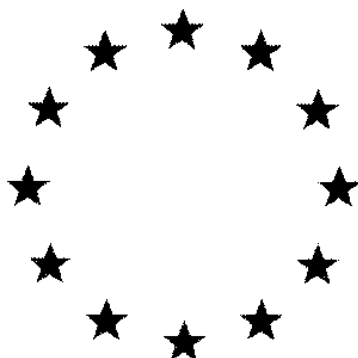


European Commission



**Draft (Renewal) Assessment Report prepared
according to the Commission Regulation (EC) No
1107/2009**

**Daminozide (ISO); 4-(2,2-
dimethylhydrazino)-4-oxobutanoic
acid; *N*-dimethylaminosuccinamic
acid**

Volume 3 - B.5 (AS)

Rapporteur Member State: Czech Republic
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April, 2018	Version 1	First draft
October, 2018	Version 2	Co-RMS, notifier comments
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TABLE OF CONTENTS

B.5. METHODS OF ANALYSIS	4
B.5.1. Methods Used for the Generation of Pre-approval Data	4
B.5.1.1. Methods for the analysis of the active substance as manufactured.....	4
B.5.1.2. Methods for risk assessment.....	15
B.5.2. Methods for Post-approval Control and Monitoring Purposes	31
B.5.3. References Relied On	47

B.5. METHODS OF ANALYSIS

Details of the literature search undertaken are available. No relevant scientifically peer-reviewed open literature reference has been identified for active substance Daminozide (ISO); 4-(2,2-dimethylhydrazino)-4-oxobutanoic acid; N-dimethylaminosuccinamic acid ('hereafter referred to as 'daminozide').

B.5.1. Methods Used for the Generation of Pre-approval Data**B.5.1.1. Methods for the analysis of the active substance as manufactured****a) Determination of pure active substance in the active substance as manufactured**

Old following studies were evaluated in original DAR (1999) and in Addendum (2002):

Author(s)	Year	Title	Owner	Original conclusion or comments
Riggs, A.S.	1994	Preliminary analysis of technical B-nine Generated by: Uniroyal research laboratory Submitted by: Uniroyal Chemical Ltd Date 05-06-1994 In accordance with GLP, excluding the analysis for 1.1-Dimethylhydrazide which were carried out under ISO 9002 Unpublished	Uniroyal DAR (1999)	A more complete description for the analytical method for daminozide in technical daminozide is required. Additional validation parameters according to modern analytical standards are required.
Wells, D.F.	1995	Daminozide - characterisation of a Technical grade Active Ingredient Generated by: Springborn laboratory Submitted by: Fine Agrochemicals Ltd Date: April 1995 GLP, Unpublished	Fine DAR (1999)	The submitted method is acceptable.
White, G.A.	1999a	Analytical Method M485: High Performance Liquid Chromatographic Determination of Daminozide in technical Material and formulations, date: 04-10-1999 GLP, Unpublished	Fine Addendum (2002)	None.
White, G.A.	1999b	Validation of Analytical Method M485, Date: 22-10-1999 GLP, Unpublished	Fine Addendum (2002)	None.

New following studies were submitted by the notifiers to cover the analysis of the active substance in the active substance as manufactured:

Reference:	Bates, G.J.D. (2012): Validation of Analytical Method M741 “High Performance Liquid Chromatographic Determination of Daminozide in Technical Material and Formulations” for the FAL 2400 Formulation
Report No.:	J19126
Guideline:	SANCO/3030/99 rev. 4 (11/07/00)
GLP:	yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Principle of the method M741 (Daminozide in Daminozide technical)

Samples of daminozide technical are weighed into vials (30 mL) and 20 mL of internal standard solution is added (diethyl phthalate at 0.6 g/L). The samples are sonicated for 10 minutes, filtered if necessary, and analysed by high performance liquid chromatography with UV detection at 220 nm, using an ACE C18 column and isocratic elution with a mobile phase water/acetonitrile/methanol. Quantification is performed using daminozide reference standard solutions containing diethyl phthalate as an internal standard.

Column	Ace 5µm C18 (250 mm x 4.6 mm)
Column temperature	30°C
Detection	UV 220 nm
Injection volume	2 µL
Flow rate	1 mL/min
Mobile phase	40/40/20 water/acetonitrile/methanol

Specificity

No interferences were observed at the retention time of interest in any control samples, demonstrating specificity of the method.

Linearity

The linearity of detector response was demonstrated using six standard solutions across the nominal concentration range of 0 to 3.5 mg/ml, with a coefficient of determination (R^2) of 0.9994 (regression line equation $y = 9.508x + 0.008$).

Precision (Repeatability)

Not determined for the technical material. For further information see partial validation of analytical method M741 below in study “Kelly, K. (2015, J20158)”.

Accuracy (Recovery)

Not a requirement. No interferences were observed.

RMS comments and conclusion

The analytical procedure has been successfully validated in terms of specificity and linearity, in accordance with all the requirements of SANCO/3030/99 rev. 4. For further information see partial validation of analytical method M741 below.

Reference:	Kelly, K. (2015c): Partial Validation of Analytical Method M741 “High Performance Liquid Chromatographic Determination of Daminozide in Technical Material and Formulations” for Technical Material
Report No.:	J20158
Guideline:	SANCO/3030/99 rev. 4 (11/07/00)
GLP:	yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Principle of the method M741 (Daminozide in Daminozide technical)

For the principle of method see study “Bates, G.J.D. (2012, J19126)” above.

Specificity

For the specificity see study “Bates, G.J.D. (2012, J19126)” above.

Linearity

For the linearity see study “Bates, G.J.D. (2012, J19126)” above.

Precision (Repeatability)

Repeatability data was generated from six determinations of technical material. The relative standard deviation (RSD) obtained was within the guideline requirement and is presented in table below.

Analyte	Number of Samples (n)	Mean Content (%)	RSD (%)	Acceptable RSD (%)
Daminozide	6	99.6	0.484	1.34

Accuracy (Recovery)

Not a requirement. The analytical procedure has been successfully validated in terms of precision.

RMS comments and overall conclusion

The analytical procedure has been successfully validated in terms of precision, in accordance with all the requirements of SANCO/3030/99 rev. 4. Thus, the method M741 is valid and suitable for determination of Daminozide in Daminozide technical. Representative chromatograms attached.

Reference:	Barker, C.H. (2015): Preliminary analysis of technical daminozide <u>Appendix II: Method GRL-GM-1280 version 1.0</u> Determination of daminozide in technical material by HPLC.
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Report No.:	GRL-13301
Guideline:	OPPTS 830.1700; Commission Regulation (EU) 283/2013
GLP:	Yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Reference:	Riggs, A.S. (2011): Validation of an Analytical Method for the Determination of Daminozide in Daminozide Technical
Report No.:	GRL-12970
Guideline:	SANCO/3030/99 rev. 4 (11/07/00)
GLP:	Yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Principle of the method GRL-GM-1280 version 1.0 (Daminozide in Daminozide technical)

Samples of the daminozide technical (100 mg) are transferred to 100 mL volumetric flasks and pyridoxine hydrochloride internal standard (20 mL) is added. The samples are made to volume with water, mixed well and analysed by HPLC-UV at 220 nm, using a Phenomenex Synergy Polar RP column and isocratic elution with a mobile phase of 0.15% phosphoric acid in water. Quantification is performed using daminozide reference standard solutions containing pyridoxine hydrochloride as an internal standard.

Column	Phenomenex 'Synergy' Polar-RP (250 mm x 4.6 mm, 4 µm particle size)
Column temperature	40°C
Detection	UV 220 nm
Injection volume	10 µL
Flow rate	1 ml/min
Mobile phase	0.1 % TFA in water, isocratic

Specificity

Analyte identity was confirmed by retention time match with an analytical standard and by comparison of UV spectra. No interferences were observed at the retention time of interest in any control samples, demonstrating specificity of the method.

Linearity

The linearity of detector response was demonstrated using five standard solutions across the concentration range of 39.6 to 160.8 mg/100 mL, with a coefficient of determination (R^2) of 0.999 (regression line equation $y = 0.01308x - 0.00624$).

Precision (Repeatability)

Repeatability data was generated from five determinations of technical material. The relative standard deviation (RSD) obtained was within the guideline requirement and is presented in table below.

Analyte	Number of Samples (n)	Mean Content (%)	RSD (%)	Acceptable RSD (%)
Daminozide	5	100	0.518	1.34

Accuracy (Recovery)

Recovery data was generated from five sample determinations. The mean percentage recovery obtained was within the guideline requirement and is presented in table below.

Analyte	Number of Samples (n)	Mean Recovery (%)	RSD (%)	Acceptable Recovery (%)
Daminozide	5	100.1	0.309	98 - 102

Limit of Quantification (LOQ)

Not a requirement. Nevertheless the LOQ for Daminozide was determined to be 0.647 mg / 100 ml.

RMS comments and conclusion

The analytical procedure has been successfully validated in accordance with all the requirements of SANCO/3030/99 rev. 4. Thus, the method GRL-GM-1280 version 1.0 is valid and suitable for determination of Daminozide in Daminozide technical. Representative chromatograms attached.

Applicability of existing CIPAC methods

Up to now there is no CIPAC method available for the determination of Daminozide in the active substance as manufactured.

b) Determination of significant and relevant impurities and additives (such as stabilisers) in the active substance as manufactured

There are no additives present in Daminozide technical.

The analytical methods regarding the significant impurities are considered confidential and are presented in the Volume 4 CA-CP.

Old following studies about relevant impurities were evaluated in original DAR (1999) and in Addendum (2002):

Author(s)	Year	Title	Owner	Original conclusion or comments
Riggs, A.S.	1994	Preliminary analysis of technical B-nine Generated by: Uniroyal research laboratory Submitted by: Uniroyal Chemical Ltd Date 05-06-1994 In accordance with GLP, excluding the analysis for 1,1-Dimethylhydrazide which were carried out under ISO 9002 Unpublished	Uniroyal DAR (1999)	This method for NDMA is validated to an acceptable level. The analytical method for 1,1-Dimethylhydrazide in technical daminozide is required including validation parameters according to modern analytical standards.

Smith, J.S.C.	1999a	Analytical Method M487, Determination of unsymmetrical Dimethylhydrazine and N-nitroso dimethylamine by Gas Chromatography, Submitted by: Fine Agrochemical Ltd, date 14-10-1999. GLP, Unpublished	Fine Addendum (2002)	None.
Smith, J.S.C.	1999b	Validation of Analytical method M487 Submitted by: Fine Agrochemical Ltd, date 27-10-1999 GLP, Unpublished	Fine Addendum (2002)	None.

New following studies were submitted by the notifiers to cover the analysis of the relevant impurities 1,1-dimethylhydrazide (UDMH) and N-nitrosodimethylamine (NDMA) in Daminozide technical:

Reference:	Kelly, K. (2015g): Validation of Analytical Method M851 “Determination of UDMH and NDMA in Daminozide by Gas Chromatography”
Report No.:	J20157
Guideline:	SANCO/3030/99 rev. 4 (11/07/00)
GLP:	Yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Principle of the method M851 (UDMH and NDMA in Daminozide technical)

Unsymmetrical Dimethylhydrazine (UDMH) and N-Nitrosodimethylamine (NDMA) are extracted from Daminozide with p-Xylene and determined in the filtrate by Temperature Controlled Gas Chromatography with Nitrogen/Phosphorus detection (GC-NPD).

Separate stock solutions of UDMH or NDMA in p-Xylene are prepared. These stocks are then used to prepare mixed Standard solutions of UDMH and NDMA in p-Xylene at concentrations based on levels expected in the technical material:

Concentration working solution (µg/mL)	
UDMH	NDMA
0.02	0.02
0.1	0.05
0.2	0.08
1.0	0.12
3.0	0.16
5.0	0.20

Aliquots of the two Standards are injected onto the GC in order to calculate the Relative Response Factors.

The Sample is prepared by mixing an accurately weighed amount with p-Xylene. A portion of the supernatant is then passed through a 45µm filter. Aliquots of the Standard and Sample solutions are then injected onto the GC and the amount of UDMH and NDMA in the Samples is calculated.

Column	Supelco Carbowax Amine (15 m x 0.53 mm, 1 µm film thickness)
Injection temperature	180°C
Detector temperature	180°C
Injection volume	5 µL
Flow rate	6 ml/min
Carrier Gas:	Nitrogen

Specificity

A solution of the Daminozide technical and a standard solution were prepared according to the method. At the same time solutions of all known impurities were prepared, at approximately the same concentration that they might be expected to be in the technical material. All the solutions were then injected onto the GC using the conditions detailed in the method. Each resulting chromatogram was then inspected for the presence of any peaks eluting at the same retention time as either the UDMH or the NDMA.

The Standard solution produced retention times as follows:

UDMH Peak 1.3 minutes

NDMA Peak 8.9 minutes

None of the impurities showed any degree of interference (if so, less than 3%) to the retention time attributable to UDMH. All of the impurities displayed a peak eluting at 9.0 minutes, close to the retention time of NDMA but it was judged to be not interfering to the method. This collectively demonstrates that the specificity of method M851 is acceptable.

Linearity

The linearity of detector response was demonstrated using six standard solutions across the concentration range for UDMH of 0.02 to 5.00 mg/kg with a coefficient of determination (R^2) of 0.9982 (regression line equation $y = 11.0164x - 0.7829$). Concentration range (five standard solutions) for NDMA is 0.02 to 0.20 mg/kg with a coefficient of determination (R^2) of 0.9947 (regression line equation $y = 21.1433x - 0.1729$).

Precision (Repeatability)

Preliminary trials during method development had shown that the technical material did not contain a measurable amount of UDMH or NDMA. A bulk sample was therefore spiked with approximately 75 µg/kg of each compound. Six replicate analyses of the spiked Daminozide technical were then prepared and analysed according to the method. The relative standard deviation (RSD) obtained was within the guideline requirement and is presented in table below.

Analyte	Number of Samples (n)	Mean Content (% w/w)	RSD (%)	Acceptable RSD (%)
UDMH	6	8.74×10^{-6}	6.88	15.5
NDMA	6	7.56×10^{-6}	4.69	15.8

Accuracy (Recovery)

This was assessed by preparing and analysing three recovery experiments at the limit of quantification (LOQ) and three recoveries at 50% higher than the LOQ. Accurately weighed amounts of the supplied technical

material were taken and transferred to six separate containers at the approximate nominal levels normally taken for the analysis.

A fortification solution of UDMH and NDMA, of suitable concentration was prepared. Aliquots of this solution were added to each container. The whole of each spiked sample weighed out was taken and analysed according to the method. The mean percentage recovery obtained was within the guideline requirement and is presented in table below.

Analyte	Number of Samples (n)	Mean Recovery (%)	SD (%)	Acceptable Recovery (%)
UDMH	2x 3	112.4	8.058	75 - 125
NDMA	2x 3	94.5	5.933	75 - 125

Limit of Quantification (LOQ)

LOQ for UDMH and NDMA was determined to be 0.05 mg/kg.

Confirmation

Confirmation was not provided. Confirmation can only be carried out by comparison retention times from recovery samples and standards. In this case the retention time was essentially the same.

RMS comments and conclusion

The analytical procedure has been successfully validated in accordance with all the requirements of SANCO/3030/99 rev. 4. Confirmation was not provided. The method M851 is valid and suitable for determination of UDMH and NDMA in Daminozide technical. Representative chromatograms attached.

Reference:	Barker, C.H. (2015): Preliminary analysis of technical daminozide <u>Appendix V: Method GRL-GM-1062 version 3.2</u> Determination of unsymmetrical dimethyl hydrazine (UDMH) in daminozide technical, B-Nine SP, Alar 85 SG and Alar 64 SP using gas chromatography.
Report No.:	GRL-13301
Guideline:	OPPTS 830.1700; Commission Regulation (EU) 283/2013
GLP:	Yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Reference:	Yu, W.S. (2000a): Validation of an analytical method for the determination of unsymmetrical dimethyl hydrazine (UDMH) in B-Nine technical and B-Nine SP formulation.
Report No.:	GRL-11423
Guideline:	-
GLP:	Yes

Published:	No
Previous evaluation:	Submitted for the purpose of renewal
Reference:	Riggs, A.S. (2009a): Validation of an Analytical Method for the Determination of UDMH in Alar 85 WG
Report No.:	GRL-12643 (GRL-12870, GRL-13222)
Guideline:	SANCO/3030/99 rev. 4 (11/07/00)
GLP:	Yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Principle of the method GRL-GM-1062 version 3.2 (UDMH in Daminozide technical)

Samples of the Daminozide (1 g) are weighed into 250 mL bottles and dissolved in 10/90, v/v, methyl ethyl ketone/ethyl alcohol (100 mL) with stirring. An aliquot (10 mL) of the samples is removed to a round bottomed flask and distilled under vacuum at 30°C. UDMH is reacted with methyl ethyl ketone (MEK) to form a volatile hydrazine. The hydrazine is separated from the Daminozide by vacuum distillation. UDMH concentration is determined by GC using external standards and a Nitrogen/Phosphorus Detector (NPD).

Column	Supelco, SPB-1 (15 m x 0.53 mm, 1.50 µm film thickness)
Detector temperature	300°C
Detection	NPD
Injection volume	1.0 µL
Carrier gas (He) flow-rate	Approx. 2.5 ml/min (constant flow mode)

Specificity

Analyte identity was confirmed by retention time match with analytical standards. No interferences were observed at the retention times of interest in any control samples, demonstrating specificity of the method.

Linearity

The linearity of detector response was demonstrated using seven standard solutions across the nominal concentration range of 44.96 - 314.72 µg/L, with a coefficient of determination (R^2) of 0.999 (regression line equation $y = 733.45x - 6114.17$).

Precision (Repeatability)

Repeatability data was generated from six sample determinations of technical material. The relative standard deviation (RSD) obtained was within the guideline requirements and is presented in table below.

Analyte	Number of Samples (n)	Mean Content (mg/kg)	RSD (%)	Acceptable RSD (%)
UDMH	6	16.94	2.60	7.00

Accuracy (Recovery)

Recovery data was generated using the standard addition technique. Samples of technical material were fortified with known amounts of UDMH. The mean recovery obtained was within the guideline requirement and is presented in table below.

Analyte	Number of Samples (n)	Mean Recovery (%)	RSD (%)	Acceptable Recovery (%)
UDMH	5	119.58	4.89	75 - 125

Limit of quantification (LOQ)

The limit of quantification (LOQ) was determined to be 5.2 mg/kg.

Confirmation

Confirmation was not provided. Confirmation can only be carried out by comparison retention times from recovery samples and standards. In this case the retention time was essentially the same.

RMS comments and conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANCO/3030/99 rev. 4. Confirmatory analysis was not provided. Nevertheless the method GRL-GM-1062 version 3.2 is suitable for determination of UDMH in Daminozide technical. Representative chromatograms attached.

Reference:	Barker, C.H. (2015): Preliminary analysis of technical daminozide <u>Appendix VI: Method GRL-GM-1144 version 3.2</u> Determination of N-nitrosodimethylamine (NDMA) in daminozide technical, Alar 85 SG (UBI 6899.00) and Alar 64 SP (UBI 6916.00) by GC/MS.
Report No.:	GRL-13301
Guideline:	OPPTS 830.1700; Commission Regulation (EU) 283/2013
GLP:	Yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Reference:	Yu, W.S. (2000b): Validation of an analytical method for the determination of N-nitrosodimethylamine (NDMA) in B-Nine technical
Report No.:	GRL-11450
Guideline:	-
GLP:	Yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Reference:	Riggs, A.S. (2009b): Validation of an Analytical Method for the Determination of NDMA in Alar 85 WG
Report No.:	GRL-12644 (GRL-12844, GRL-13223)
Guideline:	SANCO/3030/99 rev. 4 (11/07/00)
GLP:	Yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Principle of the method GRL-GM-1144 version 3.2 (NDMA in Daminozide technical)

Daminozide technical is dissolved in HPLC grade water and d₆-NDMA is added as an internal standard. The solution is adjusted to pH \geq 10 with 50% sodium hydroxide to promote NDMA extraction. The solution is then extracted with dichloromethane.

The extract is dried by filtration through a bed of anhydrous sodium sulphate and concentrated for analysis using gas chromatography and a mass spectrometer for detection.

Column	HP-5MS (30 m x 0.25 mm, 0.25 μ m film thickness)
Detector temperature	230°C
Injection temperature	175°C
Detection	MSD
Injection volume	1.0 μ L
Carrier gas (He) flow-rate	Approx. 1.0 ml/min (constant flow mode)

Specificity

Analyte identity was confirmed by retention time match with analytical standards and by comparison of mass spectra. No interferences were observed at the retention times of interest in any control samples, demonstrating specificity of the method.

Linearity

The linearity of detector response was demonstrated using seven standard solutions across the nominal concentration range of 0.051 - 1.275 μ g/vial, with a coefficient of determination (R^2) of 0.9997 (regression line equation $y = 2.050x - 0.0236$).

Precision (Repeatability)

Repeatability data was generated from six sample determinations of technical material. The relative standard deviation (RSD) obtained was within the guideline requirements and is presented in table below.

Analyte	Number of Samples (n)	Mean Content (mg/kg)	RSD (%)	Acceptable RSD (%)
NDMA	6	0.1219	9.24	14.71

Accuracy (Recovery)

Recovery data was generated using the standard addition technique. Samples of formulation were fortified with known amounts of NDMA. The mean recoveries obtained were within the guideline requirement and are presented in table below.

Analyte	Number of Samples (n)	Mean Recovery (%)	RSD (%)	Acceptable Recovery (%)
NDMA	4	75.9	13.71	75 - 125

Limit of quantification (LOQ)

The limit of quantification (LOQ) was determined to be 0.049 mg/kg.

Confirmation

Confirmation is not required because of using highly specific method GC-MSD as a primary method.

RMS comments and conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANCO/3030/99 rev. 4. For internal standard d₆-NDMA ion m/z 80.2 is used for quantification and the ion m/z 46.2 is used for confirmation and for NDMA ion m/z 74.2 is used for quantification and the ion m/z 42.2 is used for confirmation. Confirmatory analysis is not required. The method GRL-GM-1144 version 3.2 is suitable for determination of NDMA in Daminozide technical.

Representative chromatograms attached.

B.5.1.2. Methods for risk assessment

(a) Methods in soil, water, sediment, air and any additional matrices used in support of environmental fate studies

Old following environmental studies were evaluated in original DAR (1999) and in Addendum (2002):

Author	Year	Title	Owner	Original conclusion or comments
Abdel-Kader, M.H. Dzialo, D.G.	1985	Analytical method for determining daminozide residues in soils and groundwaters by a non-destructive GLC procedure. Tentative. Submitted by: Uniroyal Chemical Uniroyal file No. 85124. Date: December, 1985 Not GLP, Unpublished	Uniroyal DAR (1999)	None.
Vithala, R.V.	1999	Analytical Method for Daminozide and its Metabolites as UDMH in Soil Chyba! Záložka není definována. Uniroyal file No.: project no.: 85124 and 8647 Date: 4 February, 1999 GLP, Unpublished	Uniroyal DAR (1999)	The recovery of UDMH, the major degradate of daminozide from field dissipation studies was slightly higher than the EU requirements. The RSD satisfied the EU requirements. The number of samples for the UDMH method is very limited. Additional data for the determination of the recovery and repeatability are required.

Xu, A.X.	2001	Analytical method for determining UDMH in soil. Report Uniroyal Chemical CO. Incl., USA, no. 2000-087 Date: 21 November 2001 GLP, Unpublished	Uniroyal Addendum (2002)	The analytical method is valid for the determination residues of 1,1-dimethylhydrazine (UDMH) down to a concentration level of 0.005 mg/kg. Mean recoveries (70-110 %) and LOD values (< 0.05 mg/kg) fulfilled to the required criteria. Detection based on monitoring of ions does not satisfy the requirements of the EU directive. A justification for not applying a confirmatory method of analysis is required.
Hull, L.B.	2001	Analytical method for the determination of daminozide in water by stable isotope dilution, no.2000-137 Date: 1 September 2001 GLP, unpublished	Uniroyal Addendum (2002)	The analytical method is valid for the determination residues of daminozide in surface water at levels down to 0.5 µg/l. Mean recoveries were in the range of 70-110 % according to the criteria. Detection based on monitoring of ions does not satisfy the requirements of the EU directive. A justification for not applying a confirmatory method of analysis is required.
Meeuwssen, M.C.T.J	2001	Validation of the determination of daminozide in drinking water, no.V3455 Date: 9 March 2001 GLP, Unpublished	Uniroyal Addendum (2002)	The analytical method is valid for the determination residues of daminozide in drinking water at levels down to 0.1 µg/l. Mean recoveries were in the range of 70-110 % according to the criteria.
Bacher, R.	2000	Development and Validation of an Analytical method for the Determination of Daminozide in Air, UCC study no. 99159 GLP, Unpublished	Uniroyal Addendum (2002)	The analytical method described is valid for the determination residues of Daminozide down to a concentration level of 15µg/m ³ .

New proposed environmental monitoring methods are summarised in part B.5.2 of this document.

(b) Methods in soil, water and any additional matrices used in support of efficacy studies

No specific residues studies for which analytical methods are required are being submitted in support of the renewal of approval of daminozide.

(c) Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies

Reference:	Hart, C. (2012b): Determination of the concentration of Unsymmetrical Dimethyl Hydrazine (UDMH) in a mixture of daminozide and water over a storage period of 24 hours
Report No.:	GRL-13025
Guideline:	SANCO/3030/99 rev. 4 (11/07/00)
GLP:	Yes
Published:	No

Previous evaluation:

Submitted for the purpose of renewal

Principle of the method (UDMH in mixture of daminozide and water)

Water samples (9.0 mL) are pipetted into volumetric flasks (10 mL), formaldehyde (100 µL) is added and the samples diluted to volume with water. The samples are syringe filtered (0.45 µm) and analysed by HPLC-UV at 238 nm, using a Phenomenex Luna C18(2) column and isocratic elution with a mobile phase of methanol/pH7 sodium dihydrogen phosphate buffer solution, 20/80. Quantification is performed using external standards.

Column	Phenomenex Luna C18(2) (150 mm x 4.6 mm, 3 µm)
Column temperature	40°C
Detection	UV 238 nm
Injection volume	15 µL
Flow rate	1 ml/min
Mobile phase	20/80 methanol/pH7 sodium dihydrogen phosphate buffer solution

Specificity

Analyte identity was confirmed by retention time match with an analytical standard and by comparison of UV spectra. No interferences were observed at the retention time of interest in any control matrix samples, demonstrating specificity of the method.

Linearity

The linearity of detector response was demonstrated using seven standard solutions across the nominal concentration range of 0.028 - 4.73 mg/L, with a coefficient of determination (R^2) of 0.999 (regression line equation $y = 31052x - 153.78$).

Precision (Repeatability)

Recovery data was generated from six samples of a solution of UDMH. The mean percentage recovery obtained was within the guideline requirements and is presented in table below.

Analyte	Number of Samples (n)	Mean Content (mg/L)	RSD (%)	Acceptable RSD (%)
UDMH	6	0.060	2.73	16.4

Accuracy (Recovery)

Recovery data was generated from six samples of a solution of UDMH. The mean percentage recovery obtained was within the guideline requirements and is presented in table below.

Analyte	Number of Samples (n)	Mean Recovery (%)	RSD (%)	Acceptable Recovery (%)
UDMH	6	106	2.73	75 - 125

Limit of quantification (LOQ)

The limit of quantification (LOQ), defined as the lowest fortification where acceptable precision and accuracy data were obtained, has been demonstrated to be 0.06 mg/L.

Confirmation

Confirmation was not provided. Confirmation can only be carried out by comparison retention times from recovery samples and standards. In this case the retention time was essentially the same.

RMS comments and conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANCO/3030/99 rev. 4. Confirmatory analysis was not provided. Representative chromatograms attached.

(d) Methods in body fluids, air and any additional matrices used in support of operator, worker, resident and bystander exposure studies

New proposed monitoring methods are summarised in part B.5.2 of this document.

(e) Methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies

Considering that the use of daminozide is restricted to non-consumable crops and that residues are not defined in commodities of plant and animal origin, methods for the determination of daminozide residues in or on food and feed of plant and animal origin are not required.

(f) Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies

Reference:	(2012): Daminozide: A Reproduction Study With The Northern Bobwhite
Report No.:	616-104
Guideline:	OPPTS 850.2300, FIFRA Subdivision E Section 71-4 and OECD 206
GLP:	Yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Principle of the method (Daminozide in Avian diet)

Avian diet samples are extracted with methanol, diluted with methanol/water, 50:50, v/v and analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS), using a Thermo Aquasil C18 column (100 mm x 3 mm, 5 µm particle size) and gradient elution with mobile phases of 0.1% formic acid in water and acetonitrile. Quantification is performed using external standards. The ion transition m/z 161 > 143 is used for quantification and the ion transition m/z 161 > 101 is used for confirmation.

Column	Thermo Aquasil C18 (100 mm x 3 mm, 5 µm)
Oven temperature	40°C
Injection volume	5 µL
Flow rate	0.3 ml/min

Mobile phase

Channel A: 0.1% Formic Acid

Channel B: Acetonitrile

Specificity

No interferences were observed at the retention time of interest in control matrix samples, demonstrating the specificity of the method. Analyte identity was confirmed by retention time match with an analytical standard.

Linearity

The linearity of detector response was demonstrated using five external standard solutions across the concentration range from 0.05 to 2.00 µg/mL. The correlation coefficient (r) was determined to be 0.9994 (regression line equation $y = 2.77 \times 10^6 x + 2.49 \times 10^4$).

Precision (Repeatability)

Repeatability data was generated from five samples fortified with at the LOQ and from five samples fortified at 12x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and are presented in table below.

Matrix	Fortification Level (mg/kg)	Number of Samples (n)	Mean Recovery (%)	RSD (%)
Avian diet	100	5	83.4	4.0
	1200	5	87.4	5.0

Accuracy (Recovery)

Recovery data was generated from five samples fortified with at the LOQ and from five samples fortified at 12x LOQ. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 - 110% and are presented in table below.

Matrix	Fortification Level (mg/kg)	Number of Samples (n)	Mean Recovery (%)	RSD (%)
Avian diet	100	5	83.4	4.0
	1200	5	87.4	5.0

Limit of quantification (LOQ)

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained has been demonstrated to be 100 mg/kg.

Confirmation

Confirmation is not required because of using highly specific method HPLC-MS/MS as a primary method.

RMS comments and conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANCO/3029/99 rev. 4.

It was used 2 ion transition m/z 161 > 143 for quantification and m/z 161 > 101 for confirmation. Confirmatory analysis is not required. Representative chromatograms attached.

Reference:	(2015): Daminozide: An Early Life-Stage Toxicity Test With The Fathead Minnow (<i>Pimephales promelas</i>)
Report No.:	616A-123
Guideline:	OECD 210, OPPTS 850.1400
GLP:	Yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Principle of the method (Daminozide in Fresh water)

Fresh water samples (50 mL) are diluted with an equal volume of methanol and further diluted 2-fold with 50:50 (v/v) methanol/water. The samples are analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive ion spray mode, using an YMC-PACK ODS-AM column (150 mm x 4.6 mm, 3 μ m particle size) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Quantification is performed using external standards. The ion transition m/z 161 > 143 is used for quantification.

Column	YMC-PACK ODS-AM (150 mm x 4.6 mm, 3 μ m)
Oven temperature	40°C
Injection volume	5 μ L
Flow rate	0.4 ml/min
Mobile phase	Channel A: 0.1% Formic acid in HPLC-grade water Channel B: 0.1% Formic acid in acetonitrile

Specificity

No interferences were observed at the retention time of interest in control matrix samples, demonstrating the specificity of the method. Analyte identity was confirmed by retention time match with an analytical standard.

Linearity

The linearity of detector response was demonstrated using five external standard solutions across the concentration range from 0.025 to 0.25 mg/L. The correlation coefficient (r) was determined to be 0.9984 (regression line equation $y = 35700000x + 40700$).

Precision (Repeatability)

Repeatability data was generated from six samples fortified at the LOQ, 16x LOQ and 44x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and are presented in table below.

Matrix	Fortification Level (mg/L)	Number of Samples (n)	Mean Recovery (%)	RSD (%)
Fresh water	0.25	6	98.9	4.3
	4.00	6	98.3	6.3
	11.0	6	100.2	6.2
	Overall	18	99.1	5.4

Accuracy (Recovery)

Recovery data was generated from six samples fortified at the LOQ, 16x LOQ and 44x LOQ. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 - 110% and are presented in table below.

Matrix	Fortification Level (mg/L)	Number of Samples (n)	Mean Recovery (%)	RSD (%)
Fresh water	0.25	6	98.9	4.3
	4.00	6	98.3	6.3
	11.0	6	100.2	6.2
	Overall	18	99.1	5.4

Limit of quantification (LOQ)

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained has been demonstrated to be 0.25 mg/L.

Confirmation

Confirmation is not required because of using highly specific method HPLC-MS/MS as a primary method.

RMS comments and conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANCO/3029/99 rev. 4.

It was used only 1 ion transition m/z 161 > 143 for quantification. Confirmatory analysis is not required.

Representative chromatograms attached.

Reference:	Last, G. (2012): Chronic effects to <i>Daphnia magna</i> from exposure to daminozide and formaldehyde
Report No.:	8252736
Guideline:	OECD Chemicals Testing Guideline No. 211 <i>Daphnia magna</i> Reproduction Test (adopted 03/10/2008)
GLP:	Yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Principle of the method (Daminozide in ASTM water)

ASTM water samples are diluted with acetonitrile/water, 1/1, v/v and analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive ionisation mode, using a Waters Atlantis HILIC Silica column (100 mm x 3 mm, 3 µm particle size) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in methanol. Quantification is performed using external standards. The ion transition m/z 161.1 > 115.2 is used for quantification and the ion transition m/z 161.0 > 143.1 is used for confirmation.

Column	Waters Atlantis HILIC Silica (100 mm x 3 mm, 3 µm)
Oven temperature	30°C
Injection volume	30 µL
Flow rate	0.3 ml/min
Mobile phase	Channel A: 0.1% Formic acid in water Channel B: 0.1% Formic acid in methanol

Specificity

HPLC-MS/MS is considered to be a highly specific technique. No interferences were observed at the retention time of interest in control matrix samples.

Linearity

The linearity of detector response was demonstrated using five external standard solutions across the concentration range from 0.0025 to 0.25 µg/mL, with a coefficient of determination (R^2) of 0.9993 (regression line equation $y = 8.12317 \times 10^7 x - 28776$).

Precision (Repeatability)

Repeatability data was generated from samples fortified with daminozide at concentrations of 0.01, 1.0, 15 and 150 µg/mL. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and are presented in table below.

Matrix	Fortification Level (µg/mL)	Number of Samples (n)	Mean Recovery (%)	RSD (%)
ASTM water	0.01	5	107.0	2.11
	1.0	5	104.0	0.59
	15.0	5	99.8	2.97
	150	5	110.0	5.37

Accuracy (Recovery)

Recovery data was generated from samples fortified with daminozide at concentrations of 0.01, 1.0, 15 and 150 µg/mL. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 - 110% and are presented in table below.

Matrix	Fortification Level (µg/mL)	Number of Samples (n)	Mean Recovery (%)	RSD (%)
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ASTM water	0.01	5	107.0	2.11
	1.0	5	104.0	0.59
	15.0	5	99.8	2.97
	150	5	110.0	5.37

Limit of quantification (LOQ)

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained has been demonstrated to be 0.01 µg/mL.

Confirmation

Confirmation is not required because of using highly specific method HPLC-MS/MS as a primary method.

RMS comments and conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANCO/3029/99 rev. 4.

It was used 2 ion transition m/z 161.1 > 115.2 for quantification and m/z 161.0 > 143.1 for confirmation.

Confirmatory analysis is not required. Representative chromatograms attached.

Principle of the method (Formaldehyde in ASTM water)

ASTM water samples are diluted with water and an aliquot (0.5 mL) is added to a HPLC vial along with 0.5 mL of the derivatising agent (15.416g of ammonium acetate, 0.886 g of acetoacetanilide dissolved in ethanol/water, 8/2, v/v, 100 mL) and allowed to react for 10 minutes. The samples are analysed by high performance liquid chromatography with fluorescence detection (HPLC-FD) with excitation at 370 nm and emission at 470 nm, using a Spherisorb 5ODS1 column (150 mm x 4.6 mm, 5 µm particle size) and isocratic elution with a mobile phase of water/acetonitrile, 60/40, v/v. Quantification is performed using derivatised external standards.

Column	Spherisorb 5ODS1 (150 mm x 4.6 mm, 5 µm)
Column temperature	25°C
Injection volume	10 µL
Excitation wavelength	370 nm
Emission wavelength	470 nm
Flow rate	1.0 ml/min
Mobile phase	(60:40 v/v) HPLC water/acetonitrile

Specificity

Analyte identity was confirmed by retention time match with a derivatised analytical standard. No interferences were observed at the retention time of interest in any control matrix samples, demonstrating specificity of the method.

Linearity

The linearity of detector response was demonstrated using five external standard solutions across the concentration range from 0.005 to 0.5 µg/mL, with a coefficient of determination (R^2) of 0.9992 (regression line equation $y = 3 \times 10^6 x + 3486$).

Precision (Repeatability)

Repeatability data was generated from samples fortified with formaldehyde at concentrations of 0.005, 0.08 and 0.5 µg/mL. The relative standard deviations (RSD) obtained for each fortification level were not within the guideline requirements of less than 20% and are presented in table below.

Matrix	Fortification Level (µg/mL)	Number of Samples (n)	Mean Recovery (%)	RSD (%)
ASTM water	0.005	4	106.0	21.9
	0.08	5	118.0	6.10
	0.5	5	122.0	5.75

Accuracy (Recovery)

Recovery data was generated from samples fortified with formaldehyde at concentrations of 0.005, 0.08 and 0.5 µg/mL. The mean percentage recoveries obtained for each fortification level were not within the guideline requirements of 70 - 110% and are presented in table below.

Matrix	Fortification Level (µg/mL)	Number of Samples (n)	Mean Recovery (%)	RSD (%)
ASTM water	0.005	4	106.0	21.9
	0.08	5	118.0	6.10
	0.5	5	122.0	5.75

Limit of quantification (LOQ)

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained has been demonstrated to be 0.005 µg/mL.

Confirmation

Confirmation was not provided. Confirmation can only be carried out by comparison retention times from recovery samples and standards. In this case the retention time was essentially the same.

RMS comments and conclusion

This analytical method is not relevant, because formaldehyde was removed from definition of residues (by applicant). Formaldehyde was replaced by methanol. No methods for determination of methanol were submitted.

Reference:	Manson, P.S.; Scholey, A. (2006): Daminozide Technical: Inhibition of Growth To The Alga <i>Pseudokirchneriella subcapitata</i>
Report No.:	2242/049

Guideline:	OECD 201, Ministry of Agriculture, Forestry and Fisheries, Japan (MAFF), Notification No.12-Nousan-8147, Method No.2-7-7, Algae Growth Inhibition Studies (16 March 2005)
GLP:	Yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Principle of the method (Daminozide technical in EC Media)

EC media samples are diluted if necessary and analysed by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 215 nm, using a Luna C18(2) column (150 mm x 4.6 mm, 3 µm particle size) and isocratic elution with a mobile phase of 0.5% trifluoroacetic acid in water. Quantification is performed using external standards.

Column	Luna C18(2) (150 mm x 4.6 mm, 3 µm)
Column temperature	30°C
Injection volume	100 µL
Wavelength	215 nm
Flow rate	1.0 ml/min
Mobile phase	0.5% trifluoroacetic acid in water

Specificity

No interferences were observed at the retention time of interest in any control samples, demonstrating specificity of the method. Analyte identity was confirmed by retention time match with an analytical standard.

Linearity

The linearity of detector response was demonstrated using seven external standard solutions across the concentration range from 1.0 to 100 µg/mL. The correlation coefficient (r) was determined to be > 0.99 (regression line equation was not provided).

Precision (Repeatability)

Repeatability data was generated from five samples fortified with at the LOQ, 10x LOQ and 100x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and are presented in table below.

Matrix	Fortification Level (µg/mL)	Number of Samples (n)	Mean Recovery (%)	RSD (%)
EC Media	1.0	5	99.8	4.28
	10	5	98.1	2.25
	100	5	100	0.632

Accuracy (Recovery)

Recovery data was generated from five samples fortified with at the LOQ, 10x LOQ and 100x LOQ. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 - 110% and are presented in table below.

Matrix	Fortification Level (µg/mL)	Number of Samples (n)	Mean Recovery (%)	RSD (%)
EC Media	1.0	5	99.8	4.28
	10	5	98.1	2.25
	100	5	100	0.632

Limit of quantification (LOQ)

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained has been demonstrated to be 1.0 µg/mL.

Confirmation

Confirmation was not provided. Confirmation can only be carried out by comparison retention times from recovery samples and standards. In this case the retention time was essentially the same.

RMS comments and conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity (regression line equation was not provided), precision, accuracy and LOQ in accordance with the requirements of SANCO/3029/99 rev. 4. Confirmation was not provided. Representative chromatograms attached.

Reference:	Seeland-Fremer, A.; Mosch, W. (2014): Toxicity of Daminozide Technical To <i>Anabaena flos-aquae</i> in an Algal Growth Inhibition Test
Report No.:	87711210
Guideline:	OECD 201, EC Method C3 and SANCO 3029/99 rev 4
GLP:	Yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Principle of the method (Daminozide in Test Medium)

Test medium samples are diluted 2-fold with acetonitrile, centrifuged and analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive electrospray mode, using a Kinetex HILIC 100A column (50 mm x 2.1 mm, 2.6 µm particle size) and gradient elution with mobile phases of 0.1% formic acid in methanol and 0.1% formic acid in water. Quantification is performed using external standards. The ion transition m/z 161 > 143 is used for quantification and the ion transition m/z 161 > 115 is used for confirmation.

Column	Kinetex HILIC 100A (50 mm x 2.1 mm, 2.6 µm)
Oven temperature	30°C
Injection volume	2 µL

Flow rate	0.2 ml/min
Mobile phase	Channel A: methanol containing 0.1% formic acid Channel B: HPLC water containing 0.1% Formic acid

Specificity

No interferences were observed at the retention time of interest in control matrix samples, demonstrating the specificity of the method. Analyte identity was confirmed by retention time match with an analytical standard.

Linearity

The linearity of detector response was demonstrated using eight external standard solutions across the concentration range from 25 to 300 µg/L. The correlation coefficient (r) was determined to be 0.9982 (regression line equation $y = 8873x - 47973$).

Precision (Repeatability)

Repeatability data was generated from five samples fortified with at the LOQ, 3x LOQ and 25x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and are presented in table below.

Matrix	Fortification Level (mg/L)	Number of Samples (n)	Mean Recovery (%)	RSD (%)
Test Medium	5	5	91	2
	15	5	92	2
	125	5	97	4
	Overall	15	93	4

Accuracy (Recovery)

Recovery data was generated from five samples fortified with at the LOQ, 3x LOQ and 25x LOQ. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 - 110% and are presented in table below.

Matrix	Fortification Level (mg/L)	Number of Samples (n)	Mean Recovery (%)	RSD (%)
Test Medium	5	5	91	2
	15	5	92	2
	125	5	97	4
	Overall	15	93	4

Limit of quantification (LOQ)

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained has been demonstrated to be 5 mg/L.

Confirmation

Confirmation is not required because of using highly specific method HPLC-MS/MS as a primary method.

RMS comments and conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANCO/3029/99 rev. 4.

It was used 2 ion transition m/z 161 > 143 for quantification and m/z 161 > 115 for confirmation. Confirmatory analysis is not required. Representative chromatograms attached.

(g) Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests

Reference:	Friedlander, B. (2011): The Solubility of Technical Daminozide in Water
Report No.:	GRL-12954
Guideline:	OPPTS 830.7840
GLP:	Yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Principle of the method GRL-GM-1139 version 3.0 (Daminozide technical in water)

Aliquots of water solubility samples are transferred to 10 mL centrifuge tubes and centrifuged at 10000 rpm for 1 hour. Aliquots (2 mL) of the supernatant are transferred to 100 mL volumetric flask and made to volume with water. Aliquots (25 mL) of these samples are transferred to 100 mL volumetric flasks pyridoxine hydrochloride internal standard (20 mL) is added. The samples are made to volume with water, mixed well and analysed by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 220 nm, using a Phenomenex Prodigy ODS2 column (250 mm x 4.6 mm, 5 µm particle size) and isocratic elution with a mobile phase of 0.015% phosphoric acid in water. Quantification is performed using daminozide reference standard solutions containing pyridoxine hydrochloride as an internal standard.

Column	Phenomenex Prodigy (250 mm x 4.6 mm, 5 µm particle size)
Column temperature	40°C
Detection	UV 220 nm
Injection volume	10 µL
Flow rate	1.2 ml/min
Mobile phase	0.15 ml phosphoric acid in 1 L water

Specificity

Analyte identity was confirmed by retention time match with an analytical standard and by comparison of UV spectra. No interferences were observed at the retention time of interest in any control samples, demonstrating specificity of the method.

Linearity

The linearity of detector response was demonstrated using five standard solutions across the concentration range of 40.21 to 160.48 mg/100ml, with a coefficient of determination (R^2) of 0.999 (regression line equation $y = 0.012273x + 0.002674$).

Precision (Repeatability)

Repeatability data was generated from six water samples fortified at 1 mg/mL. The relative standard deviation (RSD) obtained was within the guideline requirements of less than 20% and is presented in table below.

Matrix	Fortification Level (mg/mL)	Number of Samples (n)	Mean Content (%)	RSD (%)
Daminozide	1.0	6	99.3	0.487

Accuracy (Recovery)

Recovery data was generated from five water samples fortified at 1 mg/mL. The mean percentage recovery obtained was within the guideline requirements of 70 - 110% and is presented in table below.

Matrix	Fortification Level (mg/mL)	Number of Samples (n)	Mean Recovery (%)	RSD (%)
Daminozide	1.0	5	99.96	0.241

Limit of quantification (LOQ)

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained has been demonstrated to be 1 mg/mL.

Confirmation

Confirmation was not provided. Confirmation can only be carried out by comparison retention times from recovery samples and standards. In this case the retention time was essentially the same.

RMS comments and conclusion

The analytical procedure (GRL-GM-1139 version 3.0) has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANCO/3029/99 rev. 4.

Confirmation was not provided. Representative chromatograms attached.

Reference:	Riggs, A.S. (2011): The Partition Coefficient (n-Octanol/Water) of Technical Daminozide
Report No.:	GRL-12953
Guideline:	OECD 107
GLP:	Yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Principle of the method GRL-GM-1275 version 1.0 (Daminozide technical in n-Octanol/Water)

Aliquots (5 mL) of the octanol and water phases from samples are transferred to 100 mL volumetric flasks and made to volume with methanol. The samples are analysed by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 200 nm, using a Waters μ Bondapak C18 column (250 mm x 4.6 mm) and isocratic elution with a mobile phase of 15/85, methanol/0.01M phosphate solution. Quantification is performed using external standards.

Column	Waters μ Bondapak C18 (250 mm x 4.6 mm)
Column temperature	40°C
Detection	UV 200 nm
Injection volume	10 μ L
Flow rate	1.5 ml/min
Mobile phase	15/85, methanol/0.01M pH 3.7 phosphate solution v/v, isocratic

Specificity

Analyte identity was confirmed by retention time match with an analytical standard and by comparison of UV spectra. No interferences were observed at the retention time of interest in any control samples, demonstrating specificity of the method.

Linearity

The linearity of detector response was demonstrated using six standard solutions across the concentration range of 1.006 to 100.6 mg/L, with a correlation coefficient of (r) of 0.99997 (regression line equation $y = 4878.8x - 5696.5$).

Precision (Repeatability)

Repeatability data was generated from six samples fortified at nominally 26 mg/L. The relative standard deviation (RSD) obtained was within the guideline requirements of less than 20% and is presented in table below.

Matrix	Fortification Level (mg/mL)	Number of Samples (n)	Mean Content (mg/L)	RSD (%)
Daminozide	26	6	25.9	0.901

Accuracy (Recovery)

Recovery data was generated from six samples fortified at nominally 26 mg/L. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 - 110% and is presented in table below.

Matrix	Fortification Level (mg/mL)	Number of Samples (n)	Mean Recovery (%)	RSD (%)
Daminozide	26	6	100.1	0.901

Limit of quantification (LOQ)

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained has been demonstrated to be 26 mg/mL.

Confirmation

Confirmation was not provided. Confirmation can only be carried out by comparison retention times from recovery samples and standards. In this case the retention time was essentially the same.

RMS comments and conclusion

The analytical procedure (GRL-GM-1275 version 1.0) has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANCO/3029/99 rev. 4.

Confirmation was not provided. Representative chromatograms attached.

B.5.2. Methods for Post-approval Control and Monitoring Purposes

(a) Methods for the determination of all components included in the monitoring residue definition as submitted in accordance with the provision of point 6.7.1 in order to enable Member States to determine compliance with established maximum residue levels (MRLs); they shall cover residues in or on food and feed of plant and animal origin

Considering that the use of daminozide is restricted to non-consumable crops and that residues are not defined in commodities of plant and animal origin, methods for the determination of daminozide residues in or on food and feed of plant and animal origin are not required.

(b) Methods for the determination of all components included for monitoring purposes in the residue definitions for soil and water as submitted in accordance with the provisions of point 7.4.2

Old following studies were evaluated in Addendum (2003) of original DAR (1999):

Author(s)	Year	Title	Owner	Original conclusion or comments
Parsons, A.H.	2002	Validation of Analytical Method M523 “Gas chromatographic determination of residues of daminozide in soil” Generated by: GC laboratories Ltd. Submitted by: Fine Agrochemicals Report No. J13923/C Date: 25 may 2002 GLP Unpublished	Fine Addendum (2003)	The LOQ of 1 mg/kg is much higher than the 0.05 mg/kg as recommended in SANCO/825/00. From the chromatograms is appears that the LOQ can be lowered to about 0.15 mg/kg. Use of a capillary column will lower the LOQ even further but that has to be demonstrated. The method is therefore not acceptable for monitoring.

Parsons, A.H.	2002	Validation of Analytical Method M524 “Gas chromatographic determination of residues of unsymmetrical dimethylhydrazine in soil” Generated by: GC laboratories Ltd. Submitted by: Fine Agrochemicals Report No. J14139 Date: 25 June 2002 GLP Unpublished	Fine Addendum (2003)	The method was validated with sufficient data points. The linearity, recovery and the repeatability are all within the limits from SANCO/825/00 rev 6. In the blank control samples no UDMH could be detected (estimated <0.005 mg/kg). The validation used a sandy loam soil. According to the report it is possible to quantify using either peak area or peak height. The method is acceptable for monitoring.
Parsons, A.H.	2002	Validation of Analytical Method M522 “Gas chromatographic determination of residues of daminozide in water” Generated by: GC laboratories Ltd. Submitted by: Fine Agrochemicals Report No. J13923/B Date: 7 October 2002 GLP Unpublished	Fine Addendum (2003)	The blank is somewhat higher than the 30% X LOQ recommended in SANCO/825/00. Because the blank is repeatable this is not considered a problem. The method is acceptable for monitoring daminozide in drinking water.

New following studies were submitted by the notifiers to cover the analysis of all components for monitoring purposes in the residue definition for soil and water:

Reference:	Munro, S. (2012): Validation of an Analytical Method for the Determination of Daminozide in Soil
Report No.:	FDD0080
Guideline:	SANCO/825/00 rev. 8.1 (16/11/2010)
GLP:	Yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Principle of the method (Daminozide in Soil)

Soil samples (10 g) are weighed into polyethylene bottles (250 mL) and pH 5 citrate buffer/ methanol, 90/10, v/v (100 mL) is added. The samples are shaken for 60 minutes and centrifuged at 3500 rpm for 5 minutes. The supernatant is filtered through a glass wool plug into a polyethylene bottle (250 mL). The residue is extracted as previously with pH 5 citrate buffer/ methanol, 90/10, v/v (100 mL) and the supernatants combined. The glass wool plug and funnel are rinsed with pH 5 citrate buffer/ methanol, 90/10, v/v (5 mL) and the total volume of the sample is adjusted to 200 mL with the same solvent mix. An aliquot (4 mL) is transferred to a graduated polypropylene tube (15 mL), diluted to 10 mL with 1% formic acid and filtered through a syringe filter (0.45 µm) into an auto sampler vial. The samples are analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive electrospray mode, using an Acquity UPLC BEH C₁₈ column (50 x 2.1 mm, 1.7 µm) and gradient elution with mobile phases of water/methanol/ formic acid, 90/10/01, v/v/v containing 0.01 M ammonium formate and methanol containing 0.1% formic acid.

Quantification is performed using external standards. The ion transition m/z 161 > 44 is used for quantitation and the ion transition m/z 161 > 61 is used for confirmation.

Column	Acquity UPLC BEH C ₁₈ (50 mm x 2.1 mm, 1.7 µm)
Injection volume	10 µL
Flow rate	0.4 ml/min
Mobile phase	Channel A: water/methanol/formic acid (90/10/0.1 v/v/v) containing 0.01M ammonium formate Channel B: methanol containing 0.1% formic acid

Specificity

HPLC-MS/MS monitoring two ion transitions is considered to be a highly specific technique. No interferences at > 30% of the LOQ were present at the retention time of interest in any of the control matrix samples.

Linearity

The linearity of detector response was demonstrated using eight external standard solutions across the concentration range of 0.25 to 20 ng/mL. The results are presented in table below.

Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Regression line equation
161 > 44	0.25 - 20	0.997	$y = 103.242x - 4.13866$
161 > 61		0.999	$y = 39.3533x - 4.36715$

Precision (Repeatability)

Repeatability data was generated from five samples fortified at the LOQ and 10x LOQ for each soil. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and are presented in table below.

Matrix	Ion transition (m/z)	Fortification Level (mg/kg)	Recovery (%)	Mean Recovery (%)	RSD (%)
Sandy Loam soil	161 > 44	0.05	75, 73, 87, 74, 74	77	7.6
		0.5	74, 75, 78, 72, 80	76	4.2
	161 > 61	0.05	76, 76, 81, 80, 73	77	4.2
		0.5	84, 80, 75, 71, 77	77	6.4
Clay/Clay Loam soil	161 > 44	0.05	85, 89, 81, 86, 81	84	4.1
		0.5	77, 73, 81, 86, 91	82	8.7
	161 > 61	0.05	88, 73, 65, 78, 81	77	11.2
		0.5	84, 71, 83, 89, 91	84	9.3

Accuracy (Recovery)

Recovery data was generated from five samples fortified at the LOQ and 10x LOQ for each soil. The mean percentage recoveries at each fortification level were within the guideline requirements of 70 - 120% and are presented in table below.

Matrix	Ion transition (m/z)	Fortification Level (mg/kg)	Recovery (%)	Mean Recovery (%)	RSD (%)
Sandy loam soil	161 > 44	0.05	75, 73, 87, 74, 74	77	7.6
		0.5	74, 75, 78, 72, 80	76	4.2
	161 > 61	0.05	76, 76, 81, 80, 73	77	4.2
		0.5	84, 80, 75, 71, 77	77	6.4
Clay/clay loam soil	161 > 44	0.05	85, 89, 81, 86, 81	84	4.1
		0.5	77, 73, 81, 86, 91	82	8.7
	161 > 61	0.05	88, 73, 65, 78, 81	77	11.2
		0.5	84, 71, 83, 89, 91	84	9.3

Limit of quantification (LOQ)

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained has been demonstrated to be 0.05 mg/kg in both soils.

Confirmation

Confirmation is not required because of using highly specific method HPLC-MS/MS with 2 ion transition as a primary method.

Extract stability

The stability of daminozide final extracts was demonstrated following frozen storage (-20°C) for 7 days, with recovery values within $\pm 10\%$ of the freshly prepared samples.

Soil characterisation

Sample ID	12/00/2501A	12/00/2503A
pH	5.1	7.7
Sand (% w/w)	73	22
Silt (% w/w)	14	43
Clay (% w/w)	13	35
pH CaCl ₂	4.5	7.3
Organic Carbon (% w/w)	1.2	4.4
Textural Class	Sandy Loam (low organic content)	Clay/Clay Loam (high organic content)
Source	Bromsgrove	Woodside Farm

RMS comments and conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANCO/825/00 rev. 8.1 (16/11/2010) - GLP study, the linearity data was acceptable (range was 0.25 to 20 ng/ml (n=8) and $r > 0.99$). A calibration curve was provided and was linear. The mean recovery (n=5) was between 70 % and 120 %, with the relative standard deviation (RSD) lower than 20 %. No interferences due to the reagent were detected. LOQ of the method (0.05 mg/kg) is

considered acceptable (SANCO/825/00 rev. 8.1 general limit: 0.05 mg/kg). It was used 2 ion transition m/z 161 > 44 for quantification and m/z 161 > 61 for confirmation. Confirmatory analysis is not required. Representative chromatograms attached. The method is suitable as enforcement method for monitoring of daminozide residues in soil.

LOQ complies with the lowest effect concentration for the most sensitive soil organism (*Eisenia fetida*: NOEC = 648 mg a.s./kg; Earthworm reproduction study 87714022, Pavić, 2014).

EU conclusions: Validated analytical method for residues of daminozide in soil

Method No.	Matrix	Analytes	LOQ (mg/kg)	Technique	Report No.	Reference
Enforcement method						
-	soil	daminozide	0.05	HPLC-MS/MS	FDD0080	Munro, S. (2012)

Reference:	Jooß, S. (2012): Validation of an Analytical Method for the Determination of Daminozide in Surface Water
Report No.:	P 2516 G
Guideline:	SANCO/825/00 rev. 8.1 (16/11/2010) and SANCO/3029/99 rev. 4 (11/07/2000)
GLP:	Yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Principle of the method (Daminozide in Surface water)

Surface water samples (10 mL) are transferred to centrifuge vials and evaporated to < 1.0 mL using a rotary evaporator. The remaining sample is diluted to 1.0 mL with water, acidified with 10% formic acid (10 µL) and analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive electrospray mode, using a Thermo Aquasil C₁₈ column (150 x 3.0 mm, 3.0 µm particle size) and gradient elution with mobile phases of water containing 0.1 % formic acid and methanol containing 0.1 % formic acid. Quantification is performed using external standards. The ion transition m/z 161 > 142.9 is used for quantification and the ion transition m/z 161 > 101.1 is used for confirmation.

Column	Thermo Aquasil C ₁₈ (150 mm x 3 mm, 3 µm)
Oven temperature	30°C
Injection volume	100 µL
Flow rate	0.5 ml/min
Mobile phase	Channel A: water containing 0.1% formic acid Channel B: methanol containing 0.1% formic acid

Specificity

HPLC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences at > 20% of the LOQ were present at the retention time of interest in any of the control matrix samples.

Linearity

Linearity of detector response was demonstrated using at least five external standard solutions across the concentration range of 0.10 to 10 (25) ng/mL. The results are presented in table below.

Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Regression line equation
161 > 143	0.10 - 10 (25)	0.9927	$y = 2.25 \times 10^5 x + 4.65 \times 10^4$
161 > 101		0.9939	$y = 8.95 \times 10^3 x + 7.57 \times 10^3$

Precision (Repeatability)

Repeatability data was generated from five samples fortified at the LOQ and 10x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and are presented in table below.

Ion transition (m/z)	Fortification Level (µg/L)	Recovery (%)	Mean Recovery (%)	RSD (%)
161 > 143	0.10	98, 111, 114, 113, 115	110	6
	1.0	80, 79, 84, 77, 76	79	4
161 > 101	0.10	107, 105, 107, 105, 101	105	2
	1.0	74, 77, 75, 75, 74	75	2

Accuracy (Recovery)

Recovery data was generated from five samples fortified at the LOQ and 10x LOQ. The mean percentage recoveries at each fortification level were within the guideline requirements of 70 - 120% and are presented in table below.

Ion transition (m/z)	Fortification Level (µg/L)	Recovery (%)	Mean Recovery (%)	RSD (%)
161 > 143	0.10	98, 111, 114, 113, 115	110	6
	1.0	80, 79, 84, 77, 76	79	4
161 > 101	0.10	107, 105, 107, 105, 101	105	2
	1.0	74, 77, 75, 75, 74	75	2

Limit of quantification (LOQ)

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained has been demonstrated to be 0.1 µg/L.

Confirmation

Confirmation is not required because of using highly specific method HPLC-MS/MS with 2 ion transition as a primary method.

Stability of water solutions

Fortification and standard solutions were stored refrigerated in amber glass bottles when not in use. Standard solutions were demonstrated to be stable for one day by consistent LC-MS/MS results. Consistent recovery results demonstrated stability of extracts during the duration of the analysis.

Surface water characterisation

Origin	Sample ID: - (was taken from river Brenz near Herbrechtingen, Germany)
pH	7.92
Calcium	97.3 mg/L
Magnesium	4.0 mg/L
Hardness	2.59 mmol/L corresponding to 14.5°dH
Conductivity	569 µS/cm at 25°C
Dissolved organic carbon (DOC)	0.87 mg/L
Silt content	< 0.5 mg/L

The surface water was kept at room temperature after collection and prior to extraction.

RMS comments and conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANCO/825/00 rev. 8.1 (16/11/2010) - GLP study, the linearity data was acceptable (range was 0.1 to 10 (25) ng/ml (n=5) and $r > 0.99$). A calibration curve was provided and was linear. The mean recovery (n=5) was between 70 % and 120 %, with the relative standard deviation (RSD) lower than 20 %. No interferences due to the reagent were detected. LOQ of the method (0.1 µg/L) is considered acceptable (SANCO/825/00 rev. 8.1 general limit: 0.1 µg/L). It was used 2 ion transition m/z 161 > 143 for quantification and m/z 161 > 101 for confirmation. Confirmatory analysis is not required. Representative chromatograms attached. The method is suitable as enforcement method for monitoring of daminozide residues in surface water.

The objective of the following study was to independently validate a previously reported method (study no. P 2516 G) for the determination of residues of daminozide in drinking water:

Reference:	Austin, R. (2015): Independent laboratory validation of method validation for daminozide in drinking water
Report No.:	BH/14/023
Guideline:	SANCO/825/00 rev. 8.1 (16/11/2010) and SANCO/3029/99 rev. 4 (11/07/2000)
GLP:	Yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Principle of the method (Daminozide in Drinking water)

Same as previously reported method (study no. P 2516 G). Drinking water was preconcentrated before analysis. Determination of final sample volumes was conducted with LC-MS/MS in the positive electrospray ionization mode monitoring the parent ion at m/z 161 and two different fragment ions at m/z 143 (quantification) and m/z 61 (confirmation). The method was validated in terms of linearity, selectivity, accuracy and precision.

Method modifications: The samples were evaporated in round bottom flasks using a rotary evaporator at 60°C instead of in centrifuge vials using a rotary vacuum concentrator. This was because a rotary vacuum concentrator was not available at Battelle UK. Transition m/z 161 > 101 was replaced with transition m/z 161 > 61 due to interference at the retention time of daminozide and a need for greater sensitivity.

Column	Thermo Aquasil C ₁₈ (150 mm x 3 mm, 3 µm)
Column temperature	35°C
Injection volume	100 µL
Flow rate	0.5 ml/min
Mobile phase	Channel A: water containing 0.1% formic acid Channel B: methanol containing 0.1% formic acid

Specificity

Chromatographic interferences at the retention time of daminozide were less than 30% of the limit of quantification in control samples, demonstrating satisfactory selectivity. In addition, LC-MS/MS monitoring two ion mass transitions is considered to be a highly specific technique.

Linearity

Linearity of detector response was demonstrated using at least five external standard solutions across the concentration range of 0.10 to 10 (25) ng/mL. The results are presented in table below.

Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Regression line equation
161 > 143	0.10 - 10 (25)	0.9997	$y = 5.49 \times 10^4 x + 2.01 \times 10^4$
161 > 61		0.9998	$y = 9.97 \times 10^3 x + 926$

Precision (Repeatability)

Repeatability data was generated from five samples fortified at the LOQ and 10x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and are presented in table below.

Ion transition (m/z)	Fortification Level (µg/L)	Recovery (%)	Mean Recovery (%)	RSD (%)
161 > 143	0.10	65, 75, 78, 83, 75	75	8.7
	1.0	78, 102, 93, 81, 120	95	18.0
161 > 61	0.10	66, 78, 79, 89, 83	79	10.7
	1.0	74, 98, 90, 79, 117	92	18.6

Accuracy (Recovery)

Recovery data was generated from five samples fortified at the LOQ and 10x LOQ. The mean percentage recoveries at each fortification level were within the guideline requirements of 70 - 120% and are presented in table below.

Ion transition (m/z)	Fortification Level (µg/L)	Recovery (%)	Mean Recovery (%)	RSD (%)
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161 > 143	0.10	65, 75, 78, 83, 75	75	8.7
	1.0	78, 102, 93, 81, 120	95	18.0
161 > 61	0.10	66, 78, 79, 89, 83	79	10.7
	1.0	74, 98, 90, 79, 117	92	18.6

Limit of quantification (LOQ)

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained has been demonstrated to be 0.1 µg/L.

Confirmation

Confirmation is not required because of using highly specific method LC-MS/MS with 2 ion transition.

Stability of daminozide in sample extract and in standard solutions

Daminozide was shown to be stable in drinking water sample extracts when stored between 1 and 8°C for at least 11 days. A stock standard solution of daminozide prepared in methanol/water (9:1) was shown to be stable when stored between 1 and 8°C for at least 11 days. A fortification standard solution of daminozide prepared in water was shown to be stable when stored between 1 and 8°C for at least 11 days. A calibration standard solution of daminozide prepared in water containing 0.1% formic acid was shown to be stable when stored between 1 and 8°C for at least 11 days.

Matrix effects

No significant matrix effects (< 20%) were observed when comparing the peak areas of solvent standards.

Therefore, solvent calibration standards were used for quantification. It is recommended to assess matrix-effect with each analytical run.

Drinking water characterisation

Origin	Sample ID: 15/002 (DRINKING H2O RES) (was taken from a drinking water tap at Battelle UK, Chelmsford)
pH	8.1
Calcium	104 ppm
Magnesium	9.5 ppm
Hardness	299 mg equivalent CaCO ₃ /L
Conductivity	0.71 mmhos/cm
Total suspended solids	6.0 ppm
Total organic carbon (TOC)	5.5 ppm
Dissolved organic carbon (DOC)	3.7 ppm
Ammoniacal nitrogen	Below detection limit of 0.2 ppm
Total nitrogen	7.0 ppm
Nitrate-nitrogen	3.9 ppm
Nitrite-nitrogen	2.4 ppm
Total phosphorus	4.8 ppm
Reactive phosphates	1.1 ppm

The water sample was stored in a freezer prior to use.

RMS comments and conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANCO/825/00 rev. 8.1 (16/11/2010) - GLP study, the linearity data was acceptable (range was 0.1 to 10 (25) ng/ml (n=5) and $r > 0.99$). A calibration curve was provided and was linear. The mean recovery (n=5) was between 70 % and 120 %, with the relative standard deviation (RSD) lower than 20 %. No interferences due to the reagent were detected. LOQ of the method (0.1 µg/L) is considered acceptable (SANCO/825/00 rev. 8.1 general limit: 0.1 µg/L). It was used 2 ion transition m/z 161 > 143 for quantification and m/z 161 > 61 for confirmation. Confirmatory analysis is not required. Representative chromatograms attached. The method is suitable as enforcement method for monitoring of daminozide residues in drinking water.

LOQ complies with the lowest effect concentration for the most sensitive aquatic organism (*Pimephales promelas*: NOEC_{chronic} = 1.7 mg/L; ELS study 616A-123, [REDACTED], 2014).

EU conclusions: Validated analytical method for residues of daminozide in surface and drinking water

Method No.	Matrix	Analytes	LOQ (µg/L)	Technique	Report No.	Reference
Enforcement method						
-	surface water	daminozide	0.1	HPLC-MS/MS	P 2516 G	Jooß, S. (2012)
-	drinking water	daminozide	0.1	LC-MS/MS (ILV)	BH/14/023	Austin, R. (2015)

(c) The analysis in air of the active substance and relevant breakdown products formed during and after application, unless the applicant shows that exposure of operators, workers, residents or bystander is negligible

Old following studies were evaluated in Addendum (2003) of original DAR (1999):

Author(s)	Year	Title	Owner	Original conclusion or comments
Parsons, A.H.	2002	Validation of Analytical Method M521 "Gas chromatographic determination of residues of daminozide in air monitoring tubes". Generated by: GC laboratories Ltd. Submitted by: Fine Agrochemicals Report No. J13923/A Date: 15 April 2002 GLP, Unpublished	Fine Addendum (2003)	In this study only the extraction recovery from the tubes is determined. No validation data on breakthrough of the air types described in SANCO/825/00 are provided. The method is not fully validated and therefore not acceptable for monitoring daminozide in air
Parsons, A.H.	2002	Determination of the capacity of the sampling tubes used for the determination of daminozide in air (breakthrough test) Generated by: GC laboratories Ltd. Submitted by: Fine Agrochemicals Report No. J14277 Date: 21 October 2002 GLP, Unpublished	Fine Addendum (2003)	The results indicate that the LOQ is 1.4 µg/m ³ (using 720 liters of air). Validation using dry air and a temperature of 25°C is not required as the results from moist air of 35°C are acceptable. This method is acceptable and can be used for monitoring daminozide in air.

New following studies were submitted by the notifiers to cover the analysis of all components for monitoring purposes in the residue definition for air:

Reference:	Miller, C. (2015): Daminozide and UDMH: Validation of Methodology for the Determination of Residues in Air
Report No.:	FDD0124
Guideline:	SANCO/825/00 rev. 8.1 (16/11/2010)
GLP:	Yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Principle of the method (UDMH in air)

A suitable sorbent type could not be found for the analysis of UDMH in air (two alternative sorbent materials investigated were XAD-2 and Anasorb CSC). For XAD-2: no residues of UDMH were detected and for Anasorb CSC: recovery values would be unacceptable in a validation test. This objective of the study could not be fulfilled.

The objective of the study could however, be fulfilled with respect to the analysis of Daminozide in air - see below.

Principle of the method (Daminozide in air)

Air cartridges were fortified with 10 µL of a standard solution of daminozide. The sorbent used was Tenax (a porous polymer). Fortifications were made directly onto the sorbent material according to the following regime: 2 untreated cartridges (control), 5 cartridges fortified at the LOQ, 160 µg/m³ of air (equivalent to 57.6 µg on cartridge) and 5 cartridges fortified at 1600 µg/m³ of air (equivalent to 576 µg on cartridge). A defined air volume (approximately 1 L/min) was passed through each Tenax air cartridge for 6 hours at nominally 35°C and 80 % relative humidity. Once the desired air volume was drawn through the tube, the sorbent material was desorbed with acetone prior to further dilution with acetonitrile. Quantitation was performed using liquid chromatography with tandem mass spectrometric detection (LC-MS/MS). The ion transition m/z 161 > 143 is used for quantification and the ion transition m/z 161 > 44 is used for confirmation.

Column	Acquity BEH C ₁₈ (50 mm x 2.1 mm, 1.7 µm)
Column temperature	45°C
Injection volume	20 µL
Flow rate	0.5 ml/min
Mobile phase	Water/methanol/formic acid (90/10/0.1 v/v/v) + 0.01 M ammonium formate

Specificity

Chromatographic interferences at the retention time of daminozide were less than 30% of the limit of quantification in control samples, demonstrating satisfactory selectivity. In addition, LC-MS/MS monitoring two ion mass transitions is considered to be a highly specific technique.

Linearity

Linearity of detector response was demonstrated using at least eight external standard solutions across the concentration range of 0.5 to 10 ng/mL. The results are presented in table below.

Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Regression line equation
161 > 143	0.5 - 10	0.9997	$y = 30503.9x + 0.03276$
161 > 44		0.9998	$y = 5163.29x + 0.00055$

Precision (Repeatability)

Repeatability data was generated from five samples fortified at the LOQ and 10x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and are presented in table below.

Ion transition (m/z)	Fortification Level (µg/m ³)	Recovery (%)	Mean Recovery (%)	RSD (%)
161 > 143	160	81, 85, 82, 77, 78	81	4.0
	1600	79, 79, 84, 82, 80	81	2.7
161 > 44	160	81, 85, 79, 76, 78	80	4.3
	1600	79, 80, 82, 82, 81	81	1.6

Accuracy (Recovery)

Recovery data was generated from five samples fortified at the LOQ and 10x LOQ. The mean percentage recoveries at each fortification level were within the guideline requirements of 70 - 120% and are presented in table below.

Ion transition (m/z)	Fortification Level (µg/m ³)	Recovery (%)	Mean Recovery (%)	RSD (%)
161 > 143	160	81, 85, 82, 77, 78	81	4.0
	1600	79, 79, 84, 82, 80	81	2.7
161 > 44	160	81, 85, 79, 76, 78	80	4.3
	1600	79, 80, 82, 82, 81	81	1.6

Limit of quantification (LOQ)

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained has been demonstrated to be 160 µg/m³ (equivalent to 57.6 µg on cartridge).

Confirmation

Confirmation is not required.

Samples extract stability and matrix effects

The final extract stability samples indicated that Daminozide was stable in the final acetonitrile extract of the Tenax sorbent material for a period of seven days when stored at approximately -20°C in the dark. There was no indication of any significant matrix effects for Daminozide in the Tenax sorbent material extract when using the validated LC-MS/MS methodology analysed.

Retention capacity

The back section of two of the cartridges fortified at the higher concentration level (1600 µg/m³ equivalent to 576 µg on cartridge) were analysed in order to check for 'breakthrough'. No residues of Daminozide were detected in the back sections of the air cartridges tested. Therefore, the retention capacity of Tenax sorbent material to Daminozide is deemed acceptable under the conditions described.

RMS comments and conclusion

The analytical procedure has been successfully validated using exposure conditions of 35°C and 80% relative humidity (RH) in terms of specificity, linearity, precision and accuracy in accordance with the requirements of SANCO/825/00 rev. 8.1 (16/11/2010) - GLP study, the linearity data was acceptable (range was 0.5 to 10 ng/ml (n=8) and $r > 0.99$). A calibration curve was provided and was linear. The mean recovery (n=5) was between 70 % and 120 %, with the relative standard deviation (RSD) lower than 20 %. No interferences due to the reagent were detected. LOQ of the method is 160 µg/m³ (equivalent to 57.6 µg on cartridge) - **LOQ is not low enough, see below**. It was used 2 ion transition m/z 161 > 143 for quantification and m/z 161 > 44 for confirmation. Confirmatory analysis is not required. Representative chromatograms attached.

LOQ not complies with the $c = 2.7 \mu\text{g}/\text{m}^3$ (concentration is calculated from $\text{AOEL}_{\text{systemic}} 0.009 \text{ mg/kg bw/day}$). Validation of the method is not sufficient (LOQ is not low enough).

Reference:	Bendig, P.; Wabbel, C. (2016): Development and Validation of an Analytical Method for the Determination of Unsymmetrical Dimethylhydrazine (UDMH) in Air
Report No.:	P 3210 G
Guideline:	SANCO/825/00 rev. 8.1 (16/11/2010) and SANCO/3029/99 rev. 4 (11/07/2000)
GLP:	Yes
Published:	No
Previous evaluation:	Submitted for the purpose of renewal

Principle of the method (UDMH in air)

Scaled (midjet) impingers are filled with aqueous phosphoric acid and methanol as trapping reagents and fortified with unsymmetrical dimethylhydrazine (UDMH). The samples are flushed with air (35°C and 80% relative humidity) at 300 mL/min for 6 hours. The trapping solutions are acidified with 4 M sodium hydroxide solution (1 mL) and derivatised using 4 mL of the derivatising reagent (2-nitrobenzaldehyde in methanol at 100 mg/mL). The impingers are filled to volume (20 mL) with methanol and placed in a drying oven at 45°C for 2 hours. After derivatization the final volume is checked and adjusted as necessary, the samples centrifuged (if final volume is turbid) and transferred to auto sampler vials for analysis. The samples are analysed by high performance liquid chromatography with tandem mass specific detection (LC-MS/MS) in negative ionisation mode, using a Phenomenex Luna Phenyl-Hexyl column (100 mm x 2 mm, 3 µm particle size) and gradient elution with mobile phases of 0.01% acetic acid in water/acetonitrile (9/1, v/v) and 0.01% acetic acid in acetonitrile. Quantification was performed using external standards. The ion transition m/z 194 > 59 is used for quantitation and the ion transition m/z 194 > 151 is used for confirmation.

Column	Phenomenex Luna Phenyl-Hexyl column: 100 mm x 2 mm x 3 µm
Column temperature	30°C
Injection volume	20 µL
Flow rate	0.5 ml/min
Mobile phases	Water/acetonitrile (9/1 v/v) + 0.01 % acetic acid Acetonitrile + 0.01 % acetic acid

Specificity

Chromatographic interferences at the retention time of UDMH were less than 30% of the limit of quantification in control samples, demonstrating satisfactory selectivity. In addition, LC-MS/MS monitoring two ion mass transitions is considered to be a highly specific technique.

Linearity

Linearity of detector response was demonstrated using six standard solutions across the concentration range of 0.025 to 2 ng/mL. The results are presented in table below.

Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Regression line equation
194 > 59	0.025 - 2.0	0.9985	$y = 1.84 \times 10^6 x - 1.96 \times 10^4$
194 > 151		0.9975	$y = 2.72 \times 10^5 x - 3.57 \times 10^3$

Precision (Repeatability)

Repeatability data was generated from five samples fortified at the LOQ and 10x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and are presented in table below.

