

ANNEX B

Metam

B.5 Methods of analysis

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B.5.0 Introductory note [added in August 2010]

This document is based on the updated version of Vol.3 B.5 of the DAR, which was peer-reviewed in June 2008 (PRAPeR 51). Evaluations presented in addendum to B.5, dated June 2008, which were peer-reviewed and accepted by PRAPeR 51 (June 2008), have been incorporated in this document as well, for the sake of completeness.

Finally, revisions were made following the evaluation of the resubmission dossier introduced in 2010 according to the legal provisions of Commission Regulation (EC) No. 33/2008 and these are highlighted in yellow.

B.5.1 Analytical methods for technical active substance and formulation analysis (Annex IIA 4.1; Annex IIIA 5.1)

B.5.1.1 Analytical methods for the determination of pure active substance in the active substance as manufactured (Annex IIA 4.1.1 and 4.1.3)

- References:

- Metamsodium All qualities – Determination active ingredient based on CS₂ content (Blom & Demuyne, 2002 – Code 09E3411 revision 2)
- CIPAC method 20/13/M/1.3 - Metam-sodium aqueous solutions (CIPAC, 1967)

GLP:

No GLP-compliance stated

Principle of the method: UCB 09E3411

The content of Metam-sodium is based on the determination of CS₂ content. By addition of an acid (H₂SO₄) to Metam-sodium, CS₂ is evolved. The gaseous mixture is carried by a stream of air through a scrubber containing lead acetate solution to remove hydrogen sulfide, and then into an absorption train containing methanolic potassium hydroxide to afford xanthate. The xanthate is titrated with standard iodine.

Findings:

Cfr. CIPAC book 1B, p. 1862: "Repeatability $r = 0.85\%$, i.e. ± 0.43 on a mean value of 45.0% w/w; Reproducibility $R = 1.59\%$, i.e. ± 0.8 on a mean value of 45.0% w/w"

Conclusions:

The method is considered equivalent to CIPAC Method 20/13/M/1.3, which is an officially accepted method for the determination of metam-sodium content in metam-sodium aqueous solutions (cfr. FAO spec.). The method was used in the 5-batch analysis (cfr. C.1.2.3 - Schouten, 2002a) and the storage stability tests (cfr. B.2.2.15 and B.2.2.19).

CIPAC methods

CIPAC method 20/13/M/1.3 (revision 1967; Book 1) and CIPAC method 20/SL/M/3 (revision 1981; Books 1B and E) are available for the determination of Metam-sodium content in Metam-sodium aqueous solutions (see above). Taken into account the non-selective principle of the CS₂-evolution method, the CIPAC method can also be applied for the determination of metam-potassium in metam-potassium aqueous solutions.

B.5.1.2 Analytical methods for the determination of significant and/or relevant impurities and additives in the active substance as manufactured (Annex IIA 4.1.2 and 4.1.3)

Methods for the determination of impurities (certified max. content ≥ 1 g/kg) in metam-sodium and metam-potassium aqueous solutions are described (incl. validation data) in Annex C, point C.1.2.4. See also B.5.1.4.

There are no additives in metam-sodium 510 g/L or metam-potassium 690 g/L that need to be considered as significant or relevant, and hence, an analytical method is not deemed to be required.

B.5.1.3 Analytical methods for the determination of the active substance in plant protection products (Annex IIIA 5.1.1 and 5.1.3)

See B.5.1.1

B.5.1.4 Analytical methods for the determination of relevant impurities and formulates in plant protection products (Annex IIIA 5.1.2 and 5.1.3)

The plant protection product (ppp) does not contain any impurities or formulates that could influence significantly the overall toxicological and/or environmental risk associated with the application of the ppp.

Added in August 2010:

MITC and DMTU are considered to be relevant impurities (cf. Vol.4 C.1.2.2). As a consequence, the relevant parts of the method validation data related to these impurities have been copied from the confidential section (Vol.4, C.1.2.4) to this non-confidential section. See here below.

Principle of the method:

Determination of N,N'-dimethylthiourea (DMTU) and methylisothiocyanate (MITC) by ion-pair reversed phase HPLC-UV(236 nm): Samples were diluted 100x with HPLC mobile phase solution and analysed within 1 hour after preparation.

Validation data:Metam-sodium TK:

Table C.1.2.4-1: Validation of methods for determination of impurities (Schouten, 2002a – Report No. V3937)

Imp.	Method	Specificity/ interferences	Linearity r^2 (conc. range)	Repeatability % RSD (conc. in % w/w)	Accuracy	
					Approximate analyte conc. in spiked samples (% w/w)	Recovery range (%)
DMTU (1)	HPLC-UV	No interferences	0.999 (12-190 mg/L)	0.8 (0.26)	0.8 1.3	98.7-101.2 93.8-99.3
MITC	HPLC-UV	No interferences	0.995 (0.4-6.2 mg/L)	10.3 (0.006)	0.024 0.044	97.7-108.3 102.9-109.6

Specificity: Identity of the impurities was confirmed on the basis of retention times. No interference peak was observed at the same retention times of the impurities. For impurities DMTU and MITC, the identity was confirmed by comparison of the chromatograms of an impurity standard solution, a technical metam-sodium sample (unspiked) containing the impurity and a technical sample spiked with the impurity.

Linearity : Linearity was determined by analyzing at least 5 calibration solutions. The analytical method was shown to be linear over the investigated concentration range. For DMTU and MITC the linear calibration range covers the concentration range in the 100-fold diluted Metam-sodium batches.

Repeatability : Five replicate sample determinations at one concentration were performed. For DMTU, the RSD was found to be lower than the corresponding Horwitz value, which is in agreement with the SANCO/3030/99 rev. 4 document criteria. However, the repeatability for MITC does not meet those validation criteria. Nevertheless, the results are accepted, as MITC content of the five batches were far less than 0.1% w/w.

Accuracy : The accuracy was investigated by performing five recovery determinations on technical Metam-sodium samples to which a known volume of a stock solution of the target impurity was added. Spiking was done at 2 concentration levels. For DMTU and MITC, 5 validation samples were prepared with approximately 3-4 times the expected actual concentration. In addition, 5 spiked samples were prepared with approximately 5 times the expected actual concentration.

Metam-potassium TK:

Table C.1.2.4-2: Validation of methods for determination of impurities (Schouten, 2002b – Report No. V4691)

Imp.	Method	Specificity/ interferences	Linearity r^2 (conc. range)	Repeatability % RSD (conc. in % w/w)	Accuracy	
					Approximate analyte conc. in spiked samples (% w/w)	Recovery range (%)
DMTU (1)	HPLC-UV	No interferences	r^2 not reported (15-196 mg/L)	0.6 (0.23)	0.7 1.2	100.3-101.3 96.4-102.0
MITC	HPLC-UV	No interferences	r^2 not reported (1-13 mg/L)	8.7 (0.017)	0.05 0.08	86.4-94.2 93.7-100.0

Specificity: Identity of the impurities was confirmed on the basis of retention times. No interference peak was observed at the same retention times of the impurities. For impurities DMTU and MITC, the identity was confirmed by comparison of the chromatograms of an impurity standard solution, a technical metam-sodium sample (unspiked) containing the impurity and a technical sample spiked with the impurity.

Linearity : Linearity was determined by analyzing at least 5 calibration solutions. The analytical method was shown to be linear over the investigated concentration range. For DMTU and MITC the linear calibration range covers the concentration range in the 100-fold diluted diluted Metam-potassium batches. Correlation coefficients were however not reported.

Repeatability : Five replicate sample determinations at one concentration were performed. For DMTU, the RSD was found to be lower than the corresponding Horwitz value, which is in agreement with the SANCO/3030/99 rev. 4 document criteria. However, the repeatability for MITC does not meet the validation criteria. Nevertheless, the results can be accepted, as MITC content of the five batches was far less than 0.1% w/w.

Accuracy : The accuracy was investigated by performing five recovery determinations on technical Metam-potassium samples to which a known volume of a stock solution of the target impurity was added. Spiking was done at 2 concentration levels. For DMTU and MITC, 5 validation samples were prepared with approximately 3-4 times the expected actual concentration. In addition, 5 spiked samples were prepared with approximately 5 times the expected actual concentration.

B.5.2 Analytical methods (residue) for plants, plant products, foodstuffs of plant and animal origin, feedingstuffs (Annex IIA 4.2.1; Annex IIIA 5.2.1)

B.5.2.1 Plant origin

Added in June 2008 (< addendum dated June 2008) – peer reviewed in June 2008:

Taminco NV has obtained ~~access rights~~ **co-ownership** ~~from~~ **with** Kanesho Soil Treatment sprl/bvba ~~to~~ **of** the following analytical method for MITC in crops. A summary of the validation data is presented here below.

References:

- Validation of the analytical method 234/2: Determination of methyl iso-thiocyanate (MITC, Reg. No. 250285) in various plant matrices and soil (Benz and Mackenroth, 2003 – Report No. 2002/1004118)
- Independent laboratory validation of analytical methodology for the determination of methyl iso-thiocyanate in lettuce and lemons (Wormald, 2005 – Report No. KST.2005/001)

GLP:

GLP-compliance stated

Principle of the method: **BASF method 234/2**

Methyl isothiocyanate (MITC) is distilled from aqueous solution into a cooled reservoir containing a known amount of ethyl acetate. The aqueous distillate collected is saturated with sodium chloride and the analyte extracted into the ethyl acetate layer. The final determination of MITC is performed by GC-MS (DB-17, 30m, 0.32mm i.d., 0.25µm film; monitoring m/z: 73, 72, 70).

Findings:

Specificity – interferences:

- GC-MS is a highly specific technique. However, due to MW of MITC, use of 3 fragment ions with m/z > 100 is not possible.
- In the ILV-study report, confirmation of residue (identity) is demonstrated by comparison of ion ratios (m/z 73/72 and 70/72) in calibration standards with those in samples.
- Blank values < 30% of LOQ (demonstrated by representative chromatograms)

Linearity: Primary validation: Linear range: 0.01 µg/mL (corresponds to residue conc. 0.00054 mg/kg) – 2.5 µg/mL; $r^2 > 0.99$ (n = 7-8);

ILV: Linear range: 0.01 µg/mL – 2.5 µg/mL; $r^2 > 0.99$ (n ≥ 8);
Representative calibration graphs provided

Recovery – precision: see Table B.5.2.1-1

Validation by an independent laboratory (ILV): Same methodology as the primary validation (only minor modifications); Acceptable recoveries and precision: see Table B.5.2.1-2.

Limit of quantification (LOQ): 0.01 mg/kg (MITC)

Table B.5.2.1-1: Recovery rates and limits of quantