Section A4.1/01

Annex Point IIA4.1

Analytical Method for Detection and Identification of DBNPA and impurities as manufactured

Please refer to the confidential section of doc IIIA4 for information on the analytical Method for Detection and Identification of DBNPA and impurities as manufactured

Section A4.2a/01

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of 2,2-Dibromo-3-nitrilopropionamide (DBNPA) in soil

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [X]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	The following information supports applicant's request for a waiver for an analytical method for measuring DBNPA in water and soil due to the compound's rapid degradation in these matrices, especially at low mg/L to sub-mg/L concentrations. As already noted, degradation of DBNPA occurs by two routes, a hydrolysis pathway and a degradation route involving reaction with nucleophiles [Exner, 1973]. Hydrolysis half-lives range from hours at neutral to alkaline pH to weeks at acidic pH. In contrast, DBNPA can react with nucleophiles or light with half-lives in the range seconds to minutes, especially with low concentrations of DBNPA present. DBNPA will react with the constituents of soil, including organic matter, microbial cells, and various nucleophiles present in the soil. For example, a series of soils with a range of soil textures were mixed in aqueous slurries containing 50 mg/L DBNPA [Exner, 1973]. Half-lives of DBNPA ranged from 4 hours in sandy loam to 25 hours in silty clay loam. At lower DBNPA concentrations, as the ratio of DBNPA to nucleophiles/organic matter decreases, the degradation rate will increase and the half-life of DBNPA will be even shorter. This point is demonstrated with the degradation of DBNPA in activated sludge, sewage, sediments, and natural waters which contain nucleophiles and reactive organic matter similar to soil. Rapid degradation of DBNPA was observed in an activated sludge die-away test (Hanstveit, 2002). Primary degradation of 0.04 mg/L [14C]DBNPA in activated sludge occurred within one hour. In a separate study, the addition of DBNPA to sewage entering a municipal STP resulted in complete disappearance of up to 10 mg/L DBNPA within 5 minutes, followed by a slower transformation of any residual DBNPA	

Section A4.2a/01
Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of 2,2-Dibromo-3-nitrilopropionamide (DBNPA) in soil

(e.g. the half-life for 23 mg/L DBNPA was 0.8 hours) [Gonsior, 2000]. Rapid degradation was also observed in natural waters and sediments [Gonsior, 2001]. Here, sub-mg/L concentrations of radiolabeled DBNPA rapidly degraded in microcosms prepared with river water and river sediments, with half-lives measured at less than one hour.

To summarize, the rapid degradation of DBNPA in environmental matrices due to the reaction with nucleophiles and reactive organic matter makes it extremely difficult to develop an analytical method to measure realistic environmental concentrations of the compound.

References

Exner, J. H., G. A. Burk, and D. Kyriacou. 1973. Rates and Products of Decomposition of 2,2 Dibromo-3-nitrilopropionamide. J. Agr. Food Chem., Vol 21, No. 5, pages 838-842.

Gonsior, S. J., and P. A. Goodwin. 2000. Evaluation of the Effect of 2,2, -Dibomonitrilopropionamide (DBNPA) on a Semi-Continuous Activated Sludge Treatment System. The Dow Chemical Company Report HET K-078141-097.

Gonsior, S. J., P. A. Goodwin, and M. K. Stock. 2001. Assessing the Biodegradability of DBNPA in Water/Sediment Mixtures. The Dow Chemical Company Report HET K-078141-098.

Hanstveit, R. and J. A. Schoonmade. 2002. 2,2,-Dibomo-3-nitrilopropionamide (DBNPA): A Definitive Die Away Test in Activated Sludge. The Dow Chemical Company Report K-078141-107.

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

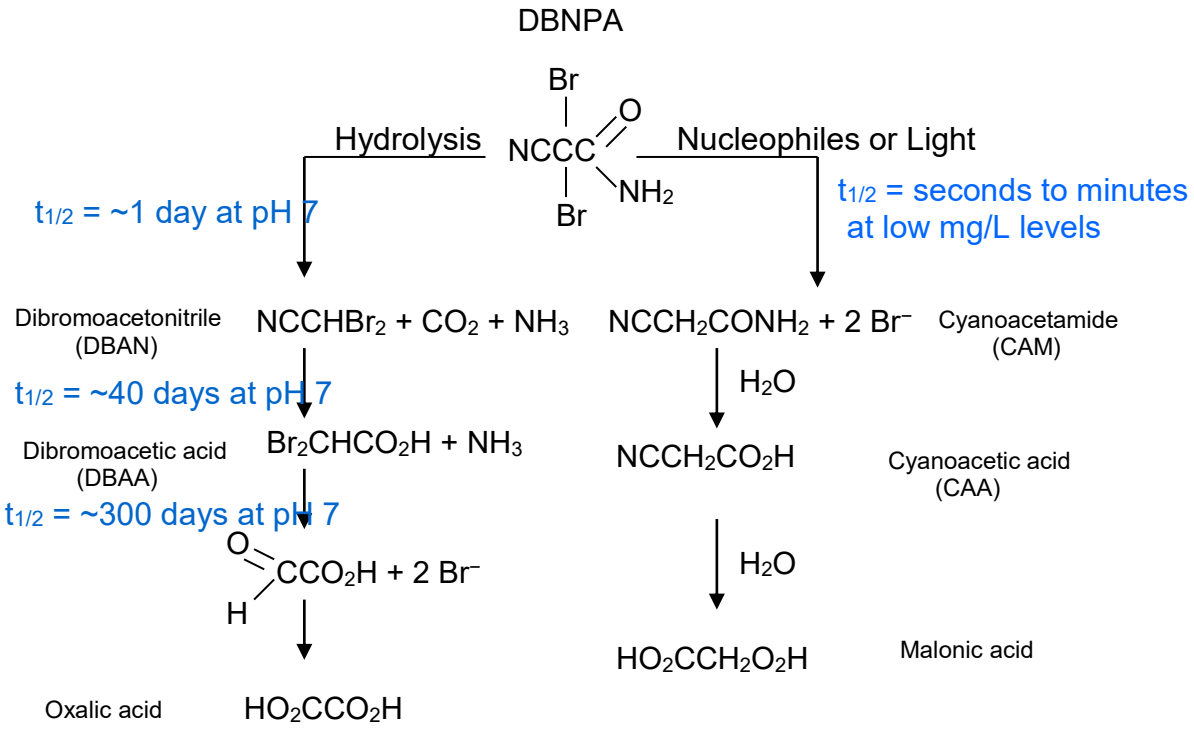
Section A4.2a/01

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of 2,2-Dibromo-3-nitrilopropionamide (DBNPA) in soil

Date	19/10/2015
Evaluation of applicant's justification	Applicant states that the analysis of DBNPA in soil is technically not feasible due to the rapid degradation in soil due to interactions with various components in the matrix. Furthermore, a method for DBAA has been developed, which serves as a marker for DBNPA.
Conclusion	Acceptable
Remarks	Acceptable
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Appendix 1 – DBNPA Degradation Pathways (Reference 4)



Section A4.2a/02

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of Dibromoacetonitrile (DBAN) in soil

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [X]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The DBAA analysis in soil has been developed and validated at EN-CAS Laboratories (Reference 1). Despite several attempts, the development of a method to determine Dibromoacetonitrile (DBAN) in soils has not been successful. Several different LC/MS/MS modes were investigated but the best DBAN detection limits were 20 times greater than the required LOQ of 50 ppb (Reference 2). GC analysis with ECD detection (based on the method published by Nikolaou, et al (Reference 3) using extraction with MTBE or water/MTBE was also attempted. The recoveries were very close to zero, indicating an ineffective method. Before further extraction schemes were pursued, we noted that the DBAN appears to convert to DBAA in the highly diluted samples. This is not unexpected based on the known stability of DBAN (Reference 4 and Appendix 1). Furthermore, in comparing the relative stability of these two degradates, the DBAA has a half-life of ~300 days as compared to a half-life of ~ 40 days for DBAN under neutral pH conditions. Because DBAN is an intermediate in the degradation of DBNPA to DBAA, and DBAA is more stable than DBAN, it is clear that the detection of DBAA would be a more representative and robust marker when evaluating the exposure of soil to DBNPA. Based on this understanding, the development of a DBAN analytical method is not essential because the DBAA is a better marker for DBNPA and we have successfully developed a method for DBAA in soils. **References** 1. Barker, W., “Validation of a Method to Measure	

Section A4.2a/02
Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of Dibromoacetonitrile (DBAN) in soil

Trace Levels of Dibromoacetic Acid (DBAA) in Soils", EN-CAS Laboratories, August 5, 2008.
2. Barker, W., "DBAN in Soil – Summary of Method Work", EN-CAS Analytical Laboratories, August 22, 2008.
3. Nikolaou, Anastasia D., et. al. 1999, "Decomposition of Dihaloacetonitriles in Water Solutions and Fortified Drinking Water Samples". Chemosphere 41 (2000) 1149-1154
4. Exner, J. H., Burk, G. A. and Kyriacou, D. (1973). Rates and Products of Decomposition of 2,2-Dibromo-3-nitrilopropionamide. J. Agri. Food Chem. 21(5), pp. 838-842.

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the

comments and views submitted

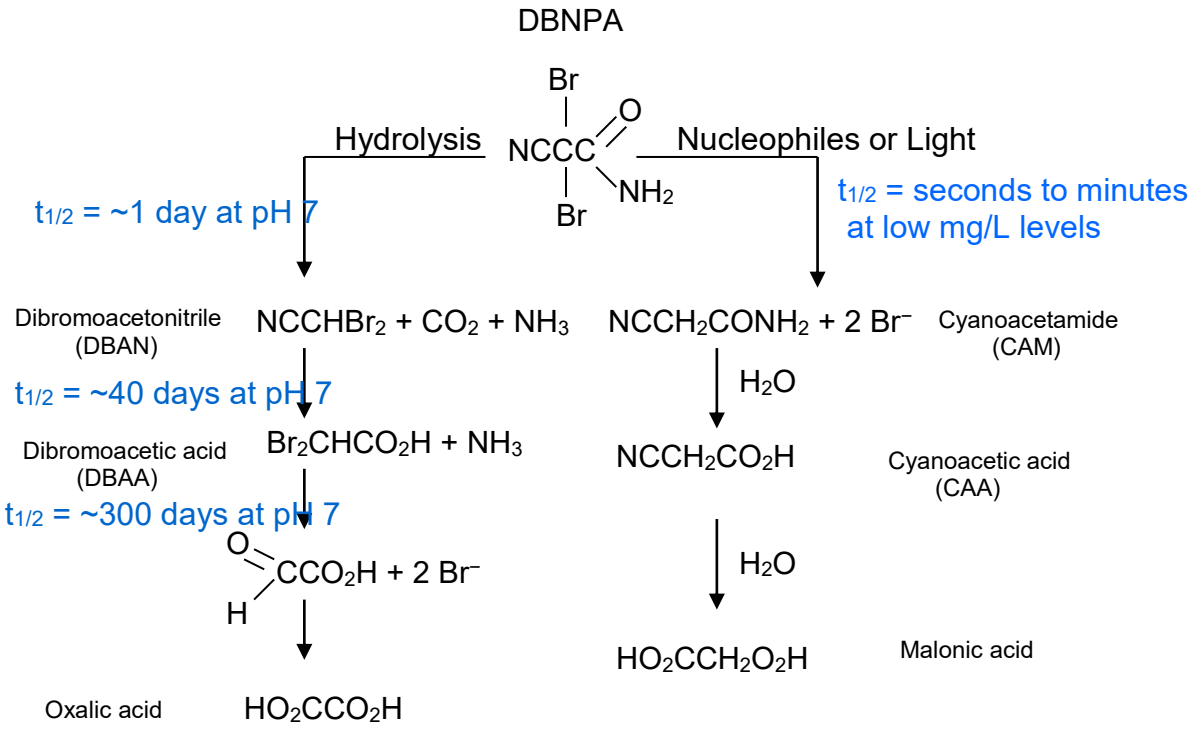
EVALUATION BY RAPPORTEUR MEMBER STATE

Date	28/05/13
Evaluation of applicant's justification	Applicant states that the analysis of DBAN in soil is technically not feasible. Different attempt to validate a method are presented along with a degradation pathway
Conclusion	Acceptable
Remarks	Acceptable

COMMENTS FROM OTHER MEMBER STATE *(specify)*

Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Appendix 1 – DBNPA Degradation Pathways (Reference 4)



Section A4.2a/03

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of Dibromoacetic acid (DBAA) in soil

			Official use only
		1 REFERENCES	
1.1	**Reference**	Barker, W. (2017), "Validation of a Method to Measure Trace Levels of Dibromoacetic Acid (DBAA) in Soils"; EN-CAS Analytical Laboratories, Winston-Salem, USA; EN-CAS Analytical Report No.: 16-0049 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dow Europe GmbH and Bromine Compounds Ltd.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data on existing active substance submitted for the first time for entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes. EC guidance document on generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414, SANCO 3029/99, rev. 4, 11/07/00, EC guidance document on pesticide residue analytical methods, SANCO/825/00 rev. 8.1, 16/11/2010.	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**	**Fortification of specimens for recovery experiments** 10 g of soil were fortified with 500 µL of either the 1.0 µg/mL or 10 µg/mL DBAA fortification solution to obtain fortification levels of 0.05 mg/kg (LOQ) or 0.50 mg/kg (10 x LOQ), respectively.	
3.1.1	Enrichment	**Extraction procedure** Weigh 10 grams of a homogenized soil weighed into a 250-mL polypropylene bottle. Fortify at the appropriate level (proposed LOQ = 0.05 mg/kg) and allow to stand for at least 15 minutes. Add 100 mL of 90:10 MeOH:1% aqueous formic acid, cap the bottle and place on a reciprocating shaker at 250 rpm for 30 minutes. Remove the specimen from the shaker and centrifuge a portion at 7000 rpm for 8 minutes. Decant the supernatant into a 16-oz French square	

Section A4.2a/03
Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of Dibromoacetic acid (DBAA) in soil

bottle.
Decant a 20-mL aliquot into a 125-mL Erlenmeyer flask. Concentrate the aliquot to near dryness (~2 mL) using a rotary evaporator with a bath temperature set at 35° C. Quantitatively transfer aliquot to a 15-mL graduated centrifuge tube and bring to 5 mL with D.I. H_2O. Condition a Strata X bond-elute with 10 mL of MeOH and 20 mL of H_2O. Add the specimen, bring to top of frit and discard solvent. Rinse the flask with 10 mL of 75:25 MeOH:H_2O and add to the column. Drain to the top of the frit. Collect the solvent in a new 15-mL graduated centrifuge tube. Bring sample volume to exactly 10 mL by adding 75:25 MeOH:H_2O as needed. Mix the sample with a Vortex mixer and transfer a portion to an auto inject vial for injection on the LC/MS/MS for analysis.

3.1.2 Cleanup — See section 3.1.1

3.2 Detection

3.2.1 Separation method

Liquid chromatography (LC) system
AB Sciex API 4000 LC system equipped with an Agilent 1100 Quat pump and autosampler, MS/MS option and Analyst 1.4.1 software. Column: Mac Mod Hydrobond AQ C8 with a 2.1 m length, 150 mm i.d., 5.0 µm particle size. Autosampler injection volume: 15 µL. Collision gas: Nitrogen at 12 units

Mobile Phase A: H_2O with 0.2% Formic acid, Mobile Phase B: MeOH

Time (min)		%A	%B	Flow Rate (mL/min)
0	85	15		0.20
9	0	100		0.20
14	0	100		0.20
14.1	85	15		0.20
19	85	15		0.20

3.2.2 Detector

MS/MS detection
MS/MS detection in the multi-reaction-monitoring mode (MRM). Apparatus: AB Sciex API 4000 mass spectrometer operated in MS/MS CAD (215 m/z to 79 m/z) with Turbo ion spray.
Data acquisition by Multi-Reaction Monitoring (MRM): Parent ion: 215 m/z. Product ion: 79 m/z, 171 m/z (confirmatory).
Retention time: ca. 4.2 min for DBAA.
Quantification: via the product ion.

3.2.3 Standard(s)

DBAA stock solution
A DBAA stock solution was prepared with the reference item in MTBE as exemplified in **Table A4.2.a/03-01**.

Section A4.2a/03

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of Dibromoacetic acid (DBAA) in soil

Table A4.2.a/03-01

Analyte	Purity [%]	Amount weighed [g]	Dilute to [mL]	Concentration of stock solution [µg/mL]
DBAA	99.7	0.10196	102	1000

DBAA fortification solutions
The following fortification solutions were prepared with DBAA in optima grade MeOH, as summarized in **Table A4.2.a/03-02**.

Table 4.2.a/03-02

Concentration of solution used [µg/mL]	Aliquot of solution [mL]	Dilute to [mL]	Obtain fortification solution with concentration of [µg/mL]
1000	1.0	100	10
10	10	100	1.0
10	1.0	100	0.1

DBAA calibration solutions
Calibration standards were prepared by adding the appropriate amount of standard to a 100-mL volumetric flask and diluting to volume with 75:25 MeoH:water (see **Table 4.2.a/03-03**). The peak areas of the DBAA were then used to establish a calibration curve.

Table 4.2.a/03-03

Section A4.2a/03
Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of Dibromoacetic acid (DBAA) in soil

Take solution with a concentration of [µg/mL]	Pipette aliquot of solution [mL]	75:25 MeOH:H_2O [mL]	Obtain calibration specimen with a concentration of DBAA [µg/mL]
10	5.0	100	0.50
10	2.5	100	0.25
10	1.0	100	0.10
10	0.50	100	0.05
1.0	2.5	100	0.025
1.0	1.0	100	0.01
1.0	0.50	100	0.005

Storage and stability of standards
All solutions were stored in a freezer when not in use. The reference item was stable throughout the study period as shown by comparison of chromatograms for analysis of standard solutions injected over the course of the study (4 weeks).

3.2.4 Interfering substance(s)
None reported. The analytical method used in this study is considered to be highly specific (see also 3.4).

