitivity to thyroid hormone perturbation (see section 6.3.6).

Genotoxicity

The genotoxicity of asulam sodium was investigated in a number of studies *in vitro* and *in vivo*. No evidence of mutagenicity was seen in two recent Ames tests, which supersede two previously evaluated bacterial reverse mutation assays as these were not conducted to modern regulatory standards. Two mouse lymphoma assays have been reported; the study previously evaluated had a significantly increased mutation frequency at the highest (cytotoxic) concentration in the presence of metabolic activation, whilst the 2009 study does not show any increase in mutations at any dose with or without S9. An equivocal result (increased proportion of cells with chromosomal aberrations at the highest (cytotoxic) concentration in the absence of metabolic activation) was seen in a study of clastogenicity *in vitro*. No evidence of unscheduled DNA synthesis was seen in a study *in vitro*, however there are concerns over the design and performance of this study. An equivocal result was also seen in the only *in vivo* study, where the proportion of micronucleated PCEs was elevated (and exceeded the historical control range) but at an intermediate concentration and statistical significance compared to the control was not attained.

Chronic toxicity and carcinogenicity

Haematological effects indicative of mild anaemia and histopathology consistent with the increased breakdown of red blood cells were seen in chronic studies in the rat and mouse; minor effects on bodyweight were also seen at high dose levels. Consistent with effects in the 90-day rat study and in the dog, increased thyroid weights and

hyperplastic change was noted in the rat. The thyroid effects were a consistent finding across the test species in short and long term studies but this was deemed not to be of relevance to risk assessment due to differences in sensitivity to thyroid hormone perturbation (see section 6.3.6). The adrenal was also identified as a target organ in the male rat, with increased incidences of medullary hyperplasia at high dose levels.

Some evidence of carcinogenicity was also seen in the male rat, with an increased incidence of phaeochromocytoma at the top dose level. Whilst the incidence of this neoplasm exceeded the laboratory's historical control data, there is information in the publically available literature on the incidence of this tumour type in Sprague Dawley rats which shows phaeochromocytomas can spontaneously occur at an incidence of up to 33%. Furthermore, this tumour is referenced in the CLP guidance as having a high spontaneous incidence rate in Sprague Dawley rats. The tumour was also not dose responsive, limited to a single sex and species, with no evidence of a multi-site response to asulam sodium and no direct evidence from the toxicology package of studies to support chemical induction of phaeochromocytomas in accordance with published literature (Greim, 2008).

Previously, no evidence of carcinogenicity was concluded for the mouse following administration of asulam sodium. Hepatocellular adenomas and carcinomas were reported in both sexes which were outside the historical control data for the conducting laboratory. In females, the hepatocellular carcinomas were elevated and outside the HCD but no dose response was observed, there was no accompanying liver toxicity i.e. histopathological changes or variation in liver weight and the lack of tumours in higher dose groups cannot be explained by toxicity to the test compound. Equally, the increase in hepatocellular adenoma was within the collated historical control data incidence, no dose response was observed and no accompanying liver histopathology was reported. In males, the incidence of hepatocellular adenomas and carcinomas was highest at the lowest tested dose and both were outside the historical control data. The incidence of these tumour types was not dose responsive, there was no accompanying liver toxicity i.e. histopathological changes or variation in liver weight and the lack of tumours in higher dose groups cannot be explained by toxicity to the test compound.

Reproductive and developmental toxicity

Evidence of a reproductive effect was seen in a rat multi-generation study as litter size was reduced at dose levels of ≥5000 ppm in the F1 generation. The underlying reason for this finding is unclear as there was no difference in the number of foetuses dead at birth across the treated groups compared with the controls. Furthermore, this effect was not dose responsive as a 5-fold increase in asulam resulted in only a further 0.3 reduction in litter loss and the effect was not reproduced in the F2 generation, particularly at the highest dose tested. There were no effects on reproductive organ weights or macroscopic findings in these organs in parental animals or offspring. Repeat dose studies in the rat, mouse and dog did not record any alterations in reproductive organs and support the results of the fertility study.

No evidence of teratogenicity was seen in developmental toxicity studies in the rat and rabbit. No evidence of maternal toxicity was seen in the rabbit; slight effects on bodyweight were seen at the top dose level in the study, however severe toxicity was

noted in a higher dose group that was sacrificed early in this study. A slight increase in minor foetal skeletal findings indicative of delayed ossification was apparent at the top dose level in the rat study; the incidence of extra ribs was also increased in treated groups in this study. No evidence of developmental toxicity was seen at the top dose level in the rabbit study.

Neurotoxicity

Whilst asulam sodium contains a carbamate group, which are known to be associated with neurotoxicity, plasma, erythrocyte or brain cholinesterase activities were measured in the eight-week mouse study, 6-month dog study and the 21-day repeat dose dermal toxicity study in the rabbit. No significant inhibition was observed across the species or sexes in any of these studies.

Immunotoxicity

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

Endocrine disrupting potential

Asulam sodium has been shown in short term studies to target the thyroid however based on published literature on the sulphonilamide moiety and differences in species sensitivity, this effect is not relevant to humans. No other effects were reported in short term studies for asulam sodium that is a concern for endocrine organs.

The sporadic finding of phaeochromocytomas (adrenal glands) is not a concern due to the effect only being observed in male rats at the highest dose tested in the carcinogenicity study. The only effect reported in the reproductive studies was a reduction in litter size in the F1 generation, however, this finding was not replicated in F2 litters. No classification for these endpoints is proposed albeit based on compromised reproductive studies. Overall, asulam sodium does not attain the criteria for endocrine disrupting potential in accordance with Regulation (EC) 1107/2009.

Table B.6.37 Summary of toxicity studies

Study	NOAEL	LOAEL	Effects at LOAEL	Reference
	ppm (mg/kg bw/d)			
Rat 90-Day	M: 2000 ppm (129)	6000 ppm (387)	Histopathology, clinical chemistry	[redacted], 2000
	F: 2000 ppm (158)	6000 ppm (479)	Histopathology	
Mouse 8-week	M: 10000 ppm (1673)	30000 ppm (5103)	↓ body weight	[redacted] 1989
	F: 30000 ppm (6835)	50000 ppm (10828)	↓body weight and weight gain	
Dog 6-month	M: 60 mg/kg bw/d	300 mg/kg bw/d	Haematology, clinical chemistry	[redacted], 1980

	F: 60 mg/kg bw/d	300 mg/kg bw/d	Clinical signs, haematology, ↑ thyroid weight	
Dog 52-week	M: 100 mg/kg bw/d	300 mg/kg bw/d	Clinical signs, ↑ thyroid weight and accompanying histopathology	[redacted] 2004
	F: 100 mg/kg bw/d	300 mg/kg bw/d	Clinical signs and thyroid histopathology	
Rabbit 21-day dermal	M: >1000 mg/kg bw/d	Not determined	Not determined	[redacted] 1989b
	F: >1000 mg/kg bw/d	Not determined	Not determined	
Rat chronic	M: 1000ppm (36)	5000 ppm (180)	Haematology, adrenal histopathology	[redacted] 1981
	F: 1000 ppm (47)	5000 ppm (243)	↓ weight gain, haematology	
Mouse chronic	M: 500 ppm (74)	5000 ppm (730)	Haematology	[redacted] 1992
	F: 500 ppm (95)	5000 ppm (938)	Haematology	
Multi-generation	reproductive			[redacted] 1981
	M: 1000ppm (46)	5000 ppm (224)	↓Litter size	
	F: 1000 ppm (58)	5000 ppm (278)		
	Adult			
	M: 5000 ppm (224)	25000 ppm (1136)	↓ bodyweight, organ weight changes	
	F: 5000 ppm (278)	25000 ppm (1456)		
	Offspring			
	25000 ppm (1136)	Not determined	↓ Survival	
Rat developmental	Maternal: 2000 mg/kg bw/d	-		[redacted] 1982
	Developmental: 1000 mg/kg bw/d	2000 mg/kg bw/d	Skeletal effects	
Rabbit developmental	Maternal: 300 mg/kg bw/d	750 mg/kg bw/d	↓ bodyweight	[redacted] 1981
	Developmental: 750 mg/kg bw/d	-		

B.6.10.1 Acceptable Daily Intake (ADI)

An ADI of 0.36 mg/kg bw/d can be derived for asulam sodium, based on the NOAEL of 36 mg/kg bw/d (1000 ppm) in male rats in the chronic rat study and applying a standard assessment factor of 100. A standard assessment factor is considered appropriate in the absence of teratogenicity; carcinogenicity was only seen at the highest dose level in the male rat (equivalent to 953 mg/kg bw/d), giving an acceptable margin of safety.

This ADI was agreed at the EU level (EFSA Journal 2010; 8(12): 1822).

B.6.10.2 Acute Reference Dose (ARfD)

Asulam sodium is of relatively low acute toxicity, however clinical signs (vomiting and salivation) were seen following gavage dosing in the dog for 6 or 12 months. An ARfD of 1 mg/kg bw/d can therefore be derived, based on the NOAEL of 100 mg/kg bw/d in the 12-month dog study. Although a lower NOAEL (60 mg/kg bw/d) was determined for the 6-month dog study, the LOAEL in both studies was the same (300 mg/kg bw/d). The use of the NOAEL from the longer term study is therefore appropriate.

This ARfD was agreed at the EU level in the previous submission (EFSA Journal 2010; 8(12): 1822).

B.6.10.3 Admissible Operator Exposure Level (AOEL)

A short-term systemic AOEL of 0.46 mg/kg bw/day can be determined, based on the NOAEL of 46 mg/kg bw/day from the rat reproduction study based on reduced litter size. A safety factor of 100 is appropriate. No correction for oral absorption is necessary as asulam sodium is extensively absorbed from the gastro-intestinal tract (>80% excreted in urine).

This was agreed in the previous EFSA conclusions (EFSA Journal 2010; 8(12): 1822).

B.6.10.4 Maximum Allowable Concentration (MAC: drinking water limit)

An MAC of 1.08 mg/l can be determined based on an average bodyweight of 60 kg, water consumption of 2 litres/day and allowing drinking water to contribute 10% of the ADI. However the maximum level of all pesticides in drinking water is limited to 0.1 µg/l under EC legislation.

B.6.10.5 Proposal for classification and labelling

Based on all the available data, Asulam-sodium is classified as a category 1 Skin sensitiser with the hazard phrase “H317- may cause skin sensitisation”. No classification is proposed for any other endpoint.

B.6.11 Acute toxicity, irritancy and skin sensitisation of the product (IIIA 7.1)

The product Asulox (code names: AE F074383 00 SL33 A1 or EXP 04668) has been tested for acute toxicity, skin and irriation and skin sensitisation. All these studies were evaluated previously and the conclusions presented.

B.6.11.1 Acute oral toxicity (IIIA 7.1.1)

Study	AE F074383 00 SL33 A103 (EXP 04668 A) - Acute oral toxicity in rats, Acute Toxic Class method
Reference	[redacted] 2002a
Date performed	17 September - 8 October 2002
Test facility	[redacted]
Report reference	[redacted]

Guideline(s)	96/54/EC, OECD 423 (1996)
Deviations from the guideline	-
GLP	Yes
Test material	Asulox (AE F074383 00 SL33 A103, EXP 04668 A) (batch 990533; 392 g/ l asulam)
Study acceptable	Yes

Fasted Wistar rats (3/sex) were gavaged with a single dose of the test material (dissolved in purified water) at a dose level of 2000 mg/kg bw and observed for 14 days.

No deaths occurred and no signs of toxicity were observed during the study period. Bodyweights were unaffected by treatment. Gross necropsy did not reveal any treatment-related findings.

The acute oral LD_{50} of the test material in the rat was found to be >2000 mg/kg bw, under the conditions of this study. The test material is not classified according to EC criteria.

[redacted] 2002a

B.6.11.2 Acute dermal toxicity (IIIA 7.1.2)

Study	AE F074383 00 SL33 A103 (EXP 04668 A) - Acute dermal toxicity in rats
Reference	[redacted] 2002b
Date performed	25 September - 9 October 2002
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	92/69/EC, OECD 402 (1987)
Deviations from the guideline	-
GLP	Yes
Test material	Asulox (AE F074383 00 SL33 A103, EXP 04668 A) (batch 990533; 392 g/ l asulam)
Study acceptable	Yes

Undiluted test material was applied for 24 hours under semi-occlusive conditions to the shorn dorsal skin of Wistar rats (5/sex) at a dose level of 2000 mg/kg bw/d. Animals were observed for 14 days.

No deaths occurred and no signs of toxicity or local dermal irritation were observed during the study period. Transient slight weight loss and transiently reduced weight gain were measured in females, however all animals gained weight over the study period. Gross necropsy did not reveal any treatment-related findings.

The acute dermal LD_{50} of the test material in the rat was found to be >2000 mg/kg bw under the conditions of this study. The test material is not classified according to EC criteria.

[redacted], 2002b

B.6.11.3 Acute inhalation toxicity (IIIA 7.1.3)

No study was submitted on the basis of the nature of the product and intended use and the low acute inhalation toxicity of the AS (LC_{50} >5.46 mg/l; [redacted] 1988a). No study is required.

B.6.11.4 Skin irritancy (IIIA 7.1.4)

Study	AE F074383 00 SL33 A103 (EXP 04668 A) - Acute dermal irritation in rabbits
Reference	[redacted], 2002c
Date performed	17th- 22nd September 2002
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	92/69/EC, OECD 404 (1992)
Deviations from the guideline	-
GLP	Yes
Test material	Asulox (AE F074383 00 SL33 A103, EXP 04668 A) (batch 990533; 392 g/ l asulam)
Study acceptable	Yes

0.5 ml of the undiluted test material was applied for 4 hours under semi-occlusive conditions to the shorn dorsal skin of three male New Zealand White rabbits. Local dermal reactions were assessed for 72 hours following dressing removal using the Draize scale.

No signs of irritation were observed in any animal at any time point.

The test material was found to be non-irritant under the conditions of this study and is not classified according to EC criteria.

[redacted], 2002c

B.6.11.5 Eye irritancy (IIIA 7.1.5)

Study	AE F074383 00 SL33 A103 (EXP 04668 A) - Acute eye irritation in rabbits
Reference	[redacted] 2002d
Date performed	24th-27th September 2002
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	92/69/EC, OECD 405 (1987)
Deviations from the guideline	-
GLP	Yes
Test material	Asulox (AE F074383 00 SL33 A103, EXP 04668 A) (batch 990533; 392 g/ l asulam)
Study acceptable	Yes

0.1 ml of the undiluted test material was instilled into one eye of three male New Zealand White rabbits. Ocular reactions were assessed for up to 72 hours, using the Draize scale.

Signs of irritation (Grade 1-2 conjunctival erythema and chemosis) were observed in two animals after 1 hour; signs decreased in severity and persisted to 48 hours in one animal. All signs had resolved by 72 hours.

Table B.6.11.5-1 Mean scores for ocular irritation

	Animal number		
	222	278	279
Average score 24-72 hours			
Corneal opacity	0	0	0
Iris effects	0	0	0
Conjunctival oedema	0	0.7	0
Conjunctival redness	0.3	0.7	0

The test material was found to be a mild eye irritant under the conditions of this study; the reactions are not of sufficient severity to warrant classification according to EC criteria.

[redacted], 2002d

B.6.11.6 Skin sensitisation (IIIA 7.1.6)

Study	AE F074383 00 SL33 A103 (EXP 04668 A) - Skin sensitisation test in guinea pigs (Modified Buehler test: nine applications)
Reference	[redacted] 2003
Date performed	30th September- 15th November 2002
Test facility	[redacted]
Report reference	[redacted]
Guideline(s)	OECD 406 (1992), EEC 96/54 B.6
Deviations from the guideline	Use of 9 applications
GLP	Yes
Test material	Asulox (AE F074383 00 SL33 A103, EXP 04668 A) (batch 990533; 392 g/ l asulam)
Study acceptable	Yes

The potential of the test material to induce delayed contact hypersensitivity was investigated in a 9-induction Buehler study using Dunkin-Hartley guinea pigs. Concentrations of the test material used for induction and challenge were based on the results of a preliminary study. An acceptable positive control study (reliability check) is also reported.

Nine induction challenges were performed on test animals (10/sex) using 6-hour occlusive applications of undiluted test material. Control animals (5/sex) were similarly treated, using purified water. After a rest period of nine days, all animals

were challenged using a 6-hour occlusive application of the undiluted test material. A second challenge was performed two weeks later, also using undiluted test material. Dermal reactions were assessed at 24 and 48 hours following dressing removal, using the Draize scale.

Dermal reactions (slight erythema and/or dryness) were observed in a number of test animals during the induction phase; no reactions were observed in controls. Following the initial challenge, dermal reactions (Grade 1 erythema) were observed in 4 test animals at 24 hours; reactions had resolved in one animal, persisted in two animals and had increased to Grade 2 in one animal at 48 hours. No reactions were observed in controls. Following the second challenge, Grade 2 erythema was observed in two test animals at 24 hours; persisting to 48 hours (with dryness) in one animal and decreasing in severity to Grade 1 in one animal. Grade 1 erythema at 24 and 48 hours was observed in one further test animal. Transient Grade 1 erythema was also observed in one test and one control animal at 24 hours.

Some evidence of skin sensitisation was seen under the conditions of this study, with reactions corresponding to a 15% sensitisation rate. Findings are consistent with the properties of the AS, which was shown to be a strong skin sensitiser in a Maximisation study ([redacted], 1987b). The test material is therefore classified according to current EC criteria.

[redacted], 2003

B.6.11.7 Summary of acute toxicity, irritation and skin sensitisation studies

'Asulox' was found to be of low acute oral and dermal toxicity. The product was found to be non-irritant to rabbit skin and was a mild eye irritant. Evidence of sensitisation was seen in a 9-induction Buehler study, consistent with the known sensitising properties of asulam-sodium. 'Asulox' is therefore classified as a category 1B skin sensitiser with the associated signal word 'Warning', and hazard statement (H317) 'May cause an allergic skin reaction'.

Table B.6.11.7-1 Summary of acute toxicity studies with 'Asulox'

Test	Species	Result	Classification (Regulation (EC) 1272/2008)	Reference
Acute oral	Rat	LD_{50} >2000 mg/kg bw	-	[redacted] 2002a
Acute dermal	Rabbit	LD_{50} >2000 mg/kg bw	-	[redacted] 2002b
Acute inhalation	-	-	-	-
Skin irritation	Rabbit	Non-irritant	-	[redacted] 2002c
Eye irritation	Rabbit	Mild irritant	-	[redacted] 2002d
Skin sensitisation	Guinea pig	Sensitiser (Buehler)	H317	[redacted] 2003

Consideration of skin sensitisation to groups exposed to the spray dilution has been completed. The tested concentrations of Asulam in water are 10, 40 and 50% in guinea pigs with sensitisation occurring at greater than or equal to 40% (see table 6.11.7-2).

Table 6.11.7-2 Summary of results from sensitisation studies

Study type and species	Sex and group size	Tested concentrations	Results	Classification	Reference
Guinea pig maximisation test	M/F 10/sex	5% Intradermal induction 50% topical induction 10 or 50% challenge concentration	10% challenge- no positive responders 50% challenge- 12/20 responders at 24h post challenge 9/20 responders at 48h post challenge	10%- not classified 50%-Sensitiser (historically R43, H317 Cat 1B)	[redacted] 1987c
Guinea Pig 9-induction Buehler method	M/F 10/sex	Undiluted Asulox (400g/L asulam) for induction and challenge	3/20 (15%) responders at challenge	Sensitiser (historically R43, H317 cat 1B)	[redacted] 2003

Data for the product Asulox suggests that 40% is the minimum concentration required to induce a sensitisation reaction as a positive response of 15% is exactly at the trigger for classification under Regulation (EC) 1272/2008 for the Buehler method. This suggests that less than 40% is not a sensitiser whilst greater than 40% would induce sensitisation. These assumptions are supported by the data in the study by Smith, 1987c; where a negative result was seen in a maximisation test with a 10% challenge concentration. The in use dilutions contain 0.3-1.3% asulam which at the lowest dilution rate is above the threshold for classification of the spray. It is, however, 30-fold lower than the predicted minimum concentration for sensitisation and therefore is not a concern for exposure groups such as bystanders and residents.

B.6.11.8 Toxicological data on non active substances (IIIA 7.4)

The information provided on the non-active substances present in 'Asulox' indicate no specific toxicological properties of concern.

B.6.11.9 Proposal for classification and labelling

'Asulox' is classified as 'Warning', with the associated hazard phrase (H317) 'May cause an allergic skin reaction', on the basis of the positive results in a Buehler study ([redacted] 2003). The precautionary phrases (P261) 'Avoid breathing dust/fume/gas/mist/vapours/spray', (P272) 'Contaminated work clothing should not be allowed out of the workplace' (P280) 'Wear protective gloves/ protective clothing/ eye protection/ face protection', (P302+P352) 'If on skin: wash with plenty of soap and water', (P333+P313) 'If skin irritation or rash occurs: get medical advice/attention', (P363) 'Wash contaminated clothing before reuse' are triggered by this classification.

B.6.11.10 Potential for interaction between ASs in the product

Not applicable.

B.6.12 Dermal absorption studies (IIIA 7.3)

No *in vivo* studies have been submitted but an *in vitro* study has been evaluated previously and the values were agreed upon in the EFSA final report for Asulam-sodium (0.5% for concentrate and 3% for in use spray dilution). The applicant reassessed the *in vitro* study in accordance with the EFSA guidance (EFSA Journal 2012; 10(4): 2665) and whilst the methodology section is the same as the DAR, the results and discussion have been updated.

B.6.12.1 Dermal absorption *in vitro*

Study	Asulox *In Vitro* Dermal Absorption Study Using Human Skin.
Reference	Odin-Feurtet, M. (2006).
Date performed (experimental phase)	25 October 2006 to 17 November 2006
Test facility	Bayer CropScience 355 rue Dostoïevski BP 153 06903 Sophia Antipolis Cedex France
Report reference	Report number SA 06280
Guidelines	OECD Guideline for the testing of chemicals. Guideline 428 skin absorption : i*n vitro* method (2004) OECD Environmental health and safety publication series on testing and assessment no. 28. Guidance document for the conduct of skin absorption studies (2004) European Commission guidance document on dermal absorption Sanco/222/2000 rev. 7 (2004)
Deviations from the guideline	None
GLP	Yes
Test material	[^{14}C]-asulam (radiochemical purity >99%, specific activity 5.16 MBq/mg) applied as: • a 400 g a.s./l SL formulation (‘Asulox’) specific activity 3.46 MBq/ml. • a 4 g a.s./l aqueous dilution of ‘Asulox’ specific activity 3.32 MBq/ml.
Study acceptable	Yes

Method

The *in vitro* dermal absorption of [^{14}C]-asulam (radiochemical purity > 99%) was investigated in human skin using a 400 g/l SL formulation ('Asulox') and an aqueous dilution of the formulation containing 4.0 g a.s./l. The dilution tested is slightly lower than that considered in the operator and bystander exposure estimates (12 g of asulam *per* litre of spray solution). Therefore, the dilution tested is likely to represent a worst case in terms of % dermal absorption considering the proposed uses.

Human skin samples from 7 donors were obtained from a tissue bank and stored at < -20 °C until used. Although the study report has no details of the age or sex of the donors, the anatomical site of the samples or the date of arrival of the samples at the laboratory, the notifier was able to produce the following information from the raw data file in response to a request for further details.

Fragment no.	Cells	Age	Race	Sex	Site	Date of arrival
TRA002200-2	H01 H06 H10	51	Caucasian	Female	Abdomen	26/10/06
TRA002194-2	H02 H08	50	Caucasian	Female	Abdomen	26/10/06
TRA002205-2	H03 H09	50	Caucasian	Female	Abdomen	26/10/06
TRA002178-2	H04	59	Caucasian	Female	Abdomen	01/03/06
TRA002190-1	H05 H07	37	Caucasian	Female	Abdomen	26/10/06
TRA002183-7	H11	60	Caucasian	Female	Abdomen	03/05/06
TRA002186	H12	60	Caucasian	Female	Abdomen	03/05/06

The thickness of the dermatomed membranes ranged from 437 – 545 µm, although no other details were provided regarding the processing of the samples.

Skin samples were mounted (presumably *stratum corneum* uppermost) in modified Franz flow-through diffusion cells with an exposed membrane area of 1.0 cm^2 in each cell. The high dose and low dose test groups each used 6 cells with skin from different donors. The diffusion cells were maintained at 32 °C using recirculating warm water. Before the start of the experiment, membrane integrity was assessed by measuring the trans-epidermal water loss (TEWL) using an evaporimeter probe on the top of the donor chamber. Skin samples with a TEWL > 40 g/hm^2 were considered to be potentially damaged (the TEWL values for the samples used were reported to range from 0.2 – 6.0 g/hm^2).

The receptor fluid of Eagle's medium supplemented with 5% bovine serum albumin and gentamycin (50 mg/l) at pH 7.3 was circulated at a flow rate of 1.5 ml/h and stirred continuously while in the receptor chamber. The solubility of asulam in the receptor fluid was determined experimentally and shown to be unlikely to limit the rate of dermal absorption in the study.

The nominal dose levels were achieved by applying the test materials at a rate of 10 $\mu l/cm^2$ to the exposed skin surface area of 1.0 cm^2 within the donor chamber. After an exposure period of 8 hours, the test compound was removed from the application site using natural sponge swabs wetted with 1% v/v Tween 80 in phosphate buffered saline (PBS) until no further radioactivity was removed (confirmed with a Geiger-Müller monitor). Samples of the receptor fluid were taken hourly for the duration of the experiment (24 hours) using a fraction collector.

Table B.6.12.1-1 Experimental details

Group	Group size *	Test material	Dose a.s. (µg/cm^2)** (applied as 10 µl of test material/cm^2)	Receptor fluid sampling (hours after application)
Concentrate	5	SL formulation 400 g a.s./l	Nominal dose 4000 µg a.s./cm^2 34.6 kBb/cm^2 Mean actual dose 3410.40 µg a.s./cm^2 31.7 kBq/cm^2	Hourly up to 24 hours
Dilution	6	Aqueous dilution 4.0 g a.s./l	Nominal dose 40 µg a.s./cm^2 33.2 kBq/cm^2 Mean actual dose 42.98 µg a.s./cm^2 39.75 kBq/cm^2	Hourly up to 24 hours

* the results from one of the cells in the high dose group (HO1) were excluded– no recovery data for this cell have been provided to support this statement.
** the actual applied dose was determined from quality control aliquots of each dose sampled before, during and after dosing each group of cells to ensure dose homogeneity. The author notes that, for the high dose group, the nominal dose values were used to calculate dose recovery values.

At the end of the study (24 hours after dosing) the skin samples were swabbed again (as described above) to remove surface residues. Radioactivity was determined by liquid scintillation counting (LSC) in the receptor fluid samples, skin wash swabs (solubilised in Soluene) and diffusion cell component washings (the components were soaked for at least 12 hours in1:1 v/v acetonitrile:distilled water). Tape stripping of the skin using Monaderm adhesive tape was carried out to remove the residual dose and the *stratum corneum.* The author concluded that the first two tape strips represented non-absorbed residual surface dose and the subsequent tape strips contained the *stratum corneum* (tape stripping was repeated an undisclosed number of times until a shiny appearance of the skin was noted indicating the removal of the *stratum corneum*). Each tape strip was individually solubilised in tetrahydrofuran and analysed by LSC. The remaining skin samples were solubilised in Soluene before LSC analysis. After the final receptor fluid sample had been taken, the remaining receptor fluid in the system was retained and analysed. All samples not analysed immediately were stored at approximately -20ºC in the dark until required. The LOD for each of the matrices was considered to be equivalent to twice the background radioactivity (dpm) for blank samples in the scintillation mixture.

Results and Discussion:

The solubility of the test article in the receptor fluid and the skin integrity were acceptable. At the end of the study period, one cell (H-01) exposed to the concentrate was removed from the analysis but no recovery data opr explanation was provided. This has no impact on the study as 5 cells from 5 different donors remained valid for the analysis.

The distribution of asulam-sodium was reported for the concentrate and in use dilution and the absorbed fraction was calculated from the skin, tape strips (3-17), receptor fluid and receptor chamber wash.

For the concentrate, the percentage absorption is 0.5% based on the absorbed fraction (0.32%) and the addition of the one standard deviation (SD=0.16) as the SD is greater than 25% of the mean total absorbed. The applicant had wished to exclude cell H-03 (see alternative mean), however, this proposal was not considered in the study report and H-03 was included in the laboratory's analysis. Whilst it is appreciated that the skin reading is high, on balance, H-03 is not dissimilar to H-04 in the final absorbed percentage and considering all 5 cells, there appears to be a high proportion of biological variation. To conclude, H-03 was included in the calculations.

Table 6.12.1-2 Distribution and percentage absorption of Asulox concentrate (400g/L)

			H-02	H-03	H-04	H-05	H-06	Mean (±SD)	Mean (±SD)[1]
a	Receptor fluid		0.000	0.010	0.012	0.000	0.124	0.029 ±0.053	0.034 ±0.060
b	Receptor compartment		0.000	0.000	0.000	0.000	0.000	0.000 ±0.000	0.000 ±0.000
c	Donor compartment		0.000	0.000	0.000	0.131	0.000	0.026 ±0.059	0.033 ±0.066
	Tape strips	1	0.018	0.255	0.111	0.088	0.013	0.097 ±0.098	0.058 ±0.049
		2	0.023	0.055	0.063	0.010	0.009	0.032 ±0.025	0.026 ±0.025
		3	0.031	0.030	0.044	0.057	0.008	0.034 ±0.018	0.035 ±0.021
		4	0.015	0.036	0.050	0.035	0.005	0.028 ±0.018	0.026 ±0.020
		5	0.012	0.015	0.026	0.041	0.006	0.020 ±0.014	0.021 ±0.016
		6	0.019	0.007	0.017	0.008	0.004	0.011 ±0.007	0.012 ±0.007
		7	0.014	0.017	0.065	0.011	0.002	0.022 ±0.025	0.023 ±0.028
		8	0.003	0.008	0.025	0.007	0.002	0.009 ±0.009	0.009 ±0.011
		9	0.009	0.017	0.010	0.008	0.002	0.009 ±0.005	0.007 ±0.004
		10	0.004	0.012	0.045	0.005	0.000	0.013 ±0.018	0.014 ±0.021
		11	0.004	-	0.012	0.005	0.001	0.006 ±0.005	0.006 ±0.005
		12	0.005	-	0.006	0.017	0.001	0.007 ±0.007	0.007 ±0.007
		13	-	-	-	0.004	-	0.004 ± n/a	0.004 ±n/a
d	**Tape strips: sub total (3-13)**		**0.116**	**0.142**	**0.300**	**0.198**	**0.031**	**0.163 ±0.010**	**0.160 ±0.010**
e	Skin wash (8h)		108.370	109.134	107.010	107.453	111.821	108.758 ±1.900	108.664 ±2.180
f	Skin wash + swabs (24h)		0.011	0.015	0.004	0.006	0.009	0.009 ±0.004	0.008 ±0.003
g	Surrounding skin		0.007	0.011	0.006	0.007	0.020	0.010 ±0.006	0.010 ±0.007
h	Skin		0.038	*0.353*	0.143	0.083	0.019	0.127 ±0.135	0.071 ±0.055
i	**Total recovery**		**108.583**	**109.975**	**107.649**	**107.976**	**112.046**	**109.252 ±1.801**	**109.191 ±1.935**
j	**Total absorption[2]**		**0.154**	**0.505**	**0.455**	**0.281**	**0.174**	**0.320 ±0.160**	**0.265 ±0.138**
	Maximal flux (µg/cm²/h)		---[3]	---[3]	---[3]	---[3]	---[3]	---[3]	---[3]

1. Mean (±SD) with outliers (H-03) excluded
2. Total absorption (j) = a + b + d + h
3. Not reported

For the in-use dilution, the percentage absorption is 2.83% based on the absorbed fraction (1.8%), corrected for normalisation (1.95%) and the addition of one standard deviation (0.887) as the SD is greater than 25% of the mean total absorbed. The applicant had wished to exclude cell H-11 (see alternative mean), however, this proposal was not considered in the study report and H-11 was included in the laboratory's analysis. Given the performing laboratory's experience it seems inappropriate to discard their conclusions, there is insufficient evidence to do so and inclusion of H-11 does not impact on the final rounded value to be used in the exposure assessments (3%).

Table 6.12.1-3 Distribution and percentage absorption of Asulox in-use dilution (4g/L)

			H-07	H-08	H-09	H-10	H-11	H-12	Mean (±SD)	Mean (±SD)[1]
a	Receptor fluid		0.016	0.062	0.008	0.106	0.000	0.004	0.033 ±0.042	0.039 ±0.044
b	Receptor compartment		0.000	0.000	0.000	0.000	0.000	0.000	0.000 ±0.000	0.000 ±0.000
c	Donor compartment		0.000	0.000	0.000	0.000	0.000	0.057	0.010 ±0.023	0.011 ±0.025
	Tape strips	1	0.155	0.110	0.209	0.296	0.117	0.085	0.162 ±0.079	0.171 ±0.084
		2	0.101	0.275	0.214	0.260	0.108	0.112	0.178 ±0.081	0.192 ±0.082
		3	0.068	0.263	0.267	0.312	0.137	0.150	0.200 ±0.095	0.212 ±0.100
		4	0.228	0.184	0.062	0.130	0.081	0.082	0.128 ±0.066	0.137 ±0.069
		5	0.197	0.156	0.232	0.168	0.092	0.085	0.155 ±0.058	0.168 ±0.055
		6	0.276	0.251	0.161	0.227	0.123	0.099	0.190 ±0.072	0.203 ±0.072
		7	0.268	0.150	0.097	0.087	0.048	0.057	0.118 ±0.082	0.132 ±0.083
		8	0.228	-	0.089	0.046	0.009	0.070	0.088 ±0.084	0.108 ±0.082
		9	0.171	-	0.074	0.022	0.042	0.046	0.071 ±0.059	0.078 ±0.065
		10	0.070	-	0.156	0.026	0.013	0.052	0.063 ±0.056	0.076 ±0.056
		11	0.073	-	0.086	0.066	0.010	0.037	0.054 ±0.031	0.066 ±0.021
		12	0.040	-	0.036	0.026	0.037	0.040	0.036 ±0.006	0.036 ±0.007
		13	0.024	-	0.015	0.034	0.003	0.025	0.020 ±0.012	0.025 ±0.008
		14	0.045	-	0.045	0.004	0.002	0.020	0.023 ±0.021	0.029 ±0.020
		15	0.029	-	0.079	-	0.005	0.078	0.048 ±0.037	0.062 ±0.029
		16	-	-	0.172	-	0.004	0.008	0.061 ±0.096	0.090 ±0.116
		17	-	-	-	-	-	0.017	0.017 ±n/a	0.017 ±n/a
d	**Tape strips: sub total (3-17)**		**1.717**	**1.004**	**1.571**	**1.148**	**0.606**	**0.866**	**1.272±0.060**	**1.693 ±0.035**
e	Skin wash (8H)		89.023	96.168	88.333	91.119	88.840	88.966	90.408 ±2.981	90.722 ±3.221
f	Skin wash + swabs (24h)		0.048	0.034	0.050	0.023	0.013	0.028	0.033 ±0.014	0.037 ±0.012
g	Surrounding skin		0.004	0.000	0.002	0.003	0.001	0.002	0.002 ±0.001	0.002 ±0.001
h	Skin		1.345	0.801	0.941	0.251	*0.031*	0.350	0.620 ±0.493	0.738 ±0.448
i	**Total recovery**		**92.409**	**98.454**	**91.328**	**93.206**	**89.716**	**90.470**	**92.717 ±3.135**	**93.349 ±3.130**
j	**Total absorption[2]**		**3.078**	**1.867**	**2.520**	**1.505**	**0.637**	**1.220**	**1.805 ±0.887**	**2.470 ±0.734**
	Maximal flux (µg/cm²/h)		---[3]	---[3]	---[3]	---[3]	---[3]	---[3]	---[3]	---[3]

1. Mean (±SD) with outliers (H-11) excluded
2. Total absorption (j) = a + b + d + h
3. Not reported

Conclusion:

When the results of this study are interpreted in conjunction with the EFSA guidance on dermal absorption (2012) the following human *in vitro* dermal absorption values were obtained for the concentrate (400 g asulam/L SL) and spray dilution (4 g asulam/L) of 0.5% and 3%, respectively.

B.6.12.2 Dermal absorption *in vivo*

No studies were submitted.

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

An in vitro dermal absorption study with human skin was submitted and absorption values for the concentrate and in use dilution of 'Asulox' have been proposed. Whilst, the approach taken by the applicant in calculating their percentages was not agreed with, the final absorption values by the RMS are not significantly different and agree with the proposals in the EFSA conclusions (2010;8 (12): 1822). For exposure assessments with 'Asulox', the dermal absorption value for the concentrate is 0.5% and for the in use dilution is 3%.

B.6.13 Toxicological data on non active substances

See B.6.11.8.