Asulam sodium

Annex B (Volume 3)

B.5 Methods of analysis

Version history

When	What
2016-04-18	Initial DAR

B.5 Methods of analysis

B.5.1 Analytical methods for formulation analysis (IIA 4.1, IIIA 5.1)

B.5.1.1 Technical active substance (IIA 4.1)

These studies were not available previously. As these studies have not been evaluated, an assessment is presented here for consideration.

Asulam sodium technical was dissolved in acetonitrile/water and the resulting solution analysed by HPLC-UV (260 nm), using a C18 column (identification was based on MS and retention times of certified standards). Validation data are shown in Table C.1.4. Representative chromatograms were submitted and were acceptable.

(Vohra, 2012a and 2012c and Desai 2014a)

[redacted] content of asulam sodium technical was determined by dissolving in acetonitrile and the resulting solution analysed by HPLC/UV (260 nm), using a C18 column (identity confirmed by co-elution with a reference standard and MS spectra). Validation data are shown in Table C.1.4. Representative chromatograms were submitted and were acceptable.

(Vohra, 2012a and 2012c and Desai 2014a)

Methanol content of asulam sodium technical was determined by dissolving the technical material in dichloromethane/dimethylformamide and the resulting solution analysed by GC/FID, using a CP capillary column. Validation data are shown in Table C.1.4. Representative chromatograms were submitted and were acceptable.

(Vohra, 2012b and Desai 2014a)

B.5.1.2 Impurities (IIA 4.1)

Please refer to the Confidential Information (Annex C/Volume 4).

B.5.1.3 Plant protection product (IIIA 5.1)

This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report, with updated validation data

<u>Asulam (CIPAC 240.011/TC/(M)3)</u>
A portion of the formulation was diluted with acetonitrile/water containing the internal standard 4-methoxybenzyl alcohol and the resulting solution analysed by HPLC-UV (270 nm), using a C18 column. Validation data are shown in Table B.5.1. Representative chromatograms were submitted and were.

(Bos, 2008b/2010a and Diepenhorst 2008b)

[redacted]<u>(CIPAC 240.011/TC/(M)3)</u>
A portion of the formulation was diluted with acetonitrile/water containing the internal standard 4-methoxybenzyl alcohol and the resulting solution analysed by HPLC-UV (270 nm), using a C18 column. Validation data are shown in Table B.5.1 below.

Representative chromatograms were submitted and were acceptable (limit of determination [redacted]).

(Bos, 2008b/2010a)

Methanol

A portion of the formulation was diluted with water and the resulting solution analysed by headspace GC-FID, using a CP-PoraBOND column. Validation data are shown in Table B.5.1 below. Representative chromatograms were submitted and were acceptable (limit of determination 3g/l).

(Bos, 2008c/2010b)

Table B.5.1 Summary of method validation (active substance and 'plant protection product')

	linearity (linear between) (g/l)	precision - repeatability	accuracy (%)	interference
Technical active substance				
Asulam	0.025-0.15 (n=6)	0.05 (n = 5)	-	No interference
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Methanol	0.002-0.06 (n=6)	0.06(n=5)	95 (n=10;fort. 0.05-1.3%)	No interference
Asulam*	0.04-0.14 (n=6)	0.2 (n = 5)	-	No interference
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Methanol*	0.001-0.02 (n=6)	0.2 (n=5)	97 (n=10;fort. 0.02-1%)	No interference
Plant protection product				
Asulam	0.01-0.08 (n=5)	0.27 (n = 10)	100(n=10;fort. 470g/l)	No interference
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Methanol	0.0004-0.003(n=5)	5.4 (n= 10)	99 (n=10;fort. 3-14g/l)	No interference

*Updated validation data (Desai 2014)

B.5.2 Analytical methods (residue) for treated plants, plant products, foodstuffs of plant and animal origin and feedingstuffs (IIA 4.2.1, IIIA 5.2)

This study has been evaluated at EU level and the conclusions presented are consistent with those in the EFSA report, with an updated method determining several additional plant metabolites

Samples were extracted three times with acidified acetonitrile/water and the resulting extracts evaporated to an aqueous remainder and analysed by LC/MS/MS (monitoring for in the case of parent asulam the precursor ion m/z 231 and the product ion m/z 156 and for its metabolite malonyl-asulam the precursor ion m/z 317 and the product ion m/z 242), using a C_{18} column. The supporting method validation data are shown in table 5.2. Representative chromatograms were submitted and were acceptable.
The method was validated by an independent laboratory.

Extraction efficiency of the method was not tested, the samples in the plant metabolism studies were extracted using the same solvent system, with the majority (77%) of the radioactivity being parent asulam. In addition at the time of development of the method extraction efficiencies were not required.

Note: To address extraction efficiency the below method was developed, using the same extraction scheme as the above method and was found to be capable of determining both asulam, its metabolites and their conjugates.

(NuBlein 2004a and b, Bardel 2004, Brumhard 2004 and Chambers 2009)

Samples were extracted three times with acidified acetonitrile/water and the resulting extracts evaporated to an aqueous remainder and analysed by LC/MS/MS (monitoring for in the case of parent asulam the precursor ion m/z 231 and the product ion m/z 156 [and 92 for confirmation]; for its metabolites acetyl asulam the precursor ion m/z 273 and the product ion m/z 198 [and 134 for confirmation]; for asulam glucosides [N-(methoxycarbonyl)-4-(α/β-D-glucopyranosylamino)benzensulfonamide] the precursor ion m/z 391 and the product ion m/z 271 [and 42 for confirmation]; acetyl sulphanilamide the precursor ion m/z 213 and the product ion m/z 64 [and 78 for confirmation]; for desamino asulam the precursor ion m/z 216 and the product ion m/z 77 [and 51 for confirmation]; for formyl asulam the precursor ion m/z 259 and the product ion m/z 184 [and 120 for confirmation]; for malonyl asulam the precursor ion m/z 317 and the product ion m/z 242 [and 178 for confirmation]; for malonyl sulphanilamide the precursor ion m/z 259 and the product ion m/z 162 [and 75 for confirmation] and for sulphanilamide the precursor ion m/z 173 and the product ion m/z 92 [and 93 for confirmation]), using a C_{18} column. The supporting method validation data are shown in table 5.2. Representative chromatograms were submitted and were acceptable.

(Chambers and Jarrot 2013a)

The above method using two ion transition (primary and confirmatory) was further validated for Asulam and Malonyl asulam, fortifying untreated grapes, cereal grain, Sunflower seeds and lettuce at 0.01 and 0.1 g/kg. Samples were extracted only once with acidified acetonitrile/water. Acceptable recoveries were obtained at both fortification levels for all four crops, albeit with a number (especially for grapes) in the 65-69% range.
On analysing residue trail samples and comparing them to the results obtained using a the initial method, were the samples are extracted three times, residues were 2 to 3 times lower – Potential Data Gap, if two ion transitions required the method needs revalidating, extracting the samples three times with acidified acetonitrile/water.

(Chambers and Jarrot 2013b)

Multi-residues (post approval monitoring)

A multi-residue method for post approval monitoring was not submitted. The notifier stated that asulam could not be determined by the German DFG method S19 (GC/MS). However, the methods submitted for the determination of asulam in plants appear to be acceptable for use in routine monitoring.

(Bacher, 2003a)

B.5.3 Analytical methods (residue) in soil, water and air (IIA 4.2.2 to 4.2.4, IIIA 5.2)

B.5.3.1 Residues in soil (IIA 4.2.2)

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

Samples were extracted with acidified acetonitrile/water and the resulting extracts centrifuged. The resulting supernatant diluted with water and analysed by LC/MS/MS (monitoring for in the case of parent asulam the precursor ion m/z 231 and the product ion m/z 156 [and 92 for confirmation] and for its metabolite sulphanilamide the precursor ion m/z 173 and the product ion m/z 156 [and 92 for confirmation]), using a C_{18} column. The supporting method validation data are shown in table 5.2. Representative chromatograms were submitted and were acceptable.

(Chambers and Jarrot 2013c)

B.5.3.2 Residues in water (IIA 4.2.3)

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

Samples were directly injected into a LC/MS/MS (monitoring for the precursor ion 231 and the product ion m/z 156 [and 92 for confirmation] for asulam and for its metabolite sulphanilamide the precursor ion m/z 173 and the product ion m/z 156 [and 92 for confirmation]), using a C18 column. The supporting method validation data are shown in Table B.5.2. Representative chromatograms were submitted and were acceptable.

(Chambers and Jarrot 2013d)

B.5.3.3 Residues in air (IIA 4.2.4)

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

Air samples were extracted using a XAD-2 adsorption tube, by drawing air through the tube at 1.5 L/min for a period of 6 hours. The tubes were then extracted with methanol and resulting extracts analysed by HPLC/MS/MS (monitoring for in the case of parent asulam the precursor ion m/z 231 and the product ion m/z 156), using a C_{18} column The supporting method validation data are shown in Table B.5.2 (recovery data were submitted on spiked XAD-2 extraction tubes that had had 1.5 L/min of air passed through them over a 6 hour period, to determine the retention capacity of the tube). Representative chromatograms were submitted and were acceptable.

(Bacher, 2001)

Table B.5.2 Summary of method description and validation (treated plants, plant products, foodstuffs, feedingstuffs, environmental samples)

Substrate	Analyte	Dissolution/ extraction	Partition, clean-up	Quantification	Limit of quantif-ication (mg/kg)	Recovery fortifica-tion level (mg/kg)	Recoveries % range (mean)	Repeatabil-ity RSD (%) (n)	Linearity demon-strated (mg/l)	Ref.
Plant										
Spinach	Asulam	Acidified acetonitrile/ water	Evaporated to aqueous remainder	LC/MS/MS m/z 156	0.1*	0.05 0.5 5	90-96 (93) 81-85 (83) 90-92 (91)	2.8 (5) 2.5 (3) 1.3 (3)	0.0003-0.5	NuBlein 2004a
	Malonyl-asulam			m/z 242		0.05 0.5 5	93-99 (97) 92-94 (93) 92-94 (93)	2.6 (5) 1.1 (3) 1.2 (3)	0.0003-0.5	
Spinach	Asulam	Acidified acetonitrile/ water	Centrifuged and the resulting supernatant diluted with water	LC/MS/MS m/z 156	0.01	0.01 0.1	78-99 (88) 94-111 (99)	8.5 (6) 6.2 (6)	0.0006-0.03	Chambers and Jarrett, 2013a
				m/z 92		0.01 0.1	75-98 (85) 95-111 (100)	12 (6) 5.9 (6)	0.0006-0.03	
	Acetyl-asulam			m/z 198	0.01	0.01 0.1	87-116 (100) 102-108 (105)	12 (6) 2 (6)	0.0006-0.03	
				m/z 134		0.01 0.1	84-114 (96) 99-108 (103)	12 (6) 3.1 (6)	0.0006-0.03	
	Asulam glucosides			m/z 271	0.01	0.01 0.1	85-108 (100) 98-105 (101)	9.8 (6) 2.8 (6)	0.0006-0.03	
				m/z 42		0.01 0.1	61-102 (82)** 93-102 (97)	18 (6) 4.1 (6)	0.0006-0.03	
	Acetyl sulphanilamide			m/z 64	0.01	0.01 0.1	90-96 (90) 81-85 (91)	7.3 (6) 3.7 (6)	0.0006-0.03	
				m/z 78		0.01	81-98 (91)	6.8 (6)	0.0006-	

Substrate	Analyte	Dissolution/ extraction	Partition, clean-up	Quantification	Limit of quantif-ication (mg/kg)	Recovery fortifica-tion level (mg/kg)	Recoveries % range (mean)	Repeatabil-ity RSD (%) (n)	Linearity demon-strated (mg/l)	Ref.
						0.1	86-95 (91)	3.7 (6)	0.03	
	Desamino asulam			m/z 77	0.01	0.01 0.1	67-99 (79) 95-99 (98)	16 (6) 1.4 (6)	0.0006-0.03	
				m/z 51		0.01 0.1	70-98 (81) 94-101 (97)	12 (6) 2.6 (6)	0.0006-0.03	
	Formyl asulam			m/z 184	0.01	0.01 0.1	84-116 (100) 89-99 (95)	13 (6) 3.9 (6)	0.0006-0.03	
				m/z 120		0.01 0.1	71-109 (90) 95-101 (97)	14 (6) 2.4 (6)	0.0006-0.03	
	Malonyl asulam			m/z 242	0.01	0.01 0.1	69-104 (82) 81-92 (88)	15 (8) 4.2 (6)	0.0006-0.03	
				m/z 178		0.01 0.1	69-108 (82) 80-93 (86)	19 (9) 5.2 (6)	0.0006-0.03	
	Malonyl sulphanilamide			m/z 162	0.05	0.05 0.5	102-111 (107) 92-99 (95)	3.2 (6) 2.5 (6)	0.003-0.15 0.003-0.15	
				m/z 75		0.05 0.5	81-115 (100) 92-102 (95)	17 (5) 4.1 (6)	0.0006-0.03	
	Sulphanilamide			m/z 92	0.01	0.01 0.1	70-91 (77) 70-76 (73)	11 (5) 4.4 (6)	0.0006-0.03	
				m/z 93		0.01 0.1	63-102(80)*** 73-82 (78)	18 (6) 4.7 (6)		
Orange	Asulam	Acidified acetonitrile/ water	Evaporated to aqueous remainder	LC/MS/MS m/z 156	0.1*	0.05 0.5	84-90 (91) 91-97 (94)	2.3 (3) 3.3 (3)	0.0005-0.01?	Bardel, 2004