3.3 Linearity

3.3.1 Calibration range
Calibrations were prepared by diluting DBAA into 75:25 MeOH:H_2O at levels ranging from 0.005 to 0.50 µg/mL DBAA, corresponding to concentrations of 0.02 to 1.5 µg/mL in 10-g soil aliquots.

3.3.2 Number of measurements
7

3.3.3 Linearity
Calculation of results was based on peak area intensity measurements. Using the Analyst software, a calibration curve and function was established as follows:
With every specimen set linear calibration functions were established by injecting calibration specimens interspersed with final specimen extracts (15-µL injection volume). Calibration functions were calculated by linear regression calculation. Regression coefficients "r^2" for all calibration curves were always > 0.99.
Representative calibration curves with calibration function are depicted in **Figure A4.2a/03-1** and **Figure A4.2a/03-2**. LC/MS/MS product ion chromatograms of calibration

Section A4.2a/03 — **Analytical Methods for Detection and Identification of Dibromoacetic acid (DBAA) in soil**

Annex Point IIA, IIA-IV.4.2

specimens are shown in **Figure A4.2a/03-3** to **Figure A4.2a/03-5**. LC/MS/MS product-ion chromatograms of control and fortified soil specimen extracts are presented in **Figure A4.2a/03-6** through **Figure A4.2a/03-8.**

3.4 Specificity: interfering substances

The analytical method utilized LC/MS/MS for the detection and quantitation of DBAA in the soil matrix. Monitoring of two transitions from the precursor ion to the product ion by LC/MS/MS is considered highly specific. The following product ions (m/z) were used for collection:

215 → 79 DBAA

215 → 171 DBAA (confirmatory)

The sample confirms when the DBAA area ratio of 215/79 to 215/171 in the samples is within one standard deviation of the ratio of the calibration standards. In this study, the ratio was 0.406 ± 0.03.

See **Figure A4.2a/03-9** for LC/MS/MS spectra.

3.5 Recovery rates at different levels

The validation of the method was conducted successfully for loamy sand with a total of two blank control specimens, five replicate specimens fortified at 0.05 mg/kg (LOQ), and five replicate specimens fortified at 10 x LOQ (0.50 mg/kg).

Method validation for the 0.05 and 0.50 mg/kg fortification levels met acceptance criteria with average recoveries of 99 and 89 % and relative standard deviations (RSD) of 8.3 and 15 %, respectively. The following recovery results (see **Table 4.2.a/03-04**) were obtained.

Table 4.2.a/03-04

Summary of Method Validation Results of DBAA in Loamy Sand					
	215/79		215/171		
Fortification Level (mg/kg)	Average Recovery	RSD	Average Recovery	RSD	n
0.05	99%	8.3%	98%	9.7%	5
0.50	89%	15%	89%	13%	5
Overall	94%	13%	93%	12%	10

Individual recovery data are summarised in **Table A4.2a/03-5**.

Average recoveries ranged between 70 and 110 % (see 4.2).

3.5.1 Relative standard deviation

The relative standard deviations (RSD) were ≤ 20 % (see 4.2).

3.6 Limit of determination

In a soil blank control specimen (see **Figure A4.2a/03-6)**, the background signal at the analyte retention would approximate a background of 0.015 mg/kg DBAA, or ca. 30 % of the LOQ. Based on the above background signals in soil the limit of detection (LOD) is estimated to be 0.015 mg/kg.

3.7 Precision

The average recoveries were in the range between 89-99 %

Section A4.2a/03 — Analytical Methods for Detection and Identification of Dibromoacetic acid (DBAA) in soil

Annex Point IIA, IIA-IV.4.2

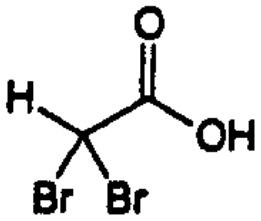
(see also 4.2).

3.7.1	Repeatability	The relative standard deviations were in the range between 8.3-15 % (see also 4.2).
3.7.2	Independent laboratory validation	No independent validation to be conducted.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

Test / reference item: Dibromoacetic acid (DBAA).



CAS No:	631-64-1
Empirical formula:	$C_2H_2Br_2O_2$
Molecular weight:	217.86 g/mol
Test substance lot no:	LC18661V
Purity:	99.7 %
Expires:	January 2019

Receipt, storage and disposal of samples
Soil sample was received at ambient temperature at EN-CAS on 22 Nov 16. Upon receipt, the sample wase assigned a unique identification number (E#) and were transferred to the main freezer for storage where it remained frozen until removed for homogenization and subsampling. Freezer storage temperatures were monitored on a daily basis and were at approximately -10 °C.

Analytical method – apparatus
Analytical balance used for analytical standards: Mettler AT201
Top loader balance: Fisher Scientific XL-3000
Ultrasonic bath: Branson 5200
Centrifuge: Sorvall RC-5B refrigerated Superspeed Centrifuge
Rotary evaporators: Rotavapor Labconco
Vacuum apparatus: Visiprep 24 Supelco
SPE tubes: Strata X Polymeric, 500 mg/6 mL (Phenomenex, Cat. # 8B-S100-HCH)
Centrifuge Tubes: 15 mL, calibrated for 10 – 20 mL (Pyrex, Cat. # 8082)
Typical glassware and laboratory equipment.

Analytical method – reagents and chemicals
Solvents:
Water, HPLC grade (Pharmco-Aaper, Cat. # 23200))
Methanol, Optima grade (Sigma Aldrich, Cat. # 34860)

Section A4.2a/03

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of Dibromoacetic acid (DBAA) in soil

Chemicals and reagants:
Aqueous formic acid, 95-97% (Sigma Aldrich, Cat. # 33015).

Soil characterization
See Appendix 1.

4.2 Conclusion

The analytical method described in this study was successfully validated for the determination of DBAA in soil with a **limit of quantification (LOQ) of 0.05 mg/kg**. The limit of detection (LOD) was estimated to be 0.02 mg/kg. The analytical method using LC/MS/MS for detection of the target analyte is regarded as highly specific and thus no additional confirmatory method needs to be demonstrated.

Summary of Method Validation Results of DBAA in Loamy Sand

Fortification Level (mg/kg)	215/79 Average Recovery	215/79 RSD	215/171 Average Recovery	215/171 RSD	n
0.05	99%	8.3%	98%	9.7%	5
0.50	89%	15%	89%	13%	5
Overall	94%	13%	93%	12%	10

4.2.1 Reliability: 1

4.2.2 Deficiencies: None.

Section A4.2a/03

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of Dibromoacetic acid (DBAA) in soil

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	24. April 2017
Materials and methods	Applicant's version acceptable.
Conclusion	Applicant's version acceptable. The second transition (215/171) is accepted as a confirmatory transition and no further method confirmation is needed.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Figure A4.2a/03-1: Representative LC/MS/MS calibration curve from the analysis of Loamy Sand Soil

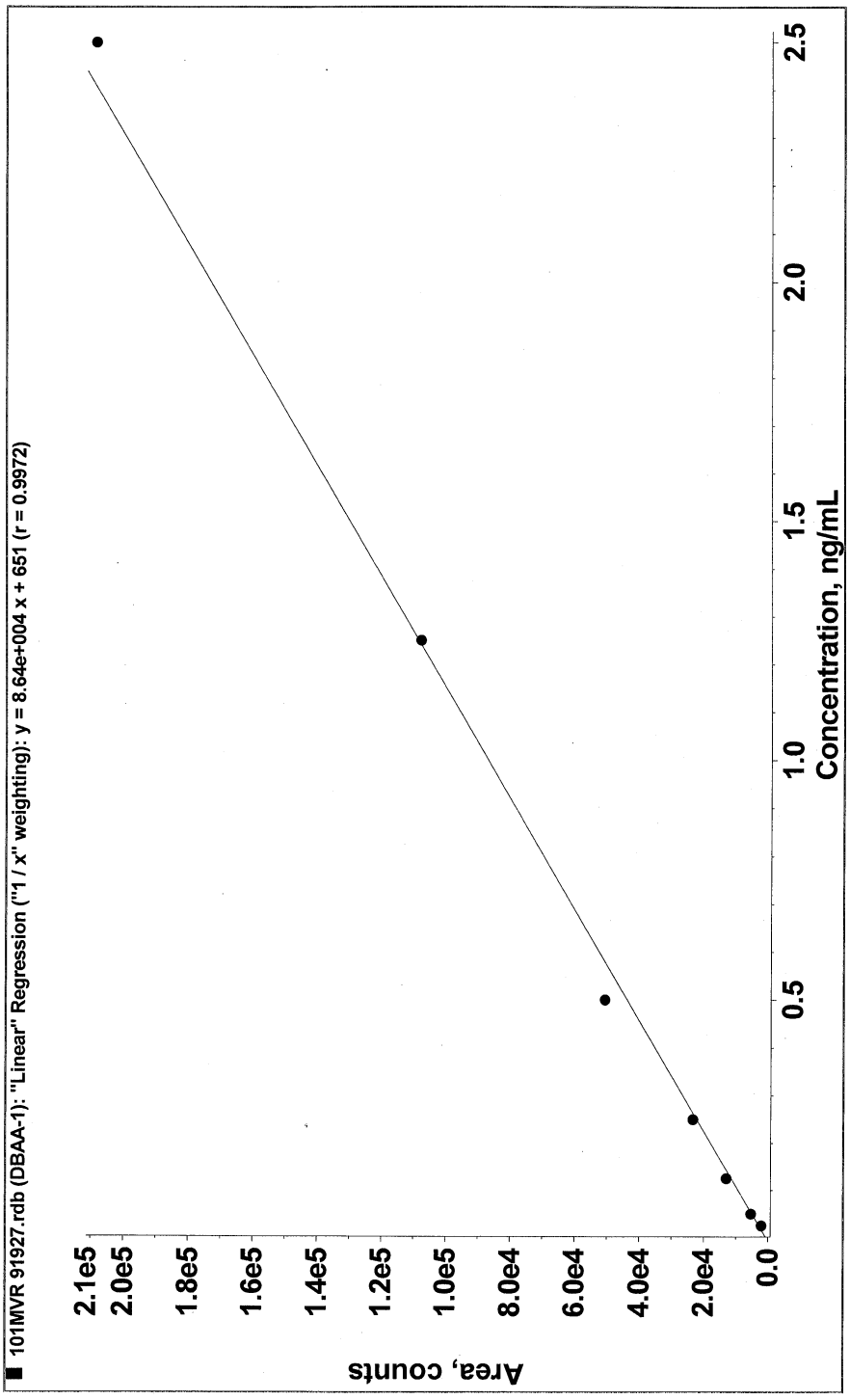


Figure A4.2a/03-2: Representative LC/MS/MS calibration curve from the analysis of Loamy Sand Soil

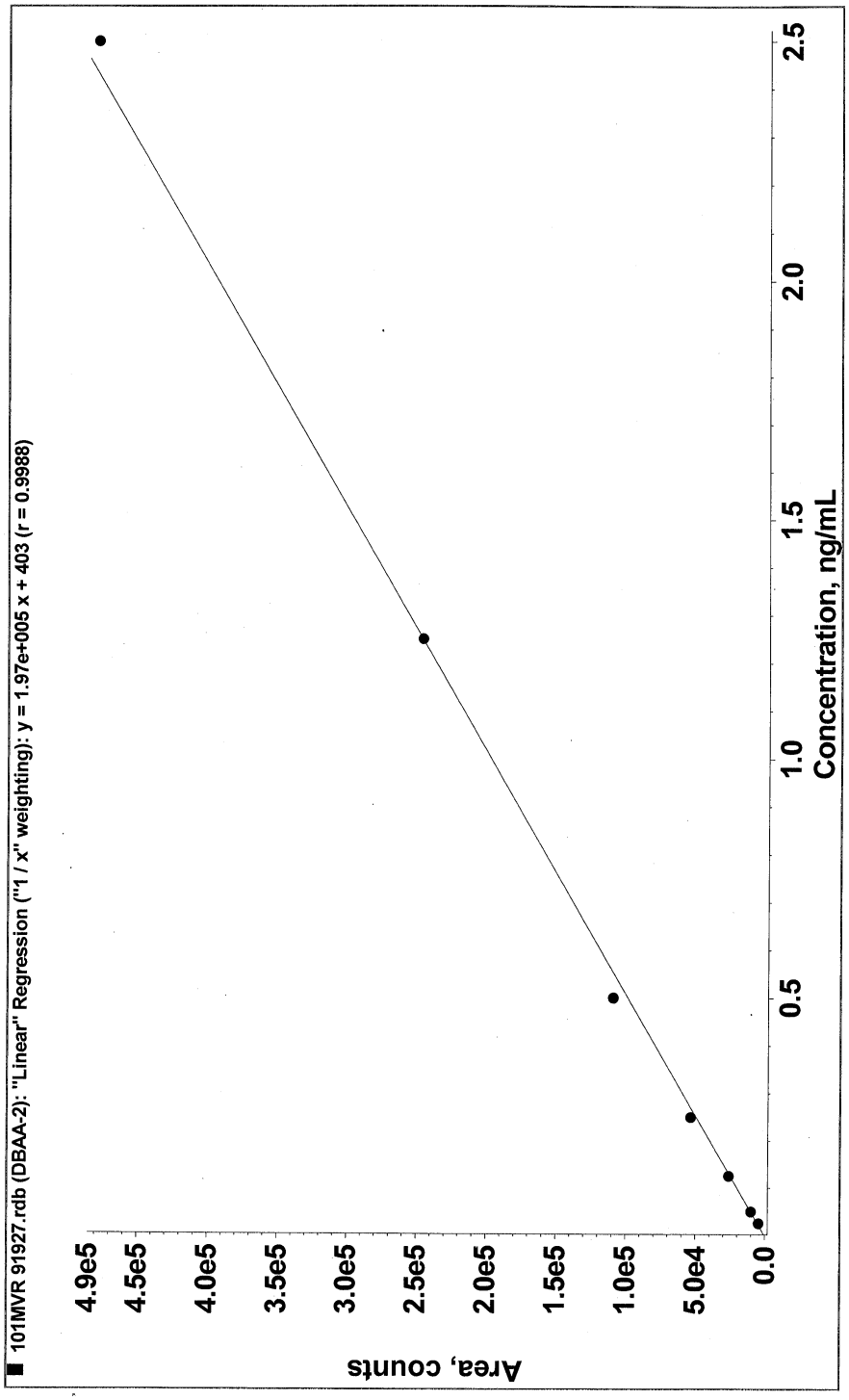


Figure A4.2a/03-3: Typical LC/MS/MS chromatogram: calibration standard corresponding to 0.005 µg/mL DBAA

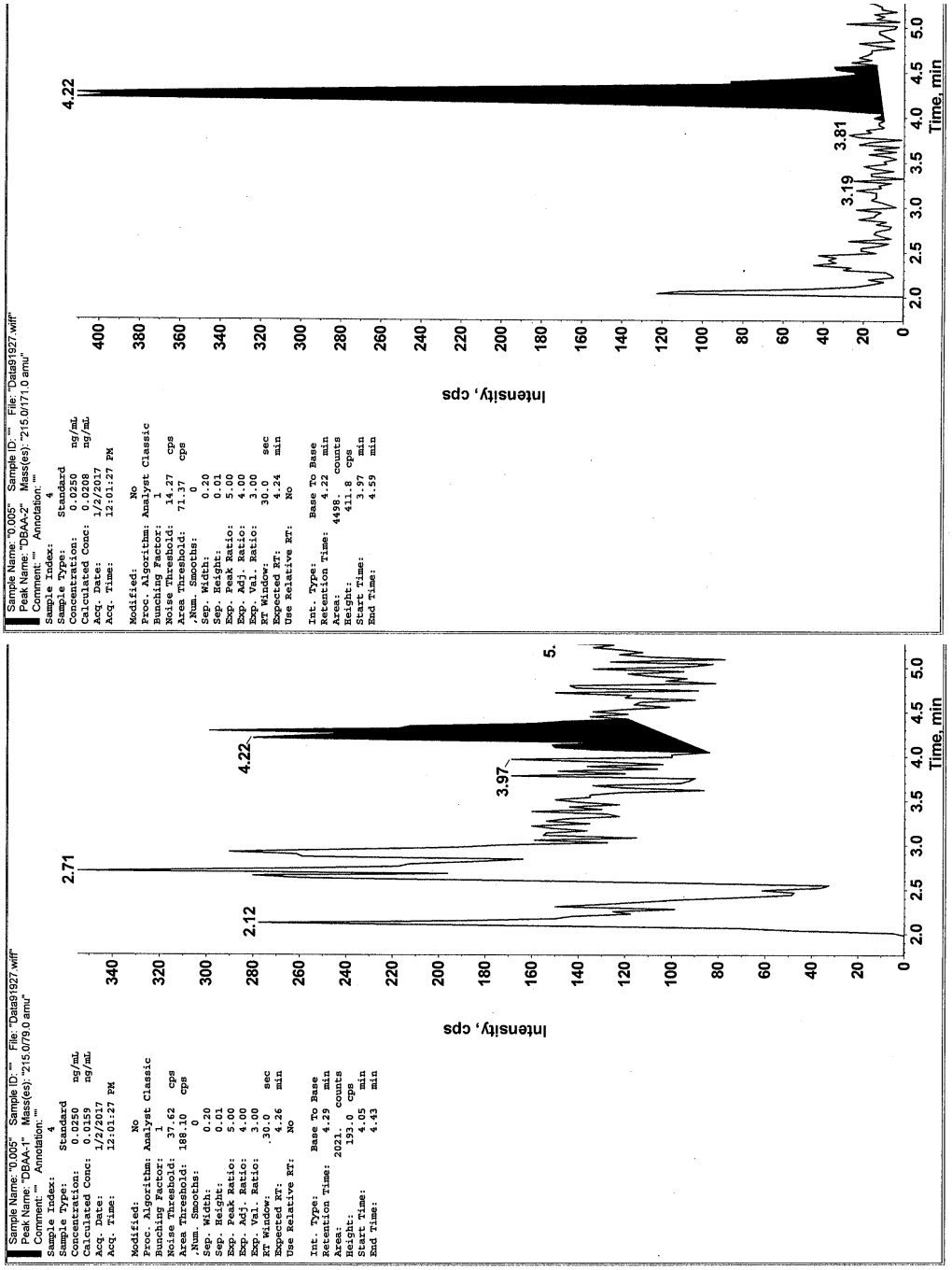


Figure A4.2a/03-4: Typical LC/MS/MS chromatogram: calibration standard corresponding to 0.05 µg/mL DBAA