Ion transition (m/z)	Fortification Level (ng/m ³)	Recovery (%)	Mean Recovery (%)	RSD (%)
194 > 59	25	108, 108, 106, 106, 103	106	2
	250	101, 103, 98, 88, 78	94	11
194 > 151	25	105, 105, 104, 101, 102	103	2
	250	101, 101, 98, 89, 76	93	11

Accuracy (Recovery)

Recovery data was generated from five samples fortified at the LOQ and 10x LOQ. The mean percentage recoveries at each fortification level were within the guideline requirements of 70 - 120% and are presented in table below.

Ion transition (m/z)	Fortification Level (ng/m ³)	Recovery (%)	Mean Recovery (%)	RSD (%)
194 > 59	25	108, 108, 106, 106, 103	106	2
	250	101, 103, 98, 88, 78	94	11
194 > 151	25	105, 105, 104, 101, 102	103	2
	250	101, 101, 98, 89, 76	93	11

Limit of quantification (LOQ)

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained has been demonstrated to be 25 ng/m³.

Confirmation

Confirmation is not required.

Samples extract stability and matrix effects

The stability of the analyte in stock and calibration solutions was demonstrated for up to 12 days (stock) and 7 days (derivatised calibration solution) respectively under frozen conditions with results in the acceptable range of 70 - 120%. Stability of the analyte in trapping solution was demonstrated for 6 days of frozen storage.

No significant effect of matrix (< 20%) was observed. Detailed results and representative chromatograms of a matrix matched standard are shown in study.

Retention capacity

The impingers were fortified with UDMH at 25 ng/m³ (LOQ) and 250 ng/m³ (10x LOQ). Subsequently, the sampling of air was performed for 6 hours with warm, humid air (approx. 35°C, > 80% relative humidity). Five replicates per fortification level were analysed using LC-MS/MS. The average recoveries at LOQ and 10x LOQ fortification levels for both mass transitions after air sampling were in the range of 70 to 120% the relative standard deviations were always ≤ 20%.

No detectable breakthrough (< 0.025 ng/mL, < LOD) in the two blank impingers connected in series with 10x LOQ fortified impingers was determined.

RMS comments and conclusion

The analytical procedure has been successfully validated using exposure conditions of 35°C and 80% relative humidity (RH) in terms of specificity, linearity, precision, accuracy and LOQ in accordance with the requirements of SANCO/825/00 rev. 8.1 (16/11/2010) - GLP study, the linearity data was acceptable (range was 0.025 to 2 ng/ml (n=6) and r > 0.99). A calibration curve was provided and was linear. The mean recovery (n=5) was between 70 % and 120 %, with the relative standard deviation (RSD) lower than 20 %. No interferences due to the reagent were detected. LOQ of the method is 0.025 µg/m³. It was used 2 ion transition m/z 194 > 59 for quantification and m/z 194 > 151 for confirmation. Confirmatory analysis is not required. Representative chromatograms attached. The method is suitable as enforcement method for monitoring of UDMH in air.

LOQ complies with the c = 0.027 µg/m³ (concentration is calculated from AOEL 0.00009 mg/kg bw/day).

EU conclusions: Validated analytical method for residues of daminozide in air

Method No.	Matrix	Analytes	LOQ (µg/m ³)	Technique	Report No.	Reference
Enforcement method						
-	air	daminozide	160	LC-MS/MS	FDD0124	Miller, C. (2015)
-	air	UDMH	0.025	LC-MS/MS	P 3210 G	Bendig, P.

						Wabbel, C. (2016)
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(d) The analysis in body fluids and tissues for active substance and relevant metabolites

According to guideline SANCO/825/00 rev. 8.1 method of analysis is not required as daminozide is not classified as either toxic or highly toxic nor is classified according to CLP as follows: Acute toxicity (cat. 1 - 3), CMR (cat. 1) or STOT (cat. 1). On the contrary under regulation 1107/2009 this method is always required. Therefore the analysis in body fluids and tissues is identified a data requirement. Method is ongoing and expected Q4 2018.

B.5.3. References Relied On

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
B.5.1.1/01	Bates, G.J.D.	2012	Validation of analytical method M741 “High performance liquid chromatographic determination of daminozide in technical material and formulations” for the FAL 2400 formulation G.C. Laboratories Ltd. Report No. J19126 GLP Unpublished	N	Y	New data for AIR3 renewal	Fine Agrochemicals Limited
B.5.1.1/02	Kelly, K.	2015c	Partial validation of analytical method M741 “High performance liquid chromatographic determination of daminozide in technical material and formulations” for technical material G.C. Laboratories Ltd. Report No. J20158 GLP Unpublished	N	Y	New data for AIR3 renewal	Fine Agrochemicals Limited
B.5.1.1/03	Barker, C.H.	2015	Preliminary analysis of technical daminozide MacDermid Agricultural Solutions Canada Company, Guelph, Canada Report: GRL-13301 (only Appendix II) GLP: Yes Published: No	N	Y	New data for AIR3 renewal	Arysta LifeScience Great Britain Limited
B.5.1.1/04	Riggs, A.S.	2011	Validation of an analytical method for the determination of daminozide in daminozide technical Chemtura Canada Co. Report No. GRL-12970 GLP Unpublished	N	Y	New data for AIR3 renewal	Arysta LifeScience Great Britain Limited
B.5.1.1/05	Kelly, K.	2015g	Validation of Analytical Method M851 UDMH and NDMA G.C. Laboratories Ltd. Report No. J20157 GLP Unpublished	N	Y	New data for AIR3 renewal	Fine Agrochemicals Limited

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
B.5.1.1/06	Barker, C.H.	2015	Preliminary analysis of technical daminozide MacDermid Agricultural Solutions Canada Company, Guelph, Canada Report: GRL-13301 (only Appendix V) GLP: Yes Published: No	N	Y	New data for AIR3 renewal	Arysta LifeScience Great Britain Limited
B.5.1.1/07	Yu, W.S.	2000a	Validation of an analytical method for the determination of unsymmetrical dimethyl hydrazine (UDMH) in B-Nine technical and B-Nine SP formulation. Uniroyal Chemical Co., Guelph, Canada Report: GRL-11423 GLP: Yes Published: No	N	Y	New data for AIR3 renewal	Arysta LifeScience Great Britain Limited
B.5.1.1/08	Riggs, A.S.	2009a	Validation of an analytical method for the determination of unsymmetrical dimethyl hydrazine (UDMH) in Alar 85 WG. Chemtura Canada Co./Cie, Guelph, Canada Report: GRL-12643 (GRL-12870, GRL-13222) GLP: Yes Published: No	N	Y	New data for AIR3 renewal	Arysta LifeScience Great Britain Limited
B.5.1.1/09	Barker, C.H.	2015	Preliminary analysis of technical daminozide MacDermid Agricultural Solutions Canada Company, Guelph, Canada Report: GRL-13301 (only Appendix VI) GLP: Yes Published: No	N	Y	New data for AIR3 renewal	Arysta LifeScience Great Britain Limited
B.5.1.1/10	Yu, W.S.	2000b	Validation of an analytical method for the determination of N-nitrodimethylamine (NDMA) in B-Nine technical Uniroyal Chemical Co., Guelph, Canada Report: GRL-11450 GLP: Yes Published: No	N	Y	New data for AIR3 renewal	Arysta LifeScience Great Britain Limited

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
B.5.1.1/11	Riggs, A.S.	2009b	Validation of an analytical method for the determination of N-Nitrosodimethylamine (NDMA) in Alar 85 WG Chemtura Canada Co./Cie, Guelph, Canada Report: GRL-12644 (GRL-12844, GRL-13223) GLP: Yes Published: No	N	Y	New data for AIR3 renewal	Arysta LifeScience Great Britain Limited
B.5.1.2/01	Hart, C.	2012b	Determination of the concentration of Unsymmetrical Dimethyl Hydrazine (UDMH) in a mixture of daminozide and water over a storage period of 24 hours Chemtura Canada Co., Canada. Report No. GRL-13025 GLP Unpublished	N	Y	New study for AIR 3 dossier	Arysta LifeScience Great Britain Limited
B.5.1.2/02	██████ ██████ ██████ ██████	2012	Daminozide: a reproduction study with the Northern bobwhite ████████████████████ Report. No. 616-104 GLP Unpublished	Y	Y	New data for AIR 3 renewal	Arysta LifeScience Great Britain Limited
B.5.1.2/03	██████████ ██████████ ██████████ ██████████	2015	Daminozide: an early life-stage toxicity test with the fathead minnow (<i>Pimephales promelas</i>) ████████████████████ Report No. 616A-123 GLP Unpublished	Y	Y	New data for AIR 3 renewal	EU Daminozide Task Force
B.5.1.2/04	Last, G.	2012	Chronic effects to <i>Daphnia magna</i> from exposure to daminozide and formaldehyde Covance Laboratories Ltd, UK. Report No. 8252736. GLP Unpublished	N	Y	New data for AIR3 renewal	EU Daminozide Task Force
B.5.1.2/05	Manson, P.S. Scholey, A.	2006	Daminozide Technical: Inhibition of growth to the alga <i>Pseudokirchneriella subcapitata</i> Covance Laboratories Ltd. Report No. 2242/049-D2149 GLP Unpublished	N	Y	New data for AIR 3 renewal	Fine Agrochemicals Limited

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
B.5.1.2/06	Seeland-Fremer, A. Mosch, W.	2014	Toxicity of daminozide technical to <i>Anabaena flos-aquae</i> in an Algal Growth Inhibition Test IBACON. Report No. 87711210 GLP Unpublished	N	Y	New data for AIR 3 renewal	EU Daminozide Task Force
B.5.1.2/07	Friedlander, B.T.	2011	The solubility of technical daminozide in water Chemtura Canada Co./Cie. Report No. GRL-12954 GLP Unpublished	N	Y	New study for AIR 3 dossier	Arysta LifeScience Great Britain Limited
B.5.1.2/08	Riggs, A.S.	2011	The partition coefficient (n-octanol/water) of technical daminozide Chemtura Canada Co./Cie. Report No. GRL-12953 GLP Unpublished	N	Y	New study for AIR 3 dossier	Arysta LifeScience Great Britain Limited
B.5.2/01	Munro, S.	2012	Validation of an analytical method for the determination of daminozide in soil Huntingdon Life Sciences, UK. Report No. FDD0080 GLP Unpublished	N	Y	New data for AIR3 renewal	Arysta LifeScience Great Britain Limited
B.5.2/02	Jooß, S.	2012	Validation of an analytical method for the determination of daminozide in surface water PTRL Europe, Germany. Report No. P 2516 G GLP Unpublished	N	Y	New data for AIR3 renewal	Arysta LifeScience Great Britain Limited
B.5.2/03	Austin, R.	2015	Independent laboratory validation of method validation for daminozide in drinking water Battelle UK Ltd. Study No. BH/14/023 GLP Unpublished	N	Y	New data for AIR3 renewal	EU Daminozide Task Force

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
B.5.2/04	Miller, C.	2015	Daminozide and UDMH: Validation of methodology for the determination of residues in air Huntingdon Life Sciences. Study No. FDD0124 GLP Unpublished	N	Y	New data for AIR3 renewal	EU Daminozide Task Force
B.5.2/05	Bendig, P. Wabbel, C.	2016	Development and Validation of an Analytical Method for the Determination of Unsymmetrical Dimethylhydrazine (UDMH) in air PTRL Europe, Germany. Report No. P 3210 G GLP Unpublished	N	Y	New data for AIR3 renewal	EU Daminozide Task Force