Substrate	Analyte	Dissolution/ extraction	Partition, clean-up	Quantification	Limit of quantif-ication (mg/kg)	Recovery fortifica-tion level (mg/kg)	Recoveries % range (mean)	Repeatabil-ity RSD (%) (n)	Linearity demon-strated (mg/l)	Ref.
						5	73-76 (75)	2 (3)		
	Malonyl-asulam			m/z 242		0.05 0.5 5	84-90 (87) 102-108 (105) 70-74 (72)	3.4 (3) 2.9 (3) 2.9 (3)	0.0005-0.01	
Barley grain	Asulam	Acidified acetonitrile/ water	Evaporated to aqueous remainder	LC/MS/MS m/z 156	0.1*	0.05 0.5 5	90-97 (94) 90-95 (92) 97-111 (90)	3.3 (5) 2.7 (3) 0.6 (3)	0.0005-0.01	Bardel, 2004
	Malonyl-asulam			m/z 242		0.05 0.5 5	94-113 (106) 91-93 (92) 81-84 (82)	7.3 (5) 1.3 (5) 1.9 (5)	0.0005-0.01	
Sunflower seeds	Asulam	Acidified acetonitrile/ water	Evaporated to aqueous remainder	LC/MS/MS m/z 156	0.1*	0.05 0.5 5	90-99 (94) 95-104 (100) 107-109(108)	5 (5) 4.5 (5) 0.9 (5)	0.0005-0.01	Bardel, 2004
	Malonyl-asulam			m/z 242		0.05 0.5 5	99-101 (100) 93-94 (93) 92-98 (96)	1 (3) 0.6 (3) 3.4 (3)	0.0005-0.01	
Spinach ILV	Asulam	Acidified acetonitrile/ water	Evaporated to aqueous remainder	LC/MS/MS m/z 156	0.1*	0.05 5	66-91 (76) 82-125(107)	12 (5) 18 (5)	0.0005-0.01	Chambers, 2009
	Malonyl-asulam			m/z 242		0.05 5	101-112 (108) 79-124(104)	4.5(5) 18 (5)	0.0005-0.01	
Orange ILV	Asulam	Acidified acetonitrile/ water	Evaporated to aqueous remainder	LC/MS/MS m/z 156	0.1*	0.05 0.5	85-94 (91) 88-102 (93)	4.2 (5) 5.9 (5)	0.0005-0.05	Brumhard 2004
	Malonyl-asulam			m/z 242		0.05 0.5	91-97 (94) 90-110 (99)	2.4 (5) 7.4 (5)	0.0005-0.05	
Barley grain		Acidified	Evaporated to	LC/MS/MS	0.1*					Brumhard

Substrate	Analyte	Dissolution/ extraction	Partition, clean-up	Quantification	Limit of quantif-ication (mg/kg)	Recovery fortifica-tion level (mg/kg)	Recoveries % range (mean)	Repeatabil-ity RSD (%) (n)	Linearity demon-strated (mg/l)	Ref.
ILV	Asulam Malonyl-asulam	acetonitrile/ water	aqueous remainder	m/z 156 m/z 242		0.05 0.5 0.05 0.5	86-92 (88) 84-92 (89) 88-95 (91) 89-99 (95)	2.8 (5) 3.3 (5) 4.9 (5) 4 (5)	0.0005-0.05 0.0005-0.05	2004
Sunflower seeds ILV	Asulam Malonyl-asulam	Acidified acetonitrile/ water	Evaporated to aqueous remainder	LC/MS/MS m/z 156 m/z 242	0.1*	0.05 0.5 0.05 0.5	83-92 (86) 90-99 (95) 83-94 (86) 97-100 (98)	4.1 (5) 3.2 (5) 9 (5) 0.9 (5)	0.0005-0.01 0.0005-0.05	Brumhard 2004
Soil										
Speyer 2.2 (Sandy loam)	Asulam Sulfanilamide	Acidified acetonitrile/ water	Centrifuged and the resulting supernatant diluted with water	LC/MS/MS m/z 156 m/z 92 m/z 156 m/z 92	0.005 0.005 0.01 0.01	0.005 0.05 0.005 0.05 0.01 0.1 0.01 0.1	82-114 (100) 95-112 (99) 81-116 (101) 93-112 (99) 67-111 (91) 77-95 (81) 80-108 (96) 77-93 (82)	14 (6) 6.7 (6) 14 (7) 7 (6) 15 (11) 8.3 (6) 11 (6) 7.3 (6)	0.0006-0.03 0.0006-0.03 0.001-0.07 0.001-0.07	Chambers and Jarrett, 2013c
Water										
Surface water (Pond water – Ongar, UK)	Asulam Sulfanilamide	-	-	LC/MS/MS m/z 156 m/z 92 m/z 156	0.05 µg/l 0.05 µg/l 0.05 µg/l	0.05 µg/l 0.5 µg/l 0.05 µg/l 0.5 µg/l 0.05 µg/l 0.5 µg/l	87-114 (102) 79-102 (96) 87-102 (96) 86-103 (98) 89-106 (98) 79-102 (86)	12 (6) 8.9 (6) 6.8 (6) 6.4 (6) 7.1 (5) 13 (6)	0.00001-0.0008 0.00001-0.0008 0.00001-0.002	Chambers and Jarrett, 2013d

Substrate	Analyte	Dissolution/ extraction	Partition, clean-up	Quantification	Limit of quantif-ication (mg/kg)	Recovery fortifica-tion level (mg/kg)	Recoveries % range (mean)	Repeatabil-ity RSD (%) (n)	Linearity demon-strated (mg/l)	Ref.
				m/z 92	0.05 µg/l	0.05 µg/l 0.5 µg/l	80-113 (101) 72-103 (89)	14 (5) 15 (6)	0.00001-0.002	
Drinking water (Ongar, UK)	Asulam	-	-	LC/MS/MS m/z 156	0.05 µg/l	0.05 µg/l 0.5 µg/l	93-113 (106) 78-114 (94)	6.8 (6) 9 (8)	0.00001-0.0008	Chambers and Jarrett, 2013d
				m/z 92	0.05 µg/l	0.05 µg/l 0.5 µg/l	76-117 (106) 81-109 (93)	15 (6) 9.6 (8)	0.00001-0.0008	
	Sulfanilamide			m/z 156	0.05 µg/l	0.05 µg/l 0.5 µg/l	77-102 (91) 76-97 (84)	11 (5) 10 (5)	0.00001-0.002	
				m/z 92	0.05 µg/l	0.05 µg/l 0.5 µg/l	71-102 (86) 71-106 (83)	14 (5) 16 (5)	0.00001-0.002	
Air 25ºC and30%RH	Asulam	XAD-2 adsorption tube	Tubes extracted with methanol	LC/MS/MS m/z 156	10 µg/m^3	10 µg/m^3 100 µg/m^3	101-108 (104) 84-97 (93)	3 (5) 6 (5)	0.02-1	Bacher, 2001
36ºC and82%RH					10 µg/m^3	10 µg/m^3 100 µg/m^3	82-110 (93) 75-100 (88)	14 (5) 11 (5)		

Recoveries were only specified if they were not in the range of 70-110%
ILV = Independent Laboratory Validation data
RH = Relative Humidity
*Sum of asulam and malonyl-asulam expressed as parent asulam
**Only one recovery below 79%
***Only one recovery below 71%

B.5.4 Analytical methods (residue) in human and animal tissues and fluids (IIA 4.2.5, IIIA 5.2)

B.5.4.1 Animal products

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

Samples were extracted with acetonitrile/acidified water (for milk methanol/acetonitrile/acidified water) and the resulting extracts acetylated using sodium acetate/acetic anhydride, converting asulam (and its metabilites acetylasulam and sulphanilamide) to its metabolite acetylasulam, which was in turn converted to acetylsulfanilamide, by acid hydrolysis. The resulting extracts were partitioned with ethyl acetate followed by acetonitrile and resulting acetonitrile extracts evaporated to dryness, reconstituted in methanol/formic acid and analysed by LC/MS/MS (monitoring for the precursor ion m/z 215 and the product ion m/z 198), using a Luna 5C column. The supporting method validation data are shown in table B.5.2. Representative chromatograms were submitted and were acceptable. No independent laboratory validation data were submitted, however no positive EU MRLs have been proposed, due to animal transfer studies indicating that positive residues would not result in animal products.

(Bacher, 2003b)

Extraction efficiency of the method of S19 were not tested, however no conjugated animal metabolites were detected in the animal metabolism studies.
Note: A study was submitted looking at extraction efficiencies, unfortunately not for the required S19 method.

B.5.4.2 In support of therapeutic and diagnostic regimes

No methods of analysis were submitted or required as asulam is not classified as toxic.

Table B.5.3 Summary of method description and validation (animal tissues and human fluids)

Substrate	Analyte	Dissolution/ extraction	Partition, clean-up	Quantification	Limit of quantif-ication (mg/kg)	Recovery fortifica-tion level (mg/kg)	Recoveries % range (mean)	Repeatabil-ity RSD (%) (n)	Linearity demon-strated (mg/l)	Ref.
Animal										
Milk	*Asulam plus its metabolite acetylsulfanilamide	Methanol/ acetonitrile/ acidified water	Extracts acetylated to acetylasulam, which was in turn converted to acetylsulf-anilamide, by acid hydrolysis	LC/MS/MS m/z 198	0.01	0.01 0.1	72-102 (84) 80-94 (85)	14 (5) 8 (5)	0.01-1	Bacher, 2003b
Egg	*Asulam plus its metabolite acetylsulfanilamide	Acetonitrile/ acidified water	Extracts acetylated to acetylasulam, which was in turn converted to acetylsulf-anilamide, by acid hydrolysis	LC/MS/MS m/z 198	0.01	0.01 0.1	74-83 (78) 70-97 (82)	5 (6) 13 (6)	0.01-0.5	Bacher, 2003b
Muscle (cattle)	*Asulam plus its metabolite acetylsulfanilamide	Acetonitrile/ acidified water	Extracts acetylated to acetylasulam, which was in turn converted to acetylsulf-anilamide, by acid hydrolysis	LC/MS/MS m/z 198	0.01	0.01 0.1	80-106 (94) 87-104 (97)	11 (6) 8 (6)	0.01-0.5	Bacher, 2003b
Kidney (cattle)	*Asulam plus its metabolite acetylsulfanilamide	Acetonitrile/ acidified water	Extracts acetylated to acetylasulam, which was in turn converted to acetylsul-fanilamide, by acid hydrolysis	LC/MS/MS m/z 198	0.05	0.01 0.1	78-83 (80) 73-98 (87)	3 (5) 11 (5)	0.01-1	Bacher, 2003b

Substrate	Analyte	Dissolution/ extraction	Partition, clean-up	Quantification	Limit of quantif-ication (mg/kg)	Recovery fortifica-tion level (mg/kg)	Recoveries % range (mean)	Repeatabil-ity RSD (%) (n)	Linearity demon-strated (mg/l)	Ref.
Fat (cattle)	*Asulam plus its metabolite acetylsulfanilamide	Acetonitrile/ acidified water	Extracts acetylated to acetylasulam, which was in turn converted to acetylsulf-anilamide, by acid hydrolysis	LC/MS/MS m/z 198	0.01	0.01 0.1	79-98 (90) 78-96 (89)	9 (5) 8 (5)	0.005-0.5	Bacher, 2003b

*50:50 mix of asulam plus its metabolite acetylsulfanilamide (residues definition = Sum of asulam and its metabolites containing the sulphanilamide moiety expressed as parent asulam)

Table B.5.4 Summary of method validation (treated plants, plant products, foodstuffs and feedingstuffs)

substrate	recovery (mg/kg/ % mean recovery)	precision - repeatability	limit of determination (mg/kg)
Plant material			
Barley grain – Asulam	0.05/94	3.3	0.05
Barley grain – Malonyl-asulam	0.05/106	7.3	0.05
Spinach - Asulam	0.05/93	2.8	0.05
Spinach - Malonyl-asulam	0.05/97	2.6	0.05
Orange – Asulam	0.05/91	2.3	0.05
Orange – Malonyl-asulam	0.05/87	3.4	0.05
Sunflower seed – Asulam	0.05/94	5	0.05
Sunflower seed – Malonyl-asulam	0.05/100	1	0.05
Environment			
Soil (Silt loam) - Asulam	0.005/100	6	0.005
Soil (Silt loam) - Sulphanilamide	0.005/91	11	0.005
Water (Surface) - Asulam	0.05µg/l/102	12	0.05µg/l
Water (Surface) - Sulphanilamide	0.05µg/l/98	7.1	0.05µg/l
Water (Drinking) - Asulam	0.05µg/l/106	6.5	0.05µg/l
Water (Drinking) - Sulphanilamide	0.05µg/l/91	11	0.05µg/l
Air (25ºC and 30%RH) – Asulam	10µg/m^3/104	3	10µg/m^3
Air (36ºC and 82%RH) – Asulam	10µg/m^3/93	14	10µg/m^3
Human and animal tissues and fluids			
Milk –Asulam (plus its metabolite acetylsulfanilamide)	0.01/84	14	0.01
Egg – Asulam (plus its metabolite acetylsulfanilamide)	0.01/78	5	0.01
Muscle - Asulam (plus its metabolite acetylsulfanilamide)	0.01/14	11	0.01
Kidney – Asulam (plus its metabolite acetylsulfanilamide)	0.01/80	3	0.01
Fat – Asulam (plus its metabolite acetylsulfanilamide)	0.01/50	9	0.01
Therapeutic regimes			
Not required as asulam is not classified as toxic	-	-	-

B.5.5 Summary of Methods of Analysis

Asulam and [redacted] were determined in the technical active substance and plant protection product by HPLC-UV, using using a C_{18} column. The limit of determination of [redacted] in technical material was [redacted] and in the product [redacted]. Acceptable validation data were submitted.
Methanol was determined in the technical active substance and plant protection product by GC-FID, using a CP capillary column. The limit of determination of Methanol in technical material was 0.02% and in the product 3g/l. Acceptable validation data were submitted.

Asulam (and its metabolite malonyl-asulam) residues in plants and plant products (Spinach, Orange, Barley Grain and Sunflower Seed) were determined by extraction with acetonitrile/water and the resulting extracts evaporated to an aqueous remainder and analysed by LC/MS/MS (monitoring for in the case of parent asulam the precursor ion m/z 231 and the product ion m/z 156 and for its metabolite malonyl-asulam the precursor ion m/z 317 and the product ion m/z 242), using a C_{18} column. The limit of determination was 0.1 mg/kg (asulam plus its metabolite malonyl-asulam expressed as

asulam). Acceptable validation data were submitted and the method was validated by an independent laboratory.

Asulam (and its metabolite sulphanilamide) residues in soil were determined by acidified acetonitrile/water and the resulting extracts centrifuged. The resulting supernatant diluted with water and analysed by LC/MS/MS (monitoring for in the case of parent asulam the precursor ion m/z 231 and the product ion m/z 156 [and 92 for confirmation] and for its metabolite sulphanilamide the precursor ion m/z 173 and the product ion m/z 156 [and 92 for confirmation]), using a C_{18} column. The limit of determination was 0.005 mg/kg for asulam and sulfanilamide. Acceptable validation data were submitted.

Asulam (and its metabolite sulphanilamide) residues in water were determined by directly injected into a LC/MS/MS (monitoring for the precursor ion 231 and the product ion m/z 156 [and 92 for confirmation] for asulam and for its metabolite sulphanilamide the precursor ion m/z 173 and the product ion m/z 156 [and 92 for confirmation]), using a C18 column. The limit of determination was 0.05 µg/l for asulam and sulphanilamide in surface and drinking water. Acceptable validation data were submitted.

Asulam residues in air were determined by drawing air through a XAD adsorption tube and extracting the tube with methanol. The resulting extracts were analysed by HPLC/MS/MS, using a C_{18} column. The limit of determination was 10 $\mu g/m^3$. Acceptable validation data were submitted.

Asulam (and its metabolites containing the sulphanilamide moiety) residues in animal products (milk, muscle, fat, kidney and egg) were determined by extracted with acetonitrile/acidified water (for milk methanol/acetonitrile/acidified water) and the resulting extracts acetylated using sodium acetate/acetic anhydride, converting asulam (and its metabilites acetylasulam and sulphanilamide) to its metabolite acetylasulam, which was in turn converted to acetylsulfanilamide. The resulting extracts were analysed by HPLC-MS/MS (monitoring for the precursor ion m/z 215 and the product ion m/z 198), using a Luna 5C column. The limit of determination was 0.01 mg/kg. Acceptable validation data were submitted.
No independent laboratory validation data were submitted, however no positive EU MRLs have been proposed, due to animal transfer studies indicating that positive residues would not result in animal products.

No methods of analysis in support of therapeutic and diagnostic regimes were submitted or required as asulam is not classified as toxic.

B.5.6 Conclusions

The information submitted with regards to methods of analysis is sufficient to support approval of asulam sodium.

B.5.7 Literature search

It is considered that the literature searches are acceptable in terms of databases searched and the search criteria applied. On checking the references list, agree with

the applicant assessment that the search did not reveal any references of relevance to this section. In addition, the applicant submitted new studies to cover the areas of concern raised in the EFSA conclusion.

B.5.8 References relied on

In every chapter (B.1, B.2, etc.) in Volume 3 (AS) the reference relied on heading should start with a paragraph indicating how the literature search was carried out and if this is considered acceptable. It should also be indicated if the RMS can agree with the justifications given by the notifier (especially for non-relevant literature).This is not expected to be a detailed study-by-study consideration. Relevant literature would be evaluated and assessed in the normal way within each section.

For (draft) renewal assessment reports the reference lists at the end of each section/chapter (sorted by data requirement) should include the newly submitted data relied upon as well as those original submitted tests and studies that are still considered relevant to support the application for renewal. However these studies should be clearly identified in the reference list as well as in the individual study sections. This could be done by consistent use of a statement for each study:
Previous evaluation: responded "N.A". for NAS, "Submitted for the purpose of renewal", or "In DAR (year)", "In addendum to DAR (year)" or any other appropriate