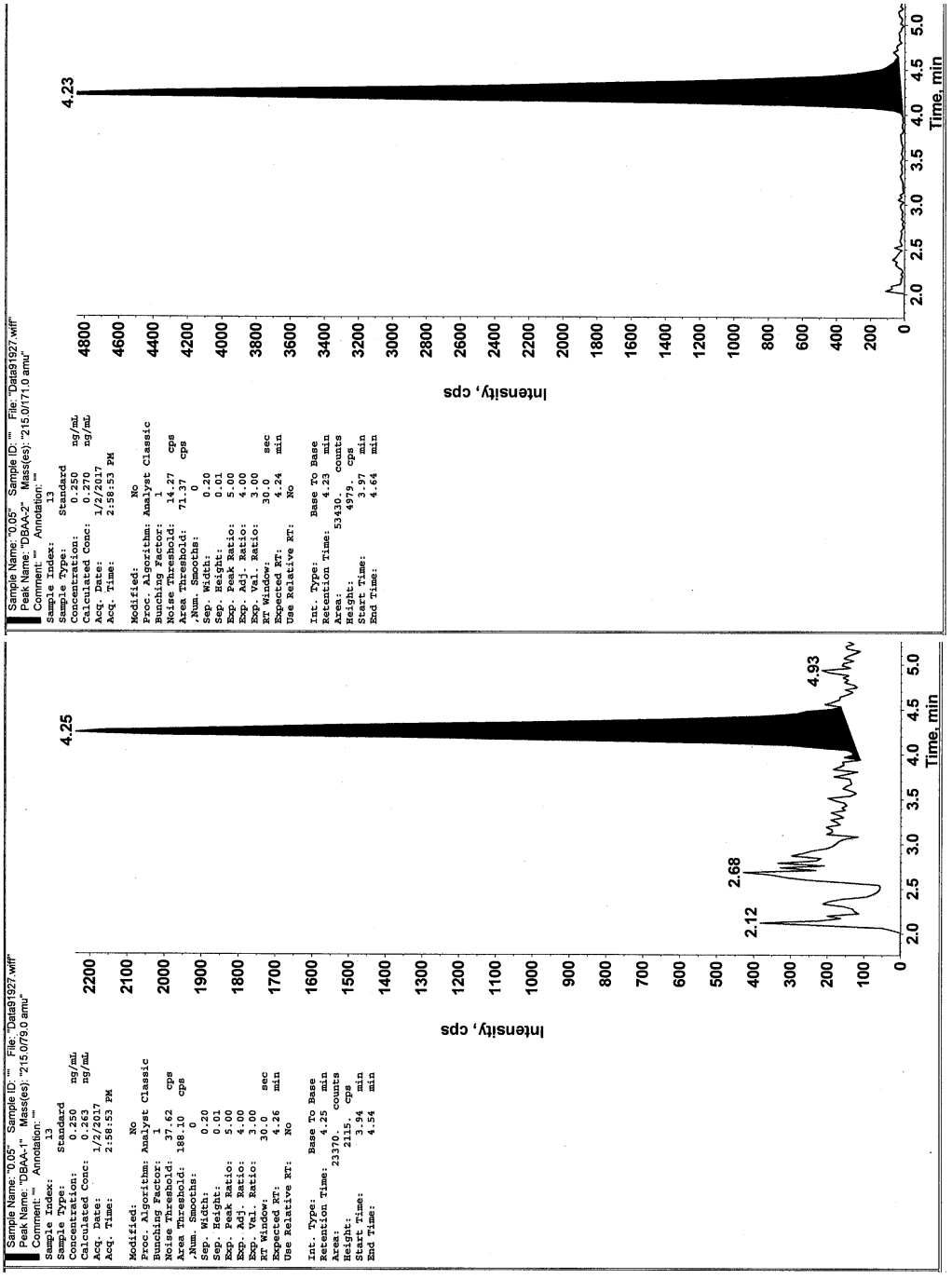


Figure A4.2a/03-5: Typical LC/MS/MS chromatogram: calibration standard corresponding to 0.50 µg/mL DBAA

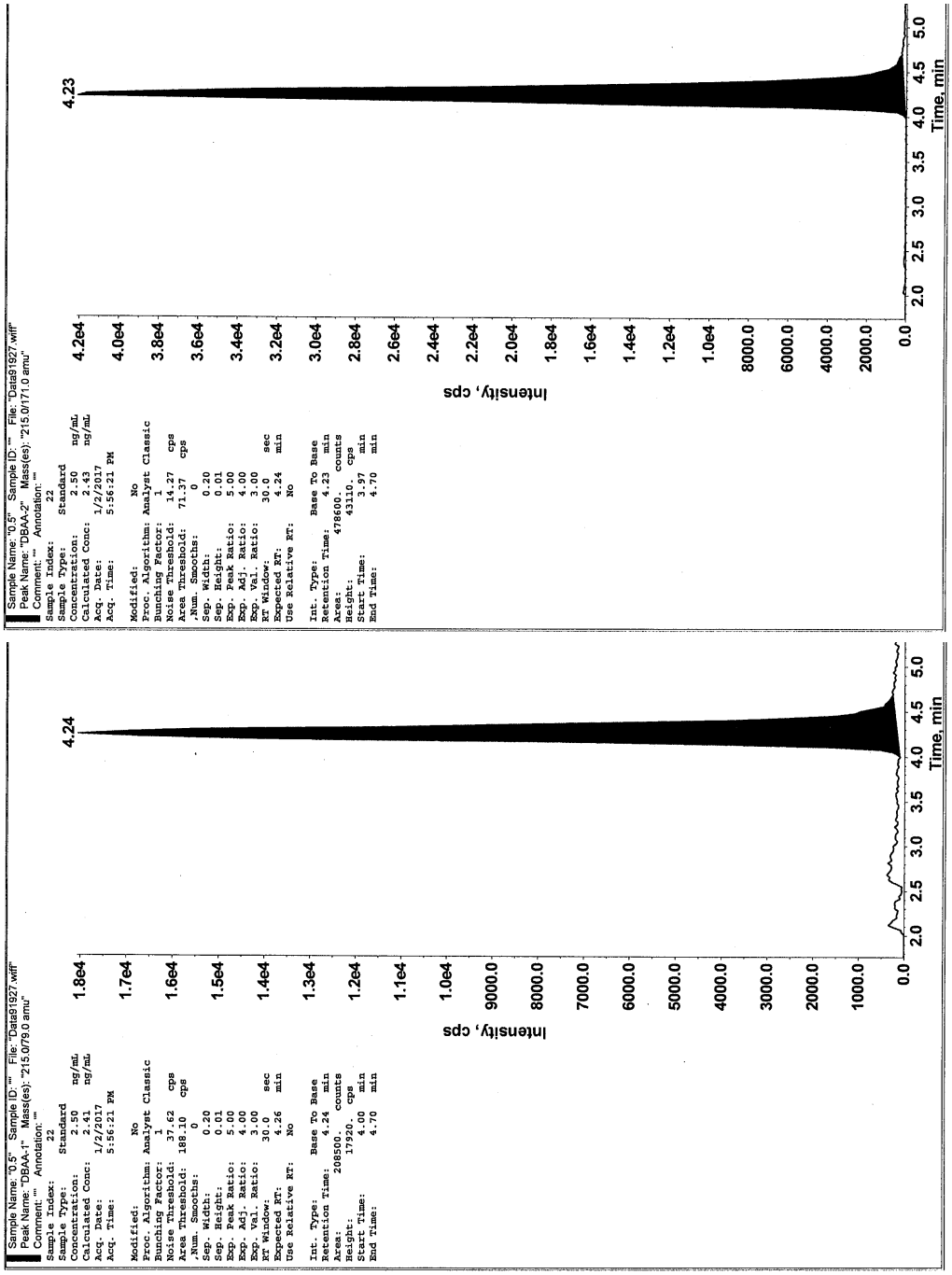


Figure A4.2a/03-6: Typical LC/MS/MS chromatogram: loamy sand soil control

Client Specimen ID: Agvise # 1460 EN-CAS Sample ID: EU11971-C1
DBAA found: <0.05 mg/kg (0.000 mg/kg)

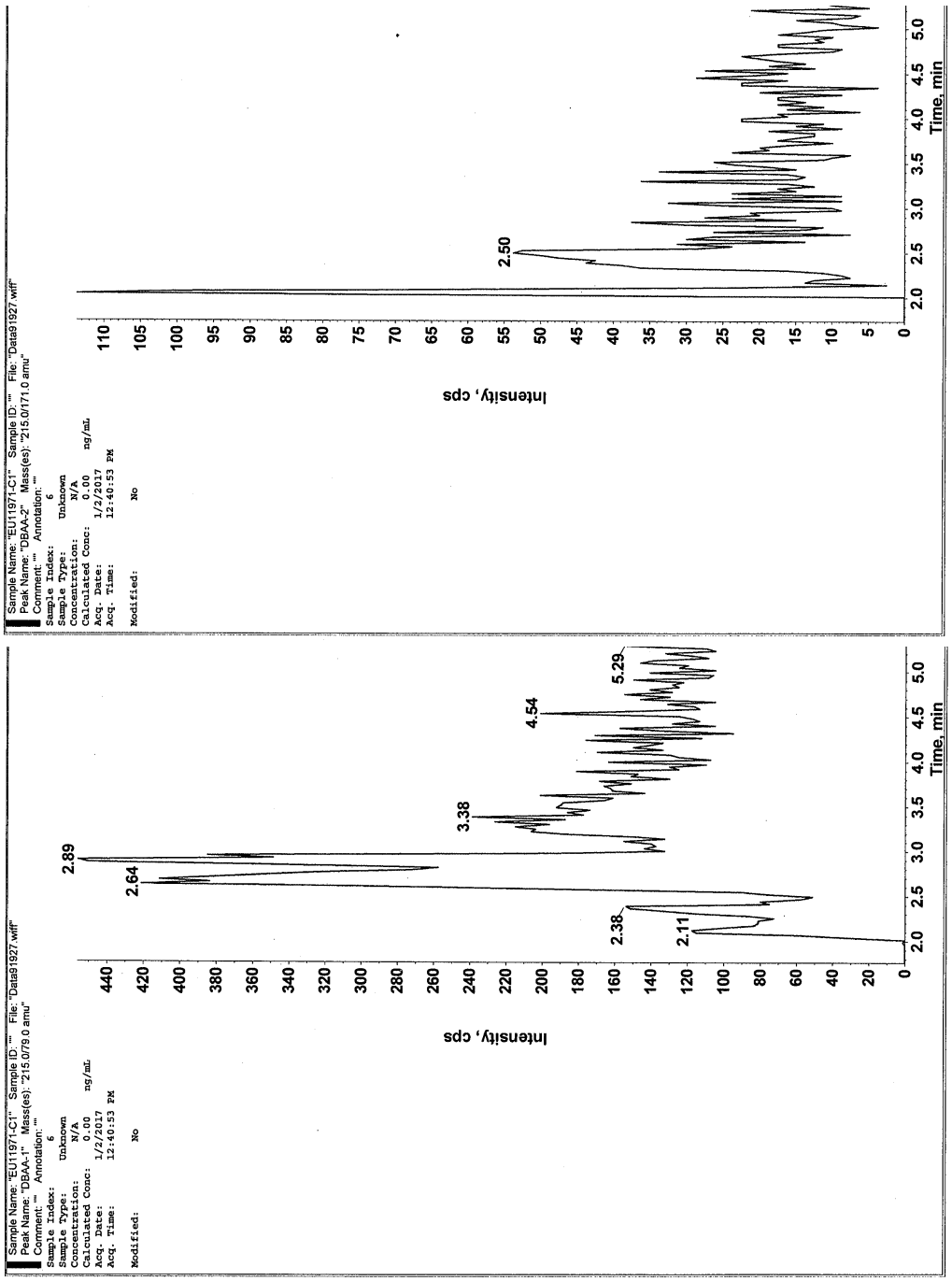


Figure A4.2a/03-7: Typical LC/MS/MS chromatogram: loamy sand soil control method validation fortified at LOQ (0.05 mg/kg DBAA)

Client Specimen ID: Agvise # 1460 EN-CAS Sample ID: EU11971-S4
DBAA found: 0.052/0.052 mg/kg Recovery: 104 %/104%

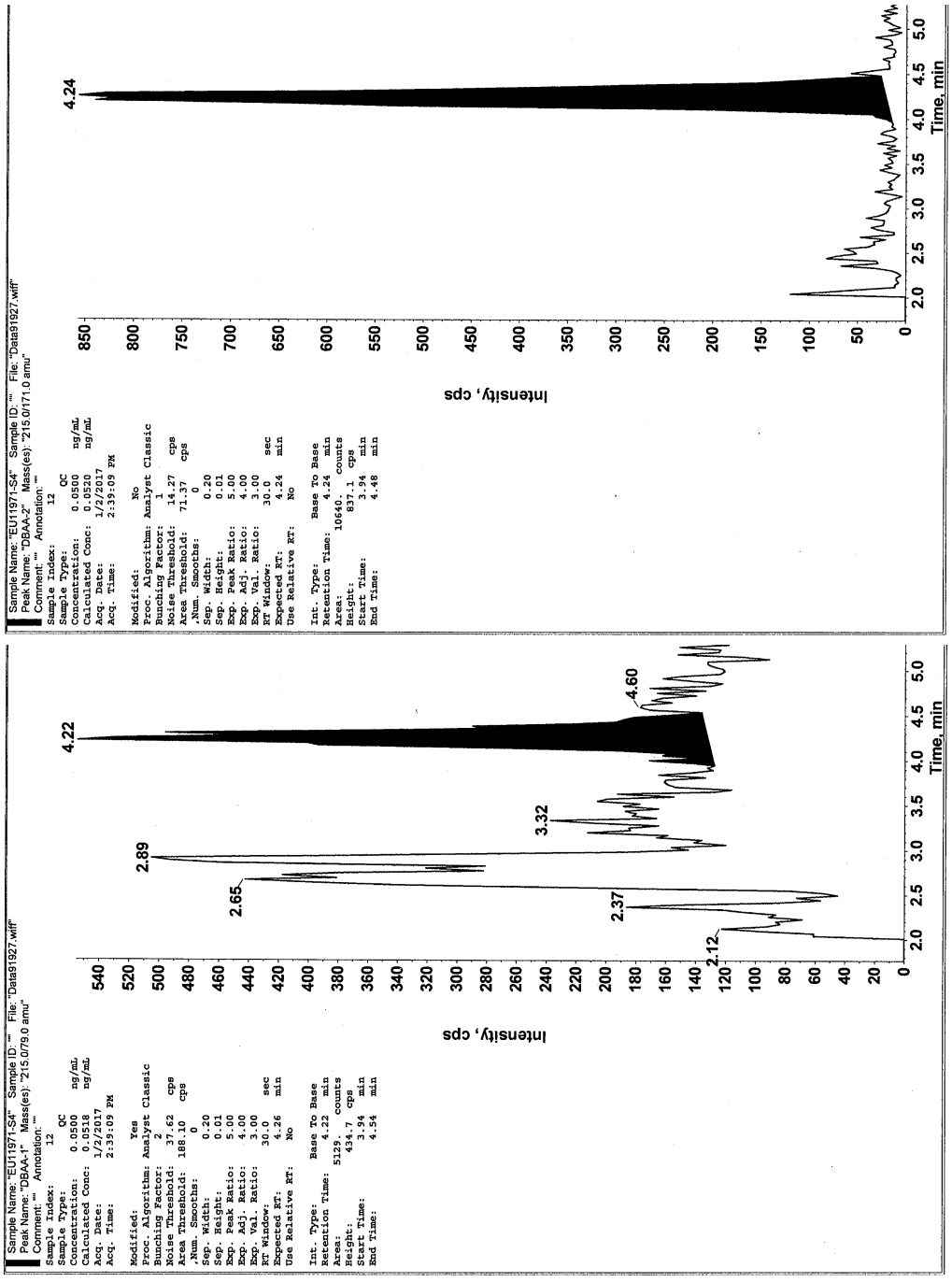


Figure A4.2a/03-8: Typical LC/MS/MS chromatogram: loamy sand soil method validation fortified at 10 x LOQ (0.50 mg/kg DBAA)

Client Specimen ID: Agvise # 1460 EN-CAS Sample ID: EU11971-S8
DBAA found: 0.433/0.451 mg/kg Recovery: 87 %/90 %

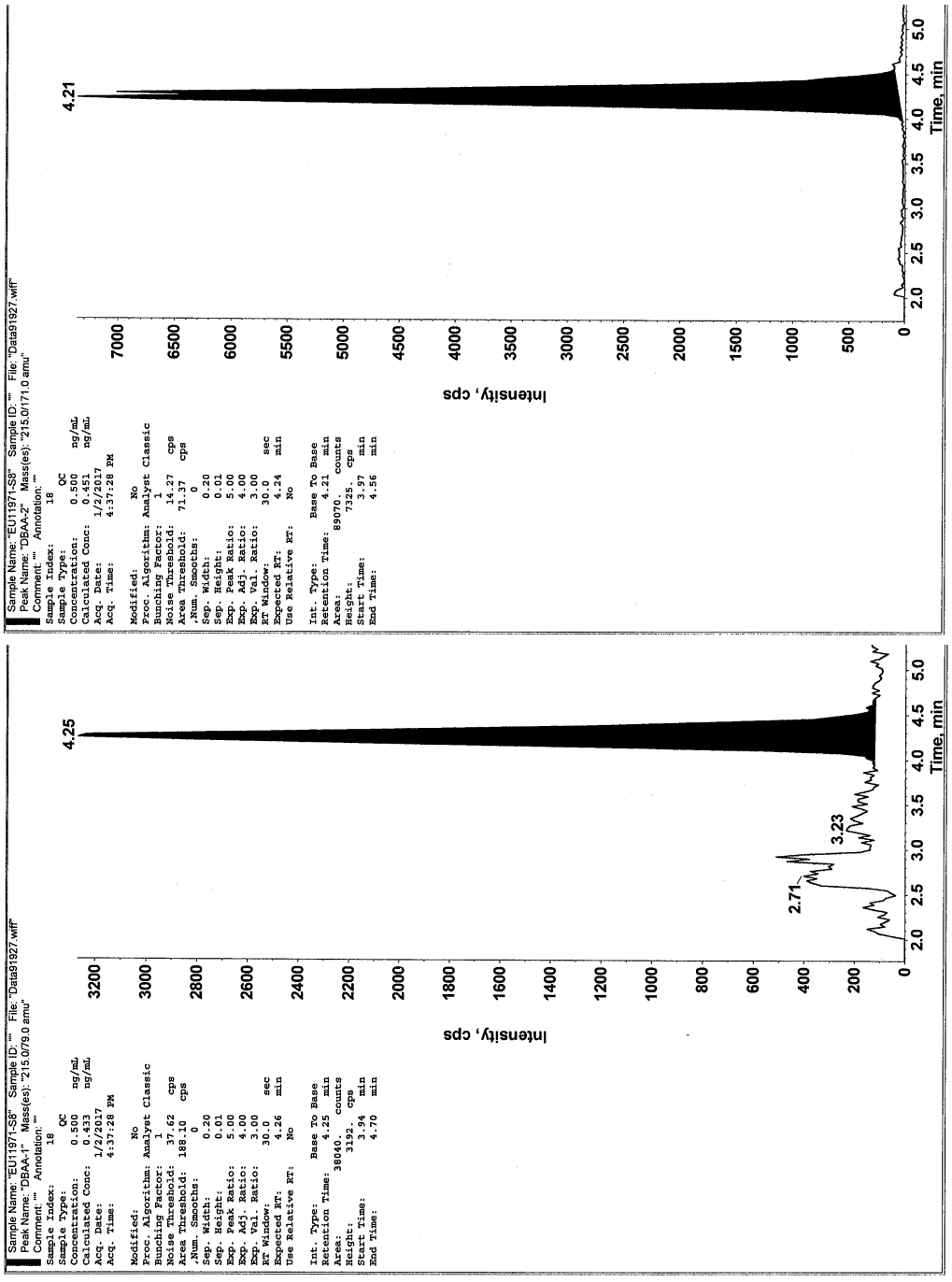
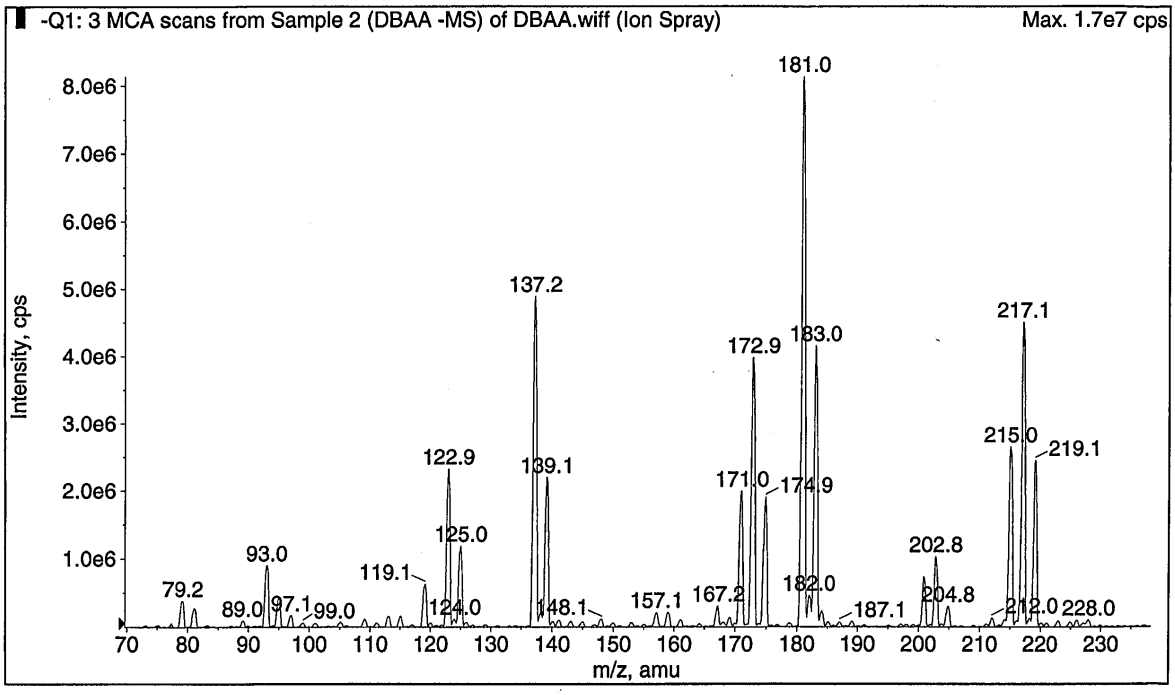


Figure A4.2a/03-9: LC/MS/MS spectra for DBAA



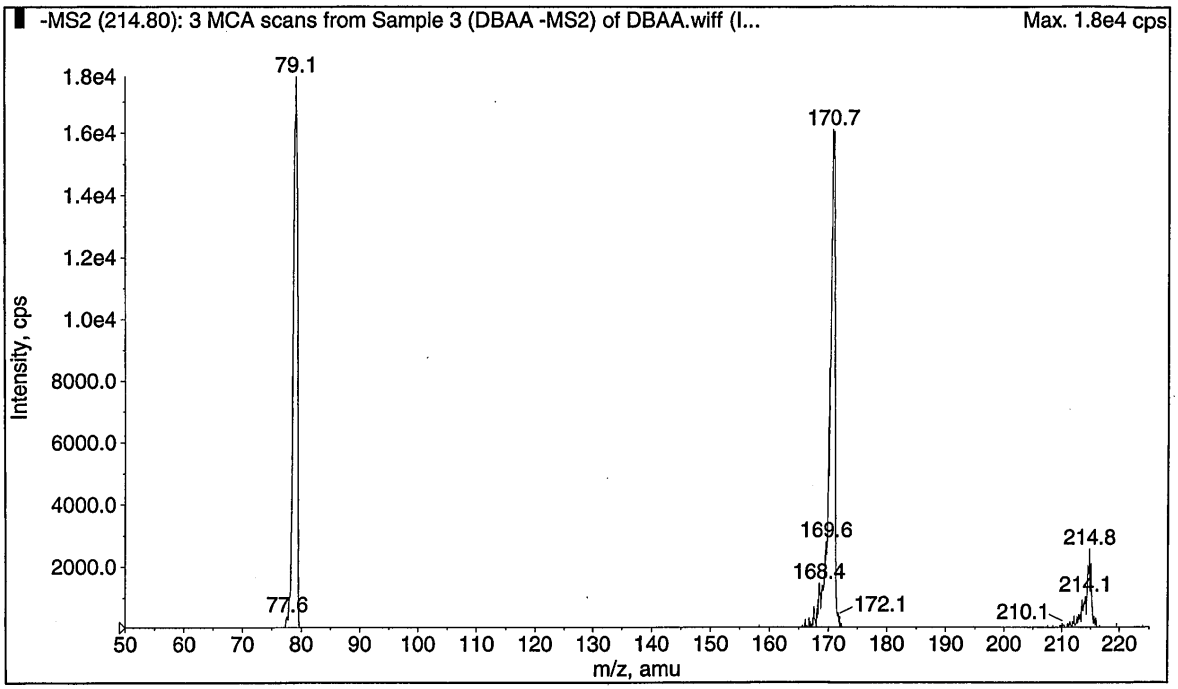


Table A4.2a/03-5: Evaluation of recovery results

Method Validation Recoveries for DBAA in Loamy Sand (MRM 215/79)

Client Sample ID	EN-CAS Sample ID	Set Number	Date Extracted	Date Analyzed	Fortification Level (mg/kg)	Residue (mg/kg)	% Recovery
Agvise # 1460	EU11971-C1	1-01-MVR	30-Dec-16	02-Jan-17	-	0.000	-
Agvise # 1460	EU11971-C2	1-01-MVR	30-Dec-16	02-Jan-17	-	0.000	-

Method Validation Recoveries for DBAA in Loamy Sand (MRM 215/171)

Agvise # 1460	EU11971-C1	1-01-MVR	30-Dec-16	02-Jan-17	-	0.000	-
Agvise # 1460	EU11971-C2	1-01-MVR	30-Dec-16	02-Jan-17	-	0.000	-

Fortifications at the Limit of Quantification LOQ (MRM 215/79)

Agvise # 1460	EU11971-S1	1-01-MVR	30-Dec-16	02-Jan-17	0.05	0.043	87
Agvise # 1460	EU11971-S2	1-01-MVR	30-Dec-16	02-Jan-17	0.05	0.048	97
Agvise # 1460	EU11971-S3	1-01-MVR	30-Dec-16	02-Jan-17	0.05	0.055	109
Agvise # 1460	EU11971-S4	1-01-MVR	30-Dec-16	02-Jan-17	0.05	0.052	104
Agvise # 1460	EU11971-S5	1-01-MVR	30-Dec-16	02-Jan-17	0.05	0.049	99

0.05 mg/kg Average % Recovery:	99
0.05 mg/kg Standard Deviation (n=5):	8.3
0.05 mg/kg % RSD:	8.3

Fortifications at the Limit of Quantification LOQ (MRM 215/171)

Agvise # 1460	EU11971-S1	1-01-MVR	30-Dec-16	02-Jan-17	0.05	0.045	89
Agvise # 1460	EU11971-S2	1-01-MVR	30-Dec-16	02-Jan-17	0.05	0.055	110
Agvise # 1460	EU11971-S3	1-01-MVR	30-Dec-16	02-Jan-17	0.05	0.044	88
Agvise # 1460	EU11971-S4	1-01-MVR	30-Dec-16	02-Jan-17	0.05	0.052	104
Agvise # 1460	EU11971-S5	1-01-MVR	30-Dec-16	02-Jan-17	0.05	0.049	98

0.05 mg/kg Average % Recovery:	98
0.05 mg/kg Standard Deviation (n=5):	9.5
0.05 mg/kg % RSD:	9.7

10XLOQ Fortifications (MRM 215/79)

Agvise # 1460	EU11971-S6	1-01-MVR	30-Dec-16	02-Jan-17	0.50	0.376	75
Agvise # 1460	EU11971-S7	1-01-MVR	30-Dec-16	02-Jan-17	0.50	0.518	104
Agvise # 1460	EU11971-S8	1-01-MVR	30-Dec-16	02-Jan-17	0.50	0.433	87
Agvise # 1460	EU11971-S9	1-01-MVR	30-Dec-16	02-Jan-17	0.50	0.387	77
Agvise # 1460	EU11971-S10	1-01-MVR	30-Dec-16	02-Jan-17	0.50	0.507	101

0.50 mg/kg Average % Recovery:	89
0.50 mg/kg Standard Deviation (n=5):	13
0.50 mg/kg % RSD:	15

Table A4.2a/03-5: Evaluation of Recovery Results (Continued)

Client Sample ID	EN-CAS Sample ID	Set Number	Date Extracted	Date Analyzed	Fortification Level (mg/kg)	Residue (mg/kg)	% Recovery
5XLOQ Fortifications (MRM 215/171)							
Agvise # 1460	EU11971-S6	1-01-MVR	30-Dec-16	02-Jan-17	0.50	0.392	78
Agvise # 1460	EU11971-S7	1-01-MVR	30-Dec-16	02-Jan-17	0.50	0.503	101
Agvise # 1460	EU11971-S8	1-01-MVR	30-Dec-16	02-Jan-17	0.50	0.451	90
Agvise # 1460	EU11971-S9	1-01-MVR	30-Dec-16	02-Jan-17	0.50	0.386	77
Agvise # 1460	EU11971-S10	1-01-MVR	30-Dec-16	02-Jan-17	0.50	0.495	99

0.50 ppb Average % Recovery:	89
0.50 ppb Standard Deviation (n=5):	11
0.50 ppb % RSD:	13
Overall Average % Recovery:	**94**
Overall Standard Deviation (n=15):	**11**
Overall % RSD:	**12**

APPENDIX 1 SOIL CHARACTERIZATION

AGVISE # 1460 Loamy Sand		
Percent Sand	80	
Percent Silt	10	
Percent Clay	10	
Bulk Density (disturbed) gm/cc	1.12	
Cation Exchange Capacity (meq/100 g)	9.9	
% Moisture at 1/3 Bar	19.7	
% Organic Carbon –Walkley Black	0.94	
% Organic Matter –Walkley Black	1.6	
pH in 1:1 soil:water ratio	5.3	
Base Saturation Data	Percent	ppm
Calcium	47.9	951
Magnesium	11.8	140
Sodium	0.4	8
Potassium	6.9	267
Hydrogen	33.1	33

Section A4.2b/01a

Annex Point IIA4.2

Analytical Methods for Detection and Identification of DBNPA in air

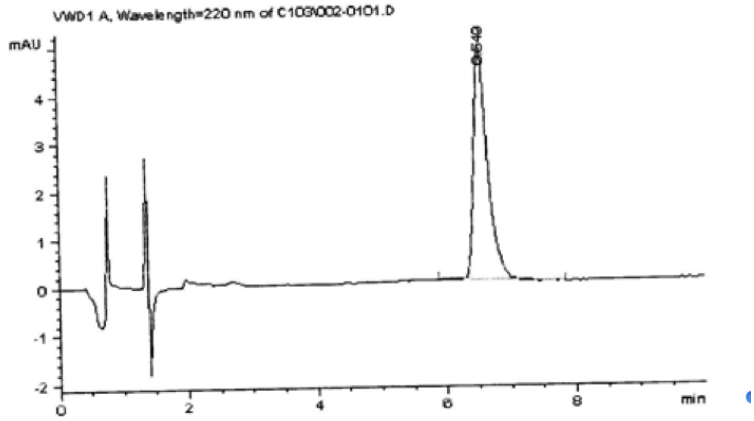
			Official use only
		1 REFERENCE	
1.1	**Reference**	Anonymous: Determination of Dibromo Nitrilopropionamide ([REDACTED]) in air. DSBG Analytical Laboratory Research and Development Branch. Doc. No. 436-004 (unpublished); C_A4.2b/03a. M.D. Kallos. Determination of [REDACTED] in Air. Supporting Data for Method 101-165-402. February 2016. ICL-IP (unpublished). M.D. Kallos. Determination of [REDACTED] in Air. Supporting Data for Method 101-165-402 (Revision #2). 21 March 2017. ICL-IP (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bromine Compounds Ltd.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for Annex I entry.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	No	
2.3	**Deviations**	Not applicable: no guideline study	
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**	--	
3.1.1	Enrichment	The method is based on the collection of airborne [REDACTED] particles using a Mixed Cellulose Ester filter from SKC. The method was tested in the range between 2 and 2000 µg on-filter. An air sample of 1000L is sufficient to ensure a deposition on-filter of enough analyte mass at detectable range. Sampling flow rate: 1 L/min Sampling time: 16.7 h minimum.	
3.1.2	Cleanup	No purification or enrichment necessary.	
3.2	**Detection**	--	
3.2.1	Separation method	HPLC Column: Hypersil C-18 5µ, 100 x 4.6 mm, or equivalent Operational column temperature: Room temperature Detector Wavelength: 220 nm. Injector volume: 10 µl	

Section A4.2b/01a

Annex Point IIA4.2

Analytical Methods for Detection and Identification of DBNPA in air

		Solvent composition: 10% Acetonitrile HPLC 90% Water HPLC Solvent flow rate: 1 ml/min
3.2.2	Detector	UV detection at 220 nm
3.2.3	Standard(s)	External Retention time: [REDACTED] 6.5 mins



Typical chromatogram ¶

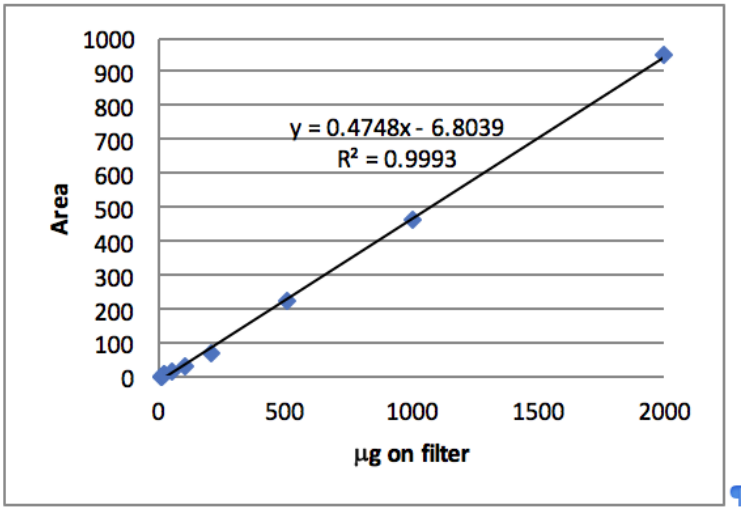
3.2.4	Interfering substance(s)	None
3.3	**Linearity**	--
3.3.1	Calibration range	A one point calibration is performed with a standard solution at a concentration of 0.005 mg DBNPA / mL. The correlation coefficient (r^2) obtained was >0.995
3.3.2	Number of measurements	One per concentration.
3.3.3	Linearity	Linearity was tested in the range between 250 ng/ml and 200 µg/ml. Each concentration was prepared by spiking unused filters with defined volumes of an aqueous solution of [REDACTED] standard at an appropriate concentration, allowed to dry, and extracted and analyzed using the procedure indicated for the samples.

Section A4.2b/01a

Annex Point IIA4.2

Analytical Methods for Detection and Identification of DBNPA in air

Spike (µg)	Area
2.5	0.826216
5	1.22975
7	1.89975
10	2.73755
15	4.35183
20	6.63919
50	17.03181
100	34.75219
200	70.83441
500	221.3642
1000	463.8027
2000	949.0458



3.4 Specifity: interfering substances

The analysis of blank sample performed by an unused filter immersed to deionized water, which did not result in any interfering substances.

3.5 Recovery rates at different levels

Due to concerns regarding the batch-to-batch reproducibility of the filters used, as well as the overall performance of any laboratory performing the tests, the work instruction describes a procedure to test the recovery on a per analysis basis, to avoid using an historical recovery coefficient which may not be appropriate. It is also a means to evaluate whether systematic errors occurred during the extraction procedure.

During development, the recovery was tested by spiking unused filters with defined volumes of an aqueous solution of [REDACTED] standard

Section A4.2b/01a

Annex Point IIA4.2

Analytical Methods for Detection and Identification of DBNPA in air

at an appropriate concentration, allowed to dry, and extracted and analyzed using the procedure indicated for the samples.

µg on filter	µg found	% Recovery calculated
5.2	4.2	80.7
10.5	7.3	69.5
21.0	17.2	81.9
50.4	50.2	99.6
100.9	85.8	85.0
201.8	170.5	84.5
500.9	487.0	97.2
1001.8	920.2	91.9
2003.5	1967.4	98.2
	Average	87.6

3.5.1 Relative standard deviation

Reproducibility: 2.48%

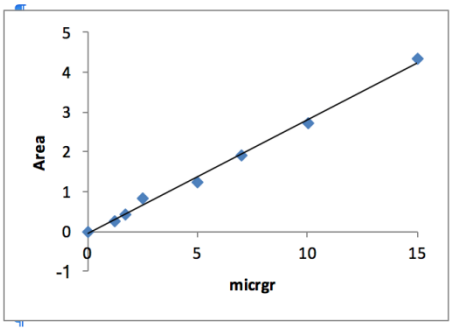
Section A4.2b/01a

Annex Point IIA4.2

Analytical Methods for Detection and Identification of DBNPA in air

3.6 Limit of determination

Calculations performed using values obtained result in a LOD of 1.2 µg on filter.



Regression Statistics	
Multiple R	0.997601
R Squared	0.995207
Adjusted R Squared	0.994408
Standard Error	0.110657
Slope	0.287272
Observations	8
LOD	1.155599

Calculations performed using the values actually obtained result in a LOQ of 3.9 µg on filter.

LOQ = (10* Standard Error of Y)/Slope

The suggested sampling volume of air is 1000 l (1 m3). Using these values, the LOQ is calculated as 0.004 mg/m3.

According to ECHA guidance LOQ must be equal or lower than the critical concentration C, defined as:

C = AEL x 0.1 x 60/20 = 0.0042 mg/m3

where: AEL that should be used for the calculation is 0.014 mg/kg bw/day

0.1= Safety factor

60 = Average normal body weight (kg)

20 = Daily air intake volume (m^3)

.

The calculated LOQ value of 0.004 mg/m3 is acceptable for a critical concentration C value of 0.0042 mg/m3

3.7 Precision

--

3.7.1 Repeatability

Reproducibility was tested by spiking six separate filters with 50 µl of a standard solution containing 1 µg/µl to obtain a deposition of 50 µg on filter. The dry spiked filters were subsequently treated like true samples, and analyzed in the HPLC under the specified conditions.

Section A4.2b/01a

Annex Point IIA4.2

Analytical Methods for Detection and Identification of DBNPA in air

Filter #	Area
1	16.24460
2	17.42432
3	17.34405
4	17.03181
5	16.95188
6	17.13598

RSD = 2.48 %

3.7.2	Independent laboratory validation	Not required

Section A4.2b/01a

Annex Point IIA4.2

Analytical Methods for Detection and Identification of DBNPA in air

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

The method is based on the collection of airborne [REDACTED] particles using a Mixed Cellulose Ester filter from SKC. This filter is submitted to the analytical laboratory for subsequent extraction and analysis by high performance liquid chromatography (HPLC).

Reagents:

- Acetonitrile HPLC
- Water HPLC.
- Deionised water at pH 3 (with HCl).
-

Standards:

All the solutions are prepared using deionsised water at pH 3.

- 50 mg of DBNPA are accurately weighed into a 50 ml volumetric bottle. Water is added to the mark and the solution is mixed to obtain a solution at 1 mg DBNPA / mL.
- 5 ml of the 1 mg DBNPA / mL solution are pipetted into a 100 ml volumetric bottle. Water is added to the mark and the solution is mixed well to obtain a solution at 0.05 mg DBNPA / mL.
- 1 ml of the 0.05 mg DBNPA / mL solution is pipetted into a 10 mL volumetric flask which is made up to the mark and mixed well to obatin a solution at a concnetration of 0.005 mg DBNPA / mL.

Preparation of samples for recovery:

All the solutions are prepared using Deionized water at pH 3.

Using a suitable syringe or fine pipette, spike 2 unused filters with 50 µl standard solution prepared in 3.4.2.

Using a suitable syringe or fine pipette, spike 2 unused filters with 100 µl standard solution prepared in 3.4.2.

Allow the filters to dry.

Insert each spiked filter into a 10 ml scintillation vial and add 10 ml deionized water pH 3. Make sure the whole filter is immersed in water. Mix sporadically during at least 2 hrs.

Sample preparation:

Insert each sample filter into a 10 ml scintillation vial. Add 10 ml deionized water pH 3. Make sure the whole filter is immersed in water.

Section A4.2b/01a — Analytical Methods for Detection and Identification of DBNPA in air

Annex Point IIA4.2

Mix sporadically during at least 2 hrs.

Analysis:

10 µL of the 0.005 mg DBNPA / mL standard solution are injected until reproducible results are obtained.

10 µL of the recovery samples are injected.

10 µL of the sample solutions are injected.

For analysis of a series of samples it is necessary to verify that the HPLC is still within calibration by re-injecting the standard.

Retention time DBNPA: 6.5 min

Calculation:

The absolute mass of material obtained for the recovery test filters is calculated as:

$$\frac{A_{sm} * W_{st} * P_{st} * 0.01}{A_{st}} = \mu g$$

where: A: area
W: weight
P: purity, expressed as percent
sm and *st* are sample and standard, respectively

The average recovery is calculated as

$$H = \frac{\sum \frac{C_i}{T_i}}{n}$$

where: H: recovery factor
C*i*: amount of DBNPA found in the recovery filter
T*i*: theoretical amount of DBNPA found in the recovery filter, which is calculated as $\frac{Wst * Pst * \mu L}{5000}$

The amount of DBNPA collected in the sample filters is calculated as

$$\frac{A_{sm} * W_{st} * P_{st} * 0.01}{A_{st} * H} = \mu g$$

where: A: area
W: weight
P: purity, expressed as percent
sm and *st* are sample and standard, respectively

Section A4.2b/01a
Annex Point IIA4.2

Analytical Methods for Detection and Identification of DBNPA in air

4.2	**Conclusion**	The method described herein allows the determination of DBNPA in air by collecting air samples on mixed cellulose ester filters. Data on reproducibilty, recovery and linearity of the method are reported in summary. The method as described requires procedure controls to be performed whenever an analysis is performed. The limit of detection is 1.2 µg DBNPA absolute as collected on the sampling filters. The limit of quantification is 0.004 mg/m^3.
4.2.1	Reliability	2
4.2.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	18 May 2017
Materials and methods	Applicant's version acceptable
Conclusion	Applicant's version acceptable
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM APPLICANT
Date	*Date*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	Corrections made as requested
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A4.2b/01b — Analytical Methods for Detection and Identification of DBNPA in air (confirmatory method)

Annex Point IIA4.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	M.D. Kallos. Determination of Dibromo Nitrilopropionamide ([REDACTED]) in Air using Liquid Chromatography and High Resolution Mass Spectrometry. Revision #2 March 2017. ICL-IP (unpublished). Dow has Letter of Access	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bromine Compounds Ltd.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for Annex I entry.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	No	
2.3	**Deviations**	Not applicable: not a guideline study	
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**	--	
3.1.1	Enrichment	The method is based on the collection of airborne [REDACTED] particles using a Mixed Cellulose Ester filter from SKC. The method was tested in the range between 2 and 1500 µg on-filter. An air sample of 1000L is sufficient to ensure a deposition on-filter of enough analyte mass at detectable range. Sampling flow rate: 1 L/min Sampling time: 16.7 h minimum.	
3.1.2	Cleanup	No purification or enrichment necessary.	
3.2	**Detection**	--	
3.2.1	Separation method	HPLC Instrument: UltiMate 3000 U-HPLC supplied by Thermo Column: Kinetix C-18 2.6µ, 100 x 4.6 mm, or equivalent Operational column temperature: 30 °C Injector volume: 100 µl Solvent composition: 30% Methanol 70% 20 mM Ammonium Acetate Solvent flow rate: 0.4 ml/min Retention time [REDACTED]: 5.54 minutes	

Section A4.2b/01b — Analytical Methods for Detection and Identification of DBNPA in air (confirmatory method)

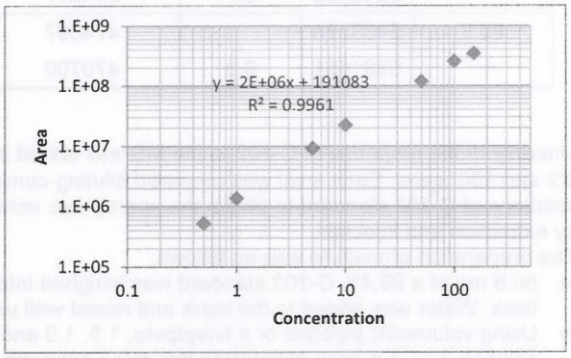
Annex Point IIA4.2

3.2.2	Detector	Exactive Plus Mass Spectrometer, supplied by Thermo	
		Scan range:	50-500 amu
		Resolution:	17500
		AGT	$5x10e^5$
		IT:	100 ms
		Probe:	Heated ESI (HESI)
		Probe temperature:	250 °C
		Sheath gas:	35 (arbitrary instrumental units)
		Auxiliary gas:	20 (arbitrary instrumental units)
		Sweep gas:	1 (arbitrary instrumental units)
		m/z:	259.8857
3.2.3	Standard(s)	External	
		Retention times:	
		[REDACTED]	
		Blank: A clean un-used filter is immersed in 10 mL deionised water at pH3 and sporadically mixed for at least 2 hours.	
3.2.4	Interfering substance(s)	None	
3.3	**Linearity**		
3.3.1	Calibration range	0.2 to 150 µg/mL.	
3.3.2	Number of measurements	One per concentration.	
3.3.3	Linearity	Linearity of the MS response was tested between 0.2 and 150 µg/mL. The correlation coefficient (r^2) was found to be 0.9961.	

Section A4.2b/01b — Analytical Methods for Detection and Identification of DBNPA in air (confirmatory method)

Annex Point IIA4.2

µg/ml	Area
0.2012	95412
0.5030	515914
1.0059	1354688
5.0296	9116756
10.059	22252624
50.296	118398641
100.59	253198979
150.89	341719640



Log-Log plot of µg/ml vs. Area

The correlation coefficient (r^2) obtained was 0.9961.

3.4 Specifity: interfering substances

No interfering substances reported.

3.5 Recovery rates at different levels

For recovery tests, sets of two unused filters are spiked with either 50 µL or 100 µL of a standard solution at a concentration of 1 mg / mL, resulting in absolute spiking levels of 0.05 mg and 0.1 mg.

[REDACTED]

Average recovery = 85.8%

3.5.1 Relative standard deviation

Average RSD = 8.7 %

Section A4.2b/01b — Analytical Methods for Detection and Identification of DBNPA in air (confirmatory method)

Annex Point IIA4.2

Fortification on-filter(µg)	Area MS	RSD (%)	Area UV Simultaneous	RSD (%)
9.98	338482		31545	
	395031	10.9	38463	14.0
49.9	3671221		282078	
	3245225	8.7	254277	7.3
99.8	5468378		474697	
	5981551	6.3	470700	0.6

3.6 Limit of determination

The LOQ, defined as the signal that produced a response with S/N≥10, was found to be 0.2 µg/mL, or 0.002 mg/m^3.

An air sample of 1000L is sufficient to ensure a deposition on-filter of enough analyte mass at detectable range.

Sampling flow rate: 1 L/min

Sampling time: 16.7 h minimum.

The critical concentration based on the AEL of 0.014 mg/kg bw/day is 0.0042 mg/m^3

LOQ of 0.002 mg/m^3 is below the critical concentration of 0.0042 mg/m^3.

3.7 Precision

3.7.1 Repeatability

The reproducibility of the method was tested on sets at levels of 1, 5, or 10 µg/mL and found to be < 10%.

The reproducibility was tested on sets at various levels, prepared by spiking clean filters with varying amounts of [REDACTED], followed by extraction and injection.

Fortification on-filter (µg)	Area MS	RSD (%)	Area UV Simultaneous	RSD (%)
9.98	338482 395031	10.9	31545 38463	14.0
49.9	3671221 3245225	8.7	282078 254277	7.3
99.8	5468378 5981551	6.3	474697 470700	0.6

3.7.2 Independent

Conformity of results between the UV method (IIIA Section 4.2b(03a))

Section A4.2b/01b — Analytical Methods for Detection and Identification of DBNPA in air (confirmatory method)

Annex Point IIA4.2

laboratory validation

and the MS method was tested by a paired t-test with n=6, using the results obtained for the samples in the recovery test. The p-value obtained at 95% Confidence Level was found to be 0.21, which indicates that the results obtained by both methods are statistically equivalent.

µg found by UV	µg found by MS
7	9
8	9
38	49
38	44
76	71
73	77

t-Test: Paired Two Sample for Means

	UV	*MS*
Mean	39.89	43.17
Variance	901.2071	852.7667
Observations	6	6
Pearson Correlation	0.984059	
Hypothesized Mean Difference	0	
df	5	
t Stat	-1.45127	
P(T<=t) one-tail	0.103206	
t Critical one-tail	2.015048	
P(T<=t) two-tail	0.206412	
t Critical two-tail	2.570582	

Section A4.2b/01b — Analytical Methods for Detection and Identification of DBNPA in air (confirmatory method)

Annex Point IIA4.2

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

The method is based on the collection of airborne [REDACTED] particles using a Mixed Cellulose Ester filter from SKC. This filter is submitted to the analytical laboratory for subsequent extraction and analysis by LC-HRMS.

The method was tested in the range between 2 and 1500 µg on-filter. An air sample of 1000L is sufficient to ensure a deposition on-filter of enough analyte mass at detectable range.

Sampling flow rate: 1 L/min

Sampling time: 16.7 h minimum.

Equipment:

- A hyphenated LC-MS instrument. The present work used an Ultimate 3000 U-HPLC and an Exactive Plus MS, both from Thermo
- A Kinetex C-18 2.6 µ, 100x4.6 mm column, or equivalent.
- Data station with the relevant instrument operating software.
- An analytical balance able to weigh to the nearest 0.1 mg.
- Miscellaneous standard laboratory glassware.

Reagents:

- Deionized water at pH3 (with HCl)
- Methanol MS grade
- Water MS grade
- Ammonium acetate AR

Preparation of blank:

Insert a clean unused filter into a 10 mL scintillation vial. Add 10 mL deionized water pH 3. Make sure the whole filter is immersed in water. Mix sporadically during at least 2 hours.

Preparation of standards:

- All the solutions are prepared using deionsised water at pH 3.
- 50 mg of DBNPA are accurately weighed into a 50 ml volumetric bottle. Water is added to the mark and the solution is mixed to obtain a solution at 1 mg DBNPA / mL.
- 5 ml of the 1 mg DBNPA / mL solution are pipetted into a 100 ml volumetric bottle. Water is added to the mark and the solution is mixed well to obtain a solution at 0.05 mg DBNPA / mL.

Section A4.2b/01b

Annex Point IIA4.2

Analytical Methods for Detection and Identification of DBNPA in air (confirmatory method)

- 1 ml of the 0.05 mg DBNPA / mL solution is pipetted into a 10 mL volumetric flask which is made up to the mark and mixed well to obatin a solution at a concnetration of 0.005 mg DBNPA / mL.

Preparation of samples for recovery:

Two sets of unused filters are spiked with either 50 µL or 100 µL of the solution at 1 mg / mL, resulting in absolute spiking levels of 0.05 mg or 0.1 mg DBNPA. The filters are allowed to dry. Recovery samples are treated in the same manner as the sample filters (see below).

Sample preparation:

Each sample filter is inserted into a 10 mL scintillation vial. 10 mL deionized water (pH3) is added, making sure the whole filter is immersed and mixed sporadically during at least two hours.

Analysis:

The blank is injected first. No signal above the S/N must be observed at the retention time of [REDACTED] (DBNPA). 100 µL of the 0.005 mg DBNPA / mL standard solution are injected until reproducible results are obtained.

100 µL of the recovery samples are injected.

100 µL of the sample solutions are injected.

Evaluations are performed on the extraced signal at m/z=259.887 (theoretical) which corresponds to the largest signal of the quasimolecular cluster $[M+NH_4]^+$. The difference between the theoretical value and the experimental m/z found must not exceed 5 ppm.

For analysis of a series of samples it is necessary to verify that the HPLC is still within calibration by re-injecting the standard every ten unknowns.

Retention time [REDACTED] (DBNPA): 5.54 min

Calculation:

The absolute mass of material obtained for the recovery test filters is calculated as:

$$\frac{A_{sm} * W_{st} * P_{st} * 0.01}{A_{st}} = \mu g$$

where: A: area
W: weight
P: purity, expressed as percent
sm and *st* are sample and standard, respectively

The average recovery is calculated as

Section A4.2b/01b — Analytical Methods for Detection and Identification of DBNPA in air (confirmatory method)

Annex Point IIA4.2

$$H = \frac{\sum \frac{C_i}{T_i}}{n}$$

where:
H: recovery factor
C*i*: amount of [REDACTED] (DBNPA) found in the recovery filter
T*i*: theoretical amount of [REDACTED] (DBNPA) found in the recovery filter, which is calculated as

$$\frac{Wst * Pst * \mu \mathrm{L}}{5000}$$

The amount of [REDACTED] (DBNPA) collected in the sample filters is calculated as

$$\frac{A_{sm} * W_{st} * P_{st} * 0.01}{A_{st} * H} = \mu \mathrm{g}$$

where: A: area
W: weight
P: purity, expressed as percent
sm and *st* are sample and standard, respectively

Limit of Quantification

The LOQ, defined as the signal that produced a response with S/N≥10, was found to be 0.2 µg/mL, or 0.002 mg/m^3.

An air sample of 1000L is sufficient to ensure a deposition on-filter of enough analyte mass at detectable range.

Sampling flow rate: 1 L/min

Sampling time: 16.7 h minimum.

The critical concentration based on the AEL of 0.014 mg/kg bw/day is 0.0042 mg/m^3

LOQ of 0.002 mg/m^3 is below the critical concentration of 0.0042 mg/m^3.

Conformity of results with UV method

Conformity of results between the UV method (IIIA Section 4.2b(03a)) and the MS method was tested by a paired t-test with n=6, using the results obtained for the samples in the recovery test. The p-value obtained at 95% Confidence Level was found to be 0.21, which indicates that the results obtained by both methods are statistically equivalent.

Section A4.2b/01b — Analytical Methods for Detection and Identification of DBNPA in air (confirmatory method)

Annex Point IIA4.2

µg found by UV	µg found by MS
7	9
8	9
38	49
38	44
76	71
73	77

t-Test: Paired Two Sample for Means

	UV	*MS*
Mean	39.89	43.17
Variance	901.2071	852.7667
Observations	6	6
Pearson Correlation	0.984059	
Hypothesized Mean Difference	0	
df	5	
t Stat	-1.45127	
P(T<=t) one-tail	0.103206	
t Critical one-tail	2.015048	
P(T<=t) two-tail	0.206412	
t Critical two-tail	2.570582	

4.2 Conclusion

The method described herein allows the determination of DBNPA in air by collecting air samples on mixed cellulose ester filters. Data on reproducibilty, recovery and linearity of the method are reported in summary. The method as described requires procedure controls to be performed whenever an analysis is performed. The limit of quantification is 0.2 µg/mL, or 0.002 mg/m^3.

4.2.1 Reliability 2

4.2.2 Deficiencies

Section A4.2b/01b — Analytical Methods for Detection and Identification of DBNPA in air (confirmatory method)

Annex Point IIA4.2

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	24. April 2017
Materials and methods	Applicant's version acceptable
Conclusion	Applicant's version acceptable
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

[REDACTED]

Section A4.2b/06

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of DBAA and DBAN in Air

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	The development of an analytical method for the determination of DBAA and DBAN in air is not required. For DBAA- In accordance with the ECHA GUIDANCE ON REGULATION (EU) No 528/2012 CONCERNING THE MAKING AVAILABLE ON THE MARKET AND USE OF BIOCIDAL PRODUCTS (BPR) version from July 2013, only if the substance is volatile (i.e. which vapour pressure > 0.01 kPa) or sprayed, or occurrence in air is otherwise probable, the analytical method in air needs to be submitted. DBAA's vapour pressure value is 2.3 x 10-2 mm Hg (which corresponds to 0.0031 kPa), which is below 0.01 kPa. The determination of DBAA in the air compartment is therefore not needed. For DBAN- DBAN is a product of hydrolysis of DBNPA, thus would only be formed in an aqueous solution. DBAN will in consequence not be directly emitted to air, for this reason the Henry constant is more relevant parameter than the vapour pressure to address potential exposure to the air compartment. DBAN has a very high water solubility (>10 g/L), a low vapour pressure (40.1 Pa) and a Henry Law's constant (0.04 Pa-m3/mole) in the range of low volatility from water solution, which support that the emissions to air are negligible. Furthermore, DBAN also converts to DBAA in highly diluted samples (which will be the case in any point of release). Therefore the determination of DBAN in the air compartment is not required as well.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	19/10/2015	

Section A4.2b/06

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of DBAA and DBAN in Air

Evaluation of applicant's justification	Applicant states that DBAA's vapour pressure value is 2.3 x 10-2 mm Hg (corresponding to 0.0031 kPa), which makes DBAA not volatile. DBAN is a product of hydrolysis of DBNPA, and would only be formed in an aqueous solution. DBAN will in consequence not be directly emitted to air. Neither DBAA or DBAN is of concern regarding inhalation toxicity, and thus it is not considered relevant to determine DBAA and DBAN in the air compartment. Applicant's justification is acceptable
Conclusion	Acceptable
Remarks	-

	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A4.2c/01

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of 2,2-Dibromo-3-nitrilopropionamide (DBNPA) in water

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [X]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	The following information supports the applicant's request for a waiver for an analytical method for measuring DBNPA in water and soil due to the compound's rapid degradation in these matrices, especially at low mg/L to sub-mg/L concentrations. As already noted, degradation of DBNPA occurs by two routes, a hydrolysis pathway and a degradation route involving reaction with nucleophiles [Exner, 1973]. Hydrolysis half-lives range from hours at neutral to alkaline pH to weeks at acidic pH. In contrast, DBNPA can react with nucleophiles or light with half-lives in the range seconds to minutes, especially with low concentrations of DBNPA present. DBNPA will react with the constituents of soil, including organic matter, microbial cells, and various nucleophiles present in the soil. For example, a series of soils with a range of soil textures were mixed in aqueous slurries containing 50 mg/L DBNPA [Exner, 1973]. Half-lives of DBNPA ranged from 4 hours in sandy loam to 25 hours in silty clay loam. At lower DBNPA concentrations, as the ratio of DBNPA to nucleophiles/organic matter decreases, the degradation rate will increase and the half-life of DBNPA will be even shorter. This point is demonstrated with the degradation of DBNPA in activated sludge, sewage, sediments, and natural waters which contain nucleophiles and reactive organic matter similar to soil. Rapid degradation of DBNPA was observed in an activated sludge die-away test (Hanstveit, 2002). Primary degradation of 0.04 mg/L [14C]DBNPA in activated sludge occurred within one hour. In a separate study, the addition of DBNPA to sewage entering a municipal STP resulted in complete disappearance of up to 10 mg/L DBNPA within 5 minutes, followed by a slower transformation of any residual DBNPA	

Section A4.2c/01
Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of 2,2-Dibromo-3-nitrilopropionamide (DBNPA) in water

(e.g. the half-life for 23 mg/L DBNPA was 0.8 hours) [Gonsior, 2000]. Rapid degradation was also observed in natural waters and sediments [Gonsior, 2001]. Here, sub-mg/L concentrations of radiolabeled DBNPA rapidly degraded in microcosms prepared with river water and river sediments, with half-lives measured at less than one hour.

To summarize, the rapid degradation of DBNPA in environmental matrices due to the reaction with nucleophiles and reactive organic matter makes it extremely difficult to develop an analytical method to measure realistic environmental concentrations of the compound.

References

Exner, J. H., G. A. Burk, and D. Kyriacou. 1973. Rates and Products of Decomposition of 2,2 Dibromo-3-nitrilopropionamide. J. Agr. Food Chem., Vol 21, No. 5, pages 838-842.

Gonsior, S. J., and P. A. Goodwin. 2000. Evaluation of the Effect of 2,2, -Dibomonitrilopropionamide (DBNPA) on a Semi-Continuous Activated Sludge Treatment System. The Dow Chemical Company Report HET K-078141-097.

Gonsior, S. J., P. A. Goodwin, and M. K. Stock. 2001. Assessing the Biodegradability of DBNPA in Water/Sediment Mixtures. The Dow Chemical Company Report HET K-078141-098.

Hanstveit, R. and J. A. Schoonmade. 2002. 2,2,-Dibomo-3-nitrilopropionamide (DBNPA): A Definitive Die Away Test in Activated Sludge. The Dow Chemical Company Report K-078141-107.

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

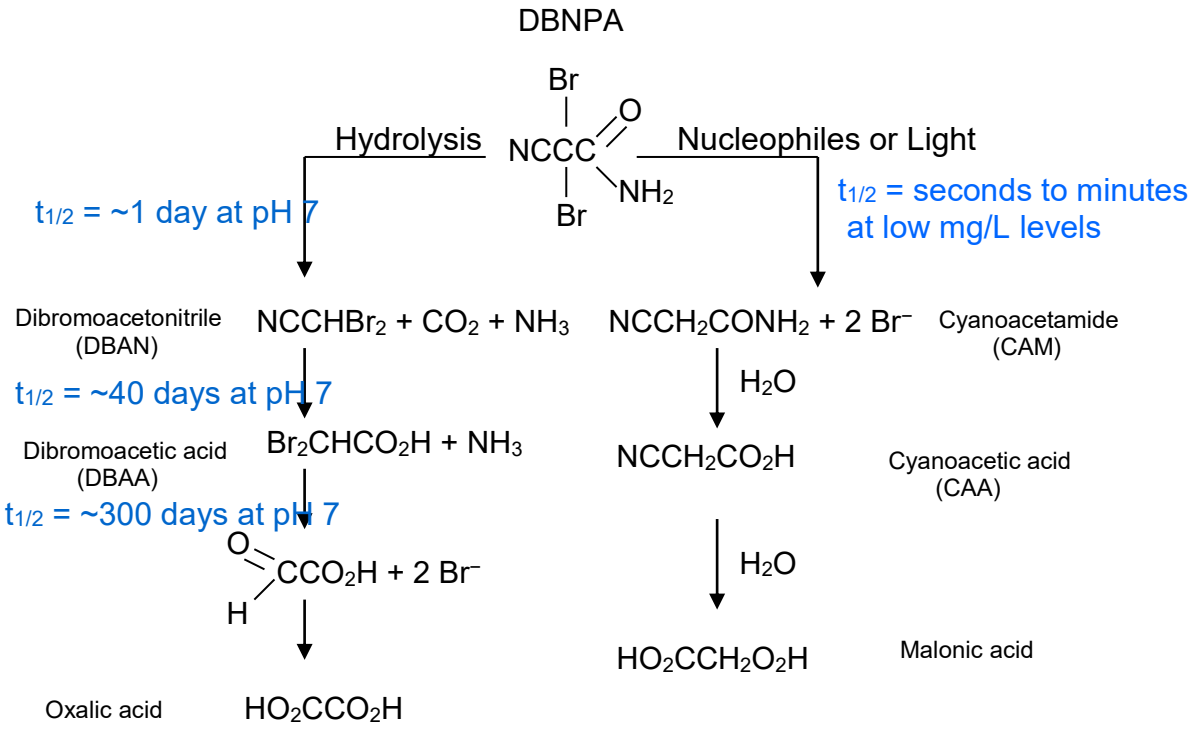
Section A4.2c/01

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of 2,2-Dibromo-3-nitrilopropionamide (DBNPA) in water

Date	19/10/2015
Evaluation of applicant's justification	Applicant states that the analysis of DBNPA in water is technically not feasible due to the rapid degradation in water due to interactions with various components in the matrix. Furthermore, a method for DBAA has been developed, which serves as a marker for DBNPA.
Conclusion	Acceptable
Remarks	Acceptable
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Appendix 1 – DBNPA Degradation Pathways (Reference 4)



Section A4.2c/02

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of Dibromoacetonitrile (DBAN) in water

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [X]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The efforts to develop a method to determine (DBAN) in water has not been successful. Several approaches were attempted including LC/MS/MS, derivatizations, and sample concentration steps, but none were successful. Detection limits achieved were 50,000 times higher than the required LOQ of 0.1 ppb (Reference 1). Another approach (Reference 2) indicated that a GC/MS based method published by Nikolaou, et al (Reference 3) might be successful. However, before fully evaluating this approach, we noted that the DBAN appears to convert to DBAA in the highly diluted samples. This is not unexpected based on the known stability of DBAN (Reference 4 and Appendix 1). Furthermore, in comparing the relative stability of these two degradates, the DBAA has a half-life of ~300 days as compared to a half-life of ~ 40 days for DBAN under neutral pH conditions. Because DBAN is an intermediate in the degradation of DBNPA to DBAA, and DBAA is more stable than DBAN, it is clear that the detection of DBAA would be a more representative and robust marker when evaluating DBNPA in water. Based on this understanding and our efforts representing for more than a year of DBAN method development work, we believe that the development of a DBAN analytical method is not essential because the DBAA is a better marker for DBNPA and we have developed a method for DBAA, which is pending validation. On the basis of these findings, we have narrowed our focus to development and validation of methods for the determination of DBAA in water, and not pursued further development of methods for DBAN in water. **References**	

Section A4.2c/02

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of Dibromoacetonitrile (DBAN) in water

1. Chamkasem, N. "Evaluation of Dibromoacetonitrile in Water by LC/MS/MS", MPI Research, August 23, 2008
2. Fishman, S., et al, "Analysis by Gas Chromatography Mass Spectrometry (GC/MS) Technique of Low Level Solutions of Morpholine, 2-Amino-2-Methyl-1-Propanol (AMP), and Dibromoacetonitrile (DBAN) in water", The Dow Chemical Company, August, 2008
3. Nikolaou, Anastasia D., et. al. 1999, "Decomposition of Dihaloacetonitriles in Water Solutions and Fortified Drinking Water Samples". Chemosphere 41 (2000) 1149-1154
4. Exner, J. H., Burk, G. A. and Kyriacou, D. (1973). Rates and Products of Decomposition of 2,2-Dibromo-3-nitrilopropionamide. J. Agri. Food Chem. 21(5), pp. 838-842.

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the

comments and views submitted

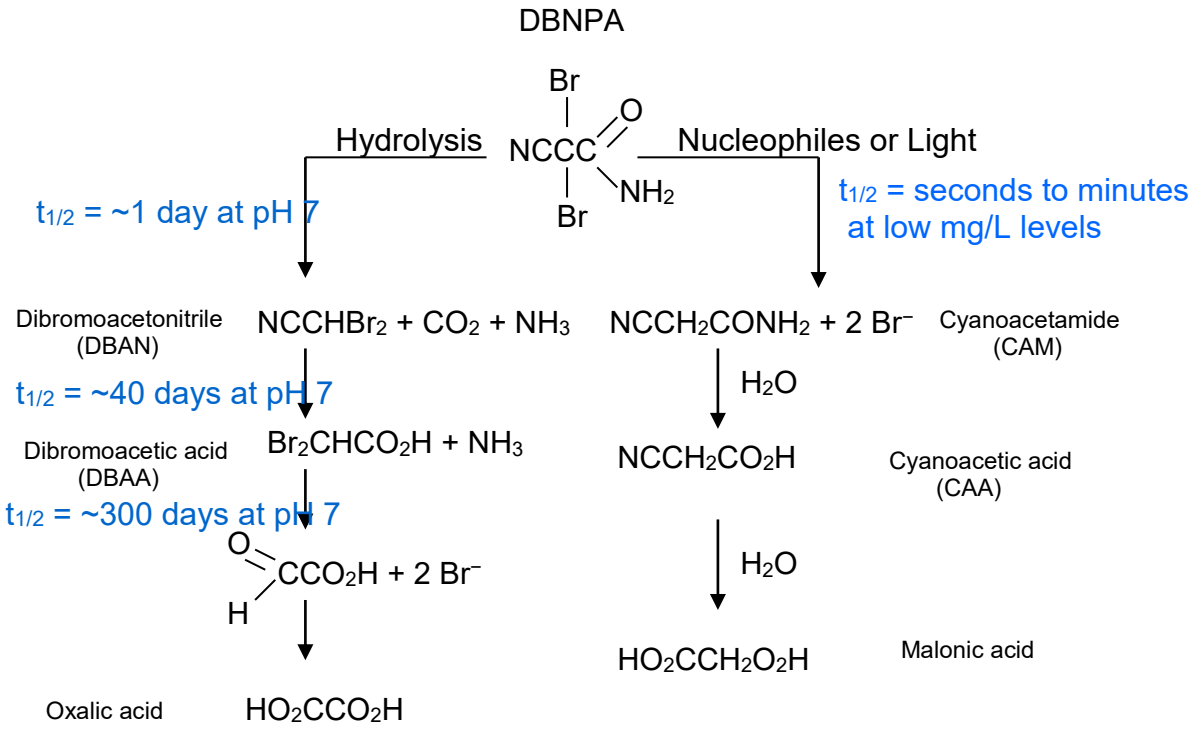
EVALUATION BY RAPPORTEUR MEMBER STATE

Date	28 May 2013
Evaluation of applicant's justification	Applicant states that the analysis of DBAN in water is technically not feasible. Different attempt to validate a method are presented along with a degradation pathway
Conclusion	Acceptable
Remarks	Acceptable

COMMENTS FROM OTHER MEMBER STATE *(specify)*

Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Appendix 1 – DBNPA Degradation Pathways (Reference 4)



Section A4.2c/03

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of Dibromoacetic acid (DBAA) in water

Official use only

1 REFERENCES

1.1	**Reference**	W. Barker, A. Watson "Validation of a Method to Measure Trace Levels of Dibromoacetic Acid in Surface and Drinking Water", EN-CAS Analytical Laboratories, 2016 , The Dow Chemical Company Report No. AL 2016-002028 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	Dow Europe GmbH and ICL Europe Coöperatief U.A.
1.2.2	Companies with letter of access	None.
1.2.3	Criteria for data protection	Data on existing active substance submitted for the first time for entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes
2.2	**GLP**	Yes
2.3	**Deviations**	Yes – GLP status of characterization of reference material was unknown, however a Mass Spectrum of the material was collected to confirm identity.

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	200 mL of sample, fortified as necessary for recovery studies, are added to a separatory funnel and the pH adjusted to < 4 using 10% sulfuric acid. The funnel is capped and shaken, then 60 mL of ethyl acetate are added, and the mixture shaken for one minute. The ethyl acetate is discarded. 10 mL concentrated sulfuric acid and 70 g sodium sulfate added and the mixture is shaken to dissolve sodium sulfate. A new 20 mL aliquot of ethyl acetate is added and shaken vigorously for 2 minutes. The ethyl acetate phase is removed and retained. The aqueous phase is retained in the funnel. A second partitioning is done with a fresh 20 mL ethyl acetate aliquot. The aqueous phase is discarded.
3.1.1	Enrichment	The first 20 mL aliquot of ethyl acetate is passed through a pad of 40 g of sodium sulphate into a 50-mL centrifuge tube and evaporated to ~5 mL. The second 20 mL aliquot of ethyl acetate is passed through a sodium sulphate pad, and added to the remaining amount of the initial aliquot. The sodium sulfate pad is rinsed with 10 mL ethyl acetate. This combined ethyl acetate is evaporated down to ~ 5 mL. The remaining ethyl acetate is transferred to a 15-mL calibrated centrifuge tube, and the 50-mL tube is rinsed with 5 mL of acetonitrile, which is transferred to the 15-mL calibrated centrifuge tube. Evaporate to ~ 0.5 mL with an

Section A4.2c/03 Analytical Methods for Detection and Identification of Dibromoacetic acid (DBAA) in water

Annex Point IIA, IIA-IV.4.2

		N-evaporator with a water bath set at ~ 45 C. Add ~1.0 mL of methanol. Concentrate to 0.8 mL and reconstitute to 2.0 mL with HPLC grade water. Bring solvent ratio to 40:60 methanol:water.
3.1.2	Cleanup	
3.2	**Detection**	
3.2.1	Separation method	HPLC Column: Mac Mod Hydrobond AQ C_8 2.1 x 150 mm, 5.0 µm particle size. Mobile Phase: Mobile phase A: water with 0.2% formic acid, Mobile Phase B: methanol Gradient: Time (min) %A %B 0 85 15 9 0 100 14 0 100 14.1 85 15 19 85 15 Flow rate: 0.2 mL/min Injection Volume: 15 µL Column temperature: 25C
3.2.2	Detector	AB Sciex API 4000 ion mass spectrometer operated in MS/MS mode with Turbo ion spray Collision gas: nitrogen at 12 units Collision energy: -35 eV (-15 eV confirmatory) Mass transition: 215 → 79 m/z 215 → 171 m/z confirmatory
3.2.3	Standard(s)	External standard calibration was performed with dibromoacetic acid standards prepared in 60:40 methanol:water; 8 standards were prepared over the range of 5-1000 ng/mL which is the equivalent of 0.005-1.0 µg/mL
3.2.4	Interfering substance(s)	Background levels in the controls were found to be below the detection limit. HPLC with tandem mass spectrometry is considered to be a highly specific technique, and no interfering substances were found. No interfering compounds were found.
3.3	**Linearity**	
3.3.1	Calibration range	8 standards were prepared over the range of 5-1000 ng/mL which is the equivalent of 0.005-1.0 µg/mL
3.3.2	Number of measurements	Each standard was injected once during each sequence run.
3.3.3	Linearity	r^2 = <0.99

Section A4.2c/03

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of Dibromoacetic acid (DBAA) in water

3.4 Specificity: interfering substances

Background levels in the controls were found to be below the detection limit. HPLC with tandem mass spectrometry is considered to be a highly specific technique, and no interfering substances were found. No interfering compounds were found.

3.5 Recovery rates at different levels

Fortification level (µg/L)	Number of replicates	Mean% Recovery	% RSD
Primary			
0.10 µg/L (surface water)	5	74%	17%
0.50 µg/L (surface water)	5	83%	12%
1.0 µg/L (surface water)	5	74%	10%
0.10 µg/L (drinking water)	5	79%	5.1%
0.50 µg/L (drinking water)	5	75%	14%
1.0 µg/L (drinking water)	5	72%	6.9%
Confirmatory			
0.10 µg/L (surface water)	5	74%	12%
0.50 µg/L (surface water)	5	83%	14%
1.0 µg/L (surface water)	5	77%	9.8%
0.10 µg/L (drinking water)	5	82%	4.1%
0.50 µg/L (drinking water)	5	73%	15%
1.0 µg/L (drinking water)	5	72%	6.1%

3.5.1 Relative standard deviation

Overall:

	N	% Mean Recovery	% RSD
Primary			
Surface Water	15	77%	13%
Drinking Water	15	75%	9.5%
Confirmatory			
Surface Water	15	78%	12%
Drinking Water	15	76%	11%

3.6 Limit of determination

Limit of quantitation (LOQ) was established at 0.1 ppb. Limit of detection was estimated from the lowest fortification sample to be ~ 0.0011 ppb.

3.7 Precision

See 3.5

3.7.1 Repeatability

No specific repeatability data to be generated.

Section A4.2c/03

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of Dibromoacetic acid (DBAA) in water

3.7.2	Independent laboratory validation	No specific repeatability data to be generated.

Section A4.2c/03

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of Dibromoacetic acid (DBAA) in water

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

An analytical method for the determination trace levels of DBAA in water was validated with a limit of quantitation established at 0.1 ppb. The method involves extraction of the DBAA from water with 2 aliquots of ethyl acetate after acidification of the sample with sulphuric acid, and addition of sodium sulphate. The ethyl acetate is dried using sodium sulphate, evaporated to near dryness, and solvent exchanged with acetonitrile and then with 40:60 methanol:water. The extract is separated by HPLC using a Mac Mod Hydrobond AQ C_8 2.1 x 150 mm id, 5.0 µm particle size column. Detection is performed using a tandem MS/MS monitoring the mass transition from m/z 215 to m/z 79. The method linearity was confirmed over a range equivalent to 0.005-1.0 µg/mL.

4.2 Conclusion

The data summarized below demonstrates the suitability of method for the analysis of dibromoacetic acid in water samples. Due to the use of an older MS/MS instrument in this study, the LOQ achieved was 0.1 ppb, which was at the practical limit of the instrument's capability, rather than the SANCO recommended 0.1 ppb limit.

Matrix: Surface Water

Fortification level (µg/L)	Average Recovery (%)	Recovery Range (%)	RSD (%)	n
Primary				
0.10	74	62-96	17	5
0.05	83	72-94	12	5
1.0	74	66-83	10	5
Confirmatory				
0.10	74	62-87	12	5
0.05	83	72-101	14	5
1.0	77	68-88	9.8	5

Matrix: Drinking Water

Fortification level (µg/L)	Average Recovery (%)	Recovery Range (%)	RSD (%)	n
Primary				
0.10	79	72-82	5.1	5
0.50	75	60-88	14	5
1.0	72	64-77	6.9	5
Confirmatory				
0.10	82	78-87	4.1	5

Section A4.2c/03

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of Dibromoacetic acid (DBAA) in water

0.50	73	56-85	15	5	
1.0	72	66-78	6.1	5	

4.2.1	Reliability	1
4.2.2	Deficiencies	The characterization of the reference material was taken from the supplier's Certificate of Analysis. The GLP status is unknown, however the supplier is considered a highly reliable supplier of characterized materials. Also, the test facility analyzed the reference substance by GC/MS which confirmed it to be the correct material. Therefore this deficiency is not expected to have a significant impact on the quality or integrity of the study.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	19 December 2016
Materials and methods	Water samples were adjusted to a pH<4, extracted with ethyl acetate and passed through sodium sulphate. The ethyl acetate is evaporated and residues reconstituted in 40:60 acetonitrile water followed by LC/MS/MS analysis In section 3.3.1 it is stated that eight standards were prepared. According to doc IV 4.02c (03) figure 1 only seven standards were injected, however this is not mentioned.
Conclusion	The average recovery is between 70-110% and the precision is below 20% for each fortification level. Linearity has been demonstrated in the range 0.005-1.0 µg/mL. Validation data is provided for both the primary and the confirmatory transition. The method is therefore considered adequately validated. The method is suitable for the determination of DBAA in water with an LOQ of 0.1 µg/L which complies with the requirement according to the Drinking Water Directive. No PNEC value for surface water has been derived for DBAA.
Reliability	1
Acceptability	Acceptable
Remarks	-
	COMMENTS FROM...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4.2-2 Summary of validation of DBAA in water

Surface water			
Fortification level [µg/L]	Average Recovery [%]	RSD [%]	n
0.2	90	15	5
1.0	81	6.6	5
2.0	77	3.8	5
Drinking water			
Fortification level [µg/L]	Average Recovery [%]	RSD [%]	n
0.2	95	10	5
1.0	87	16	5
2.0	91	5.6	5

Section A4.2d/01

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of DBNPA and 2-Cyanoacetamide in Rat Blood and Tissue

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [X]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	**DBNPA** Attempts at validating an analytical method for the determination of dibromonitrilopropionamide (DBNPA) in rat blood and liver tissue were unsuccessful due to the instability of DBNPA in those matrices. As summarized in Appendix A of Study 081159 of The Dow Chemical Company (Doc. No. 437-001), replicate samples of rat blood and liver were fortified with DBNPA (0.5 µg/g in blood; 2.0 µg/g in liver). Samples were extracted immediately after fortification with acetonitrile. Quantitation incorporated isotopically labelled internal standard ($^{13}C_3$-DBNPA). The resulting extracts were analyzed by high performance liquid chromatography (HPLC), negative ion electrospray ionization (-ESI), with mass spectrometry detection (MS). DBNPA was not detected in any of the samples thereby verifying the instability of DBNPA in the biological matrices. Refer to Appendix A of Report 081159 (Doc. No. 437-001) for complete details on the sample preparation, extraction, and analytical instrumentation employed in the attempt to validate a method for the determination of DBNPA in rat blood and liver tissue. The full report 081159 will be provided in a post submission. **2-Cyanoacetamide** An analytical method for the determination of 2-cyanoacetamide (CAM) in rat blood was successfully completed at a concentration 0.5 µg CAM/g blood. Attempts were also made, but were unsuccessful in determining CAM in blood at 0.05 (the target LOQ for this matrix, as per European Commission Guidance Document	

Section A4.2d/01

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of DBNPA and 2-Cyanoacetamide in Rat Blood and Tissue

	on Residue Analytical Methods, SANCO/825/00 – rev 7, March 20, 2004), 0.1 and 0.2 µg CAM/g blood. An analytical method for the determination of CAM in rat liver was successfully completed at concentrations 0.5 and 1.0 µg CAM/g liver. Attempts were also made, but were unsuccessful in determining CAM in liver at 0.1 (the target LOQ for this matrix, as per European Commission Guidance Document on Residue Analytical Methods, SANCO/825/00 – rev 7, March 20, 2004) and 0.2 µg/g liver. The lowest quantifiable concentration of CAM in rat blood and liver was 0.5 µg/g matrix. That limit of quantitation (LOQ) was the limiting factor in preventing methods to be completed at the targeted lower concentrations of 0.05 µg CAM/g blood and 0.1 µg CAM/g liver. The complete details of the method validation are reported in Study 081159 of The Dow Chemical Company (also identified as File# K-078141-128), which will be provided in a post submission.
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	28 May 2013
Evaluation of applicant's justification	The applicant states that attempts at validating an analytical method for the determination of dibromonitrilopropionamide (DBNPA) in rat blood and liver tissue were unsuccessful due to the instability of DBNPA in those matrices, this is acceptable. Validation of CAM is given in Section A4.2d/02.
Conclusion	Acceptable
Remarks	-
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*

Section A4.2d/01

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of DBNPA and 2-Cyanoacetamide in Rat Blood and Tissue

Remarks

Section A4.2d/02

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of DBNPA and Cyanoacetamide in Rat Blood and Tissue

Official use only

1 REFERENCE

1.1 Reference

Rick D. L., McClymont E. L.: DBNPA: DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF DIBROMONITRILOPROPIONAMIDE AND 2-CYANOACETAMIDE IN RAT BLOOD AND LIVER TISSUE., , Dow Chemical report K-078141-128, Study ID 081159 (unpublished); report to be post submitted

Dibromonitrilopropionamide (DBNPA) – Parent compound found to be unstable; waiver requested (please refer to Doc III A section 4.2d/01, Doc. No. 437-001)

2-Cyanoacetamide (CAM) – residue method successfully completed; see information below and referenced report

1.2 Data protection: Yes

1.2.1 Data owner: The Dow Chemical Company

1.2.2 Companies with letter of access: None

1.2.3 Criteria for data protection: Data on existing a. s. submitted for the first time for entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study: Yes

2.2 GLP: Yes

2.3 Deviations: The chemical purity assay of 2-cyanoactamide was not conducted under GLPs (vendor Certificate of Analysis purity used).

3 MATERIALS AND METHODS

3.1 Preliminary treatment

3.1.1 Enrichment

Fortified blood samples were prepared in 0.5 g aliquots of rat blood at concentrations of 0.1, 0.2 and 0.5 µg CAM/g of rat blood (n=5 at each concentration). Stock solutions of CAM were prepared in acetonitrile at concentrations of 2.5, 5.0 and 12.5 µg CAM/mL acetonitrile. ^{13}C-CAM was prepared in acetonitrile at 101 µg ^{13}C-CAM/mL. All fortified samples were prepared using 0.5 ± 0.02 g rat blood that was weighed into 2-mL micro-centrifuge tubes. Fortification of the samples was achieved via addition of 20 µL aliquots of the stock solutions.

^{13}C-CAM (20 µL of 101 µg ^{13}C-CAM/mL) was added to each of the fortified rat blood samples and to one of the control rat blood samples. Acetonitrile (0.5 mL) was added to all samples which were then vortex-

Section A4.2d/02

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of DBNPA and Cyanoacetamide in Rat Blood and Tissue

mixed briefly, followed by centrifugation at 15000 x g for 15 minutes. Aliquots of the supernatants were transferred to a 30000 molecular weight cut-off centrifuge filter and centrifuged for 30 minutes at 15000 x g. The resulting supernatants were transferred to clean vials for analysis by HPLC/MS/MS.

Fortified liver samples were prepared in 0.5 g aliquots of rat liver homogenate at concentrations of 0.1, 0.2, 0.5 and 1 µg CAM/g of rat liver (n=5 at each concentration). The liver homogenate contained either 0.3 or 0.5 g liver/g homogenate. Stock solutions of CAM were prepared in acetonitrile at concentrations of 2.5, 5.0 and 12.5 µg CAM/mL acetonitrile. ^{13}C-CAM was prepared in acetonitrile at 101 µg ^{13}C-CAM/mL. All fortified samples were prepared using 0.5 ± 0.5 g rat liver that was weighed into 1-dram vials. Fortification of the samples was achieved via addition of 20 µL - 40 µL of the stock solutions.

^{13}C-CAM (29 µL of 101 µg ^{13}C-CAM/mL) was added to each of the fortified rat liver samples and to one of the control rat liver samples. Acetonitrile (0.25 mL) was added to all samples which were then vortex-mixed briefly, followed by centrifugation at 863 x g for 10 minutes. Aliquots of the supernatants were transferred to a 30000 molecular weight cut-off centrifuge filter and centrifuged for 30 minutes at 15000 x g. The resulting supernatants were transferred to clean vials for analysis by HPLC/MS/MS.

3.1.2 Cleanup

See section 3.1.1

3.2 Detection

3.2.1 Separation method

HPLC Conditions:

Analytical Column: Whatman Partisil 10 SCX; 4.6 x 250 mm
HPLC Eluent: A = 0.05 M ammonium acetate + 0.1% formic acid
B = Milli Q Water + 0.1% formic acid

Gradient:

Time	%A	%B	Flow (mL/min)
0.00	0	100	0.5
1.00	0	100	0.5
4.00	90	10	0.5
6.00	90	10	0.5
7.00	0	100	0.5
10.0	0	100	0.5

Injection Volume: 100 µL

Column Temperature: 25 °C

3.2.2 Detector

Detection by negative ion electrospray (turbo spray) ionization and tandem mass spectrometry detection, operating in the multiple reaction monitoring (MRM) mode.

Precursor and product ions:

Section A4.2d/02

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of DBNPA and Cyanoacetamide in Rat Blood and Tissue

		CAM: Q1 mass = 82.9 amu, Q3 mass = 65.1 amu ^{13}C-CAM: Q1 mass = 83.9 amu, Q3 mass = 66.1 amu Dwell Time: 500 msec/ion/scan
3.2.3	Standard(s)	Quantitation of CAM in blood and liver extracts was performed using an internal standard technique employing a stable isotope labeled standard. Quantitative standards were prepared in 50/50 Milli-Q water/acetonitrile.
3.2.4	Interfering substance(s)	No interfering substances were found in extracts of control blood or control liver.
3.3	**Linearity**	
3.3.1	Calibration range	Standards of CAM were prepared at a total of eight concentrations, ranging from 0.00905 to 0.468 µg CAM/mL diluent (50/50 Milli-Q water/acetonitrile) and containing the same amount of internal standard as the samples (2 µg/mL).
3.3.2	Number of measurements	Each of the eight calibration CAM standards was injected three times throughout the analytical sequence. The average refit concentrations ranged from 87.6% to 117%. The relative standard deviations of refits were 3.44% to 15.0%.
3.3.3	Linearity	Standards of CAM at eight concentrations of 0.00905 to 0.468 µg CAM/mL diluent were analyzed in triplicate. The resulting calibration curve yielded a correlation coefficient (r^2) of 0.9948.

Section A4.2d/02

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of DBNPA and Cyanoacetamide in Rat Blood and Tissue

3.4 Specifity: interfering substances

HPLC/MS/MS affords a highly specific method for both quantitation and confirmation of residue identity by retention time matching in conjunction with monitoring the specific MRM transition of CAM and the ^{13}C labeled internal standard. No interferences were observed.

3.5 Recovery rates & Standard deviations at different levels

Recovery of CAM from fortified blood and liver samples:

Matrix	Fortification Level (µg/g)	Number of Spikes Analyzed	Average Recovery (%)	Relative Standard Deviation (%)
Blood	0.0	2	NQ	NA
	0.1	5	NQ	NA
	0.2	5	NQ	NA
	0.5	5	106	6.6
Liver	0.0	2	NQ	NA
	0.1	5	NQ	NA
	0.2	5	NQ	NA
	0.5	5	92.3	10.8
	1.0	5	98.5	10.8

NQ = Not Quantifiable above 0.5 µg/g
NA = Not Applicable

3.5.1 Relative standard deviation

See section 3.5

3.6 Limit of determination

The limit of quantitation (LOQ) for the determination of CAM in blood and liver was set equal to the lowest quantifiable fortified 'spike' prepared; 0.5 µg/g matrix. No recoveries at lower concentrations (0.1 and 0.2 µg/g blood or liver) were obtained.

3.7 Precision

See section 3.5

3.7.1 Repeatability

No specific repeatability data to be generated.

3.7.2 Independent laboratory validation

No independent validation to be conducted.

Section A4.2d/02

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of DBNPA and Cyanoacetamide in Rat Blood and Tissue

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

The method for analysis of 2-cyanoacetamide (CAM) described in DBNPA: DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF DIBROMONITRILOPROPIONAMIDE AND 2-CYANOACETAMIDE IN RAT BLOOD AND LIVER TISSUE., D. L. Rick, E. L. McClymont., Dow Chemical report K-078141-128, is valid for the determination of 2-cyanoacetamide (CAM) in blood and liver at 0.5 and 0.5-1.0 µg/g, respectively.

Blood and liver homogenate sample aliquots (0.5 g) were fortified with CAM and the ^{13}C labeled internal standard and then extracted with acetonitrile. Following centrifugation and filtration using ultracentrifugation, the supernatants were analyzed for CAM by HPLC/MS/MS.

LC/MS/MS affords a highly specific method for both quantitation and confirmation of residue identity by retention time matching in conjunction with monitoring the specific MRM ions for the analyte and internal standard. The method response was linear with a calibration curve correlation coefficient (r^2) of 0.9948. The average recovery of CAM from fortified blood matrix was 106% for blood concentration of 0.50 µg/g. Recovery of CAM from fortified liver matrix averaged 92.3% and 98.5% for liver concentrations of 0.50 and 1.0 µg/g, respectively.

4.2 Conclusion

The data summarized in Section 3.5 demonstrates the suitability of methods for the analysis of 2-cyanoacetamide (metabolic product of dibromonitrilopropionamide) in blood and liver. In both matrices, the limit of quantitation was determined to be 0.5 µg/g. The desired quantitation limits of 0.05 µg/g in blood and 0.1 µg/g in liver were not attainable due to instrumental sensitivity limits and matrix effects from the biological extracts.

4.2.1 Reliability 1

4.2.2 Deficiencies None.

Section A4.2d/02

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of DBNPA and Cyanoacetamide in Rat Blood and Tissue

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	19 December 2016
Materials and methods	-
Conclusion	DBNPA: For an evaluation of the detection of DBNPA in blood and tissue, see RSS for A4.2d/01. CAM: Samples fortified with concentrations below 0.5 mg CAM/L rat blood (i.e. 0.1 and 0.2 mg/L blood) or 0.5 mg CAM/kg rat liver (i.e. 0.1 and 0.2 mg/kg liver) were non-quantifiable due to matrix interferences. According to ECHAs Guidance on the BPR: Vol 1 Part A, Vers 1.1 (Nov 2014), the LOQ should be set at 0.05 mg/L for body fluids and 0.1 mg/kg for tissues. Data for LOQ in rat blood and in tissue is not in line with the ECHA guidance. Acceptable recovery and linearity was however found at the reported LOQs. The RMS finds the justification from the applicant acceptable regarding interference as it is not possible to quantify lower concentrations due to instrumental sensitivity limits and matrix effects from the biological extracts. The determined LOQs for fluids and tissue are therefore accepted. The method is considered adequately validated.
Reliability	2
Acceptability	Acceptable
Remarks	
	COMMENTS FROM...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A4.3/01

Annex Point IIA, IIA-IV.4.2

Dibromoacetic Acid (DBAA): Analytical Method Determination of Test Material in Milk and Beef

Official use only

1 REFERENCE

1.1	**Reference**	Rick D. L., McClymont E. L.: DBNPA: DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF DIBROMONITRILOPROPIONAMIDE, DIBROMOACETONITRILE AND DIBROMOACETIC ACID IN MILK AND BEEF MUSCLE TISSUE, The Dow Chemical report K-078141-129, Study ID 081160 (unpublished). Marty, G. T.: Dibromoacetic Acid (DBAA): Analytical Method Determination of Test Material in Milk and Beef, The Dow Chemical Report K-008458-005, Study ID 161075 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	The Dow Chemical Company
1.2.2	Companies with letter of access	None
1.2.3	Criteria for data protection	Data on existing a. s. submitted for the first time for entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes
2.2	**GLP**	Yes
2.3	**Deviations**	The chemical purity assays of DBAA was not conducted under GLPs (vendor Certificates of Analysis purities used).

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	Fortified milk samples were prepared with weighed 1-mL aliquots of commercially available 1% fat-free milk. Stock solutions of dibromoacetic acid (DBAA) were prepared at 1.0 and 10.0 µg/mL in acetonitrile and 10 µL aliquots of these were used to fortify the milk samples to 0.01 µg/g and 0.10 (µg/g), respectively. Internal standard was then added to the samples (20 µL of 100 µg/mL ^{13}C-DBAA). The mixed samples were acidified with 20 µL of formic acid and allowed to stand at room temperature for approximately 5 minutes, followed by centrifugation at 14000 x *g* for 15 minutes. The supernatant was analysed by HPLC/-ESI/MS/MS.

Section A4.3/01

Annex Point IIA, IIA-IV.4.2

Dibromoacetic Acid (DBAA): Analytical Method Determination of Test Material in Milk and Beef

Fortified meat samples were prepared from weighed 5-g aliquots of commercially available ground beef (approximately 10% fat). Stock solutions of dibromoacetic acid (DBAA) were prepared at 1 and 10 µg/mL in acetonitrile and 50 µL aliquots of these were used to fortify the meat samples to 0.01 µg/g and 0.10 (µg/g), respectively. Internal standard was then added to the samples (20 µL of 1000 µg/mL ^{13}C-DBAA) along with 10 mL of ultrapure water and the samples were vortex-mixed for approximately 30 seconds, followed by shaking on a flat-bed shaker for 15 minutes. To separate layers, the samples were centrifuged 10 minutes at 863 x *g* and approximately 1.5 mL of the supernatant was transferred to a micro-centrifuge tube and further centrifuged at 14000 x g for 15 minutes. To filter these extracts, aliquots of the supernatants were transferred to a 30000 molecular weight cut-off centrifuge filter and centrifuged for 15 minutes at 14000 x g. The resulting supernatants were transferred to clean vials for analysis by HPLC/-ESI/MS/MS.

3.1.2 Cleanup — See section 3.1.1

3.2 Detection

3.2.1 Separation method

HPLC Conditions:
Analytical Column: Agilent Technologies Zorbax XDB-C_8, 4.6 x 150 mm
HPLC Eluent A: Ultrapure Water + 0.5% Formic Acid
HPLC Eluent B: Acetonitrile + 0.5% Formic Acid
Gradient:

Time (min)	% A	% B	Flow Rate (mL/min)
0.00	98.0	2.00	0.5
0.10	98.0	2.00	0.5
5.50	2.00	98.0	0.5
7.50	2.00	98.0	0.5

Injection Volume : 10 µL
Column Temperature : 30 °C

3.2.2 Detector

Detection by negative ion electrospray (Jet Stream) ionization and tandem mass spectrometry detection, operating in the multiple reaction monitoring (MRM) mode.

Precursor and product ions:

DBAA: Q1 mass = 216.9, Q3 mass = 172.9

^{13}C-DBAA internal standard: Q1 mass = 217.9, Q3 mass = 172.9

Dwell Time: 200 msec/ion/scan

3.2.3 Standard(s)

Quantitation of DBAA in milk and meat extracts was performed using an internal standard technique employing a stable isotope labeled

Section A4.3/01

Annex Point IIA, IIA-IV.4.2

Dibromoacetic Acid (DBAA): Analytical Method Determination of Test Material in Milk and Beef

		standard. Quantitative standards were prepared in Ultrapure water.
3.2.4	Interfering substance(s)	No interfering substances were found in extracts of control milk or control meat.
3.3	**Linearity**	
3.3.1	Calibration range	Standards of DBAA were prepared at a total of seven concentrations, ranging from 0.001 to 1.00 µg DBAA/mL diluent and containing the same amount of internal standard as the samples (~2.00 µg/mL).
3.3.2	Number of measurements	Seven calibration standards were injected three times throughout the analytical sequence. The lowest calibration standard was discarded due to a low signal response and was not needed to bracket the analyzed sample responses. Upon the sequence initiation, the instrument exhibited erratic chromatographic signal and retention time responses then stabilized after the fourth analyzed sample, the third standard of the calibration curve. The first four analytical injections will not be used. Average refit concentrations ranged from 87.8% to 114%. Relative standard deviations of refits of the 4 highest concentration standards, 0.010, 0.05, 0.2 and 1.00 µg/mL, were 10.4, 4.50, 0.546 and 0.525 % RSD, respectively. The percent relative error for the lowest two utilized calibration standards, 0.002 and 0.005, were 11.6 and 2.16 % RE, respectively.
3.3.3	Linearity	Quantitation of DBAA standards at six concentrations of 0.002 to 1.00 µg DBAA/mL diluent were analyzed in triplicate. The resulting calibration curve yielded a correlation coefficient (r^2) of 0.9995.

Section A4.3/01

Annex Point IIA, IIA-IV.4.2

Dibromoacetic Acid (DBAA): Analytical Method Determination of Test Material in Milk and Beef

3.4 Specifity: interfering substances

HPLC/MS/MS affords a highly specific method for both quantitation and confirmation of residue identity by retention time matching in conjunction with monitoring the specific MRM transition of DBAA and the ^{13}C labeled internal standard. No interferences were observed.

3.5 Recovery rates & Standard deviations at different levels

Recovery of DBAA from fortified milk and meat samples:

Matrix	Fortification Level (µg/g)	Number of Spikes Analyzed	Average Recovery (%)	Relative Standard Deviation (%)
Milk	0.0100	5	93.4%	6.96%
	0.100	5	93.9%	4.41%
	0.000	2	NQ	

3.5.1 Relative standard deviation

See section 3.5

3.6 Limit of determination

The limit of quantitation (LOQ) for the determination of DBAA in milk and meat was set equal to the lowest fortified 'spike' prepared; 0.0100 µg/g matrix. The chromatographic signal/noise for DBAA was approximately 20.6:1 for milk and 13.5:1 for meat extracts.

3.7 Precision

See section 3.5

3.7.1 Repeatability

No specific repeatability data to be generated.

3.7.2 Independent laboratory validation

No independent validation to be conducted.

Section A4.3/01

Annex Point IIA, IIA-IV.4.2

Dibromoacetic Acid (DBAA): Analytical Method Determination of Test Material in Milk and Beef

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

The method for analysis of dibromoacetic acid (DBAA) described in DBNPA: DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF DIBROMONITRILOPROPIONAMIDE, DIBROMOACETONITRILE AND DIBROMOACETIC ACID IN MILK AND BEEF MUSCLE TISSUE., D. L. Rick, E. L. McClymont., The Dow Chemical report K-078141-129, is valid for the determination of dibromoacetic acid (DBAA) in milk and meat over the concentration range of 0.1 to 1.0 µg/mL and 0.1 milk and 1.0 µg/g beef.

The method for analysis of dibromoacetic acid (DBAA) described in Dibromoacetic Acid (DBAA): Analytical Method Determination of Test Material in Milk and Beef, G. T. Marty, The Dow Chemical Report K-008458-005, Study ID 161075, is valid for the determination of dibromoacetic acid (DBAA) in milk and meat over the concentration range of 0.01 to .10 µg/mL milk and of 0.01 to .10 µg/g beef.

Milk sample aliquots (1 g) fortified with DBAA and the ^{13}C labeled internal standard were acidified. Following centrifugation, the supernatants were analyzed for DBAA by HPLC/MS/MS.

Meat sample aliquots (5 g) were fortified with DBAA and the ^{13}C labeled internal standard and vortex-mixed. Ultrapure water was added and the samples were again vortex-mixed followed by shaking on a flat-bed shaker. The samples were centrifuged, filtered and then analyzed by HPLC/MS/MS.

LC/MS/MS affords a highly specific method for both quantitation and confirmation of residue identity by retention time matching in conjunction with monitoring the specific MRM ions for the analyte and internal standard. The method response was very linear with a calibration curve correlation coefficient (r^2) of 0.9999. Recovery of DBAA from fortified milk matrix averaged 93.4% and 93.9% for milk concentrations of 0.0100 and 0.100 µg/g, respectively. Recovery of DBAA from fortified meat matrix averaged 73.8% and 82.0% for meat concentrations of 0.0100 and 0.100 µg/g, respectively.

4.2 Conclusion

The data summarized in Section 3.5 demonstrates the suitability of methods for the analysis of dibromoacetic acid (degradation product of dibromonitrilopropionamide) in milk and meat.

4.2.1 Reliability 1

4.2.2 Deficiencies None.

Section A4.3/01

Annex Point IIA, IIA-IV.4.2

Dibromoacetic Acid (DBAA): Analytical Method Determination of Test Material in Milk and Beef

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	19 December 2016
Materials and methods	Milk sample aliquots (1 g) fortified with DBAA and the ^{13}C labeled internal standard were acidified. Following centrifugation, the supernatants were analyzed for DBAA by LC/MS/MS. Meat sample aliquots (5 g) were fortified with DBAA and the ^{13}C labeled internal standard and vortex-mixed. De-ionized water was added and the samples were again vortex-mixed followed by shaking on a flat-bed shaker. The samples were centrifuged, filtered and then analyzed by LC/MS/MS.
Conclusion	The method is suitable for the determination of dibromoacetic acid (degradation product of dibromonitrilopropionamide) in milk and meat with an LOQ of 0.01 µg/g. which is in line with the requirements according to ECHAs guidance on the BPR, Vol 1, part A, annex II.
Reliability	1
Acceptability	Acceptable
Remarks	The study was post submitted in 2010.
	COMMENTS FROM...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4.2-3 Recovery of DBAA from fortified milk and meat samples:

Milk			
Fortification level [µg/g]	Average Recovery [%]	RSD [%]	n
0.1	99.5	6.92	5
1.0	102	6.19	5
Meat			
Fortification level [µg/g]	Average Recovery [%]	RSD [%]	n
0.1	97.2	5.86	5
1.0	96.6	0.597	5

Section A4.3/02

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of DBNPA and DBAN in Milk and Meat

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [X]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	**DBNPA** Attempts at validating an analytical method for the determination of dibromonitrilopropionamide (DBNPA) in milk and beef muscle tissue were unsuccessful due to the instability of DBNPA in those matrices. As summarized in Appendix A of Study 081160 of The Dow Chemical Company (Doc. No. 437-002), replicate samples of milk and meat were fortified with DBNPA (1.0 µg/mL in milk; 1.0 µg/g in meat). Samples were extracted immediately after fortification with acetonitrile (milk) or 50/50 water/acetonitrile (beef). Quantitation incorporated isotopically labelled internal standard (13C3-DBNPA). The resulting extracts were analyzed by high performance liquid chromatography (HPLC), negative ion electrospray ionization (-ESI), with mass spectrometry detection (MS). DBNPA was not detected in any of the samples thereby verifying the instability of DBNPA in the biological matrices. Refer to Appendix A of Report 081160 (Doc. No. 437-002) for complete details on the sample preparation, extraction, and analytical instrumentation employed in the attempt to validate a method for the determination of DBNPA in milk and beef. The full report 081160 will be provided in a post submission. **DBAN** Attempts at validating an analytical method for the determination of dibromoacetonitrile (DBAN) in milk and beef muscle tissue were unsuccessful due primarily to matrix interference and signal suppression (variability) in the analytical system [high performance liquid chromatography	

Section A4.3/02

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of DBNPA and DBAN in Milk and Meat

	(HPLC), negative atmospheric pressure photoionization (-APPI), with mass spectrometry detection (MS)]. As summarized in Appendix B of Study 081160 of The Dow Chemical Company (Doc. No. 437-002), a variety of extraction procedures, analytical instrumentation platforms, and analysis conditions were attempted in the unsuccessful attempts at developing this method. In general, negative APPI appeared to afford the best sensitivity, especially when analyzing DBAN prepared in simple standard matrices. However, once actual sample extracts were analyzed, peak area response was dramatically suppressed due to matrix effect and peak response became very variable. Refer to Appendix B of Report 081160 (Doc. No. 437-002) for complete details on the sample preparation, extraction, and analytical instrumentation employed in the attempt to validate a method for the determination of DBAN in milk and beef. The full report 081160 will be provided in a post submission.
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	28 May 2013
Evaluation of applicant's justification	The applicant states that attempts at validating an analytical method for the determination of dibromonitrilopropionamide (DBNPA) in milk and beef muscle tissue were unsuccessful due to the instability of DBNPA in those matrices. This is acceptable Furthermore the applicant states that attempts at validating an analytical method for the determination of dibromoacetonitrile (DBAN) in milk and beef muscle tissue were unsuccessful due primarily to matrix interference and signal suppression (variability) in the analytical system. This is acceptable. The report was received in post-submission in 2010.
Conclusion	Acceptable
Remarks	-
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*

Section A4.3/02

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification of DBNPA and DBAN in Milk and Meat

Remarks