Active substance

*details to be added during Peer review

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or Unpublished	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	*Previous evaluation
CA 1.11.1/01	Vohra, Y.	2012a	Analysis of five representative production batches of asulam sodium (50% w/v) aqueous concentrate grade active ingredient to determine % asulam sodium and to quantify its associated impurities United Phosphorus Limited Report No.: 227-2-12-4961 JAI Research Foundation, India GLP, Unpublished Confidential information	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or Unpublished	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	*Previous evaluation
CA 1.11.1/03	Desai	2014a	Method validation for of asulam sodium aqueous concentrate grade active ingredient and its associated impurities United Phosphorus Limited Report No.: 258-2-12-8132 JAI Research Foundation, India GLP, Unpublished Confidential information	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA 4.2.1/01	Vohra, Y.	2012c	Method Validation for of Asulam Sodium (50% w/v) Aqueous Concentrate Grade Active Ingredient and its Associated Impurities United Phosphorus Limited Report No.: 228-2-12-4962 JAI Research Foundation, India GLP, Unpublished ⇒ Document J, CA 2.5.2/03	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA 4.2.3/02	Vohra, Y.	2012b	Determination of methanol content in five representative production batches of asulam sodium (50% w/v) aqueous concentrate United Phosphorus Limited Report No.: 258-2-12-4963 JAI Research Foundation, India GLP, Unpublished Confidential information	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA 4.3.1.1/01	Chambers, J.G., Jarrett, H.	2013a	Asulam and Major Metabolites: Method Validation in Spinach United Phosphorous Limited Report No. NZ/13/005 Battelle UK Ltd., United Kingdom GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or Unpublished	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	*Previous evaluation
CA 4.3.1.1/02	Nüßlein, F.	2004a	Method 00831 for the determination of residues of Asulam and Malonyl-asulam in/on spinach by HPLC-MS/MS United Phosphorus Limited Report No.: C043214 Bayer CropScience Germany GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA 4.3.1.1/03	Chambers, J.G.	2009	Determination of Residues of Asulam and Malonyl-asulam in Spinach – Independent Laboratory Validation United Phosphorus Limited Report No.: NZ/08/004 Battelle UK Ltd. United Kingdom GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA 4.3.1.1/05	Chambers, J.G	2007	Determination of Residues of Asulam and Malonyl Asulam in Spinach – Method validation United Phosphorus Limited Report No.: OU/07/003 Battelle UK Limited, United Kingdom GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA 4.3.1.1/04	Nüßlein, F.	2004b	Method 00843 for the determination of residues of malonylsulfanilamide (AE 1422567) in/on spinach (leaves and washings) by HPLC-MS/MS United Phosphorus Limited Report No.: C043661 Bayer CropScience Germany GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA 4.3.1.1/07	Chambers, J.G., Jarrett, H.	2013b	Asulam and Malonyl Asulam: Method Validation in Crops United Phosphorous Limited Report No. NZ/13/002 Battelle UK Ltd. United Kingdom GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or Unpublished	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	*Previous evaluation
CA 4.3.1.1/08	Bardel, P.	2004	Enforcement method 00831 modification M001 for the determination of residues of asulam and malonyl-asulam in/on barley (grain), sunflower (seeds) and orange (fruit) by HPLC-MS/MS. United Phosphorus Limited Report No.: C042941 Bayer CropScience Germany GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA 4.3.1.1/09	Brumhard, B.	2004	Independent Laboratory Validation of Method 00831/M001 for the Determination of Residues of Asulam and Malonyl-Asulam in/on Matrices of Plant Origin by HPLC-MS/MS United Phosphorus Limited Report No.: C042966 Bayer CropScience Germany GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA 4.3.1.1/10	Bacher, R.	2003a	Assessment and Possible Validation of the Multi-Residue Enforcement Method DFG S19 (Extended Revision) for the Determination of Asulam in Spinach United Phosphorus Limited Report No.: C030948 PTRL Europe, Germany GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA 4.3.1.2/01	Bacher, R.	2003b	Validation of a Common Moiety Method for the Determination of Asulam and its Metabolite Acetylsulfanilamide in Foodstuffs of Animal Origin United Phosphorus Limited Report No.: C030961 PTRL Europe, Germany GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or Unpublished	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	*Previous evaluation
CA 4.4/02	Chambers, J.G., Jarrett, H.	2013c	Asulam and Sulfanilamide: Method Validation in Soil United Phosphorus Limited Report No. NZ/13/004 Battelle UK Ltd., United Kingdom GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA 4.5/02	Chambers, J.G., Jarrett, H.	2013d	Asulam and Sulfanilamide: Method Validation in Surface Water and Drinking Water United Phosphorus Limited Report No. NZ/13/003 Battelle UK Ltd., United Kingdom GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	
CA 4.7/01	Bacher, R.	2001	Analytical Method for the determination of Asulam in Air United Phosphorus Limited Report No.: C015406 PTRL Europe, Germany GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	

Plant Protection Product – Asulox

*details to be added during Peer review

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or Unpublished	Vertebrate study Y/N	Data protection claimed Y/N	Owner	Justification if data protection is claimed	*Previous evaluation
CP 5.2.1/02	Bos, M.W.S.	2008b	Validation of SOP DLA-267.2 "Asulam technical and formulations asulam and [redacted] content by HPLC" Cerexagri B.V., The Netherlands United Phosphorus Limited Report No.: DL 08-020 GLP, Unpublished ⇒ Document J CP 5.2.4/03	N	Y	UPL	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	
CP 5.2.1/03	Diepenhorst, P.C.	2008b	Validation of SOP DLA-267.1 "Asulam-sodium technical and formulations determination of active ingredient by HPLC" Cerexagri B.V., The Netherlands United Phosphorus Limited Report No.: DL 07-073 GLP, Unpublished	N	Y	UPL	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	
CP 5.2.1/04	Bos, M.W.S.	2010a	Revalidation of SOP DLA-267.2 "Asulam technical and formulations asulam and [redacted] content by HPLC" Cerexagri B.V., The Netherlands United Phosphorus Limited Report No.: DL 10-014 GLP, Unpublished ⇒ Document J CP 5.2.4/04	N	Y	UPL	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	
CP 5.2.4/01	Bos, M.W.S.	2008c	Validation of SOP DLA-267.3 "Asulam technical and formulations determination of methanol content by headspace GC-FID" Cerexagri B.V., The Netherlands United Phosphorus Limited Report No.: DL 08-025 GLP, Unpublished	N	Y	UPL	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or Unpublished	Vertebrate study Y/N	Data protection claimed Y/N	Owner	Justification if data protection is claimed	*Previous evaluation
CP 5.2.4/02	Bos, M.W.S.	2010b	Revalidation of SOP DLA-267.3 "Asulam technical and formulations determination of methanol content by headspace GC-FID" Cerexagri B.V., The Netherlands United Phosphorus Limited Report No.: DL 10-013 GLP, Unpublished	N	Y	UPL	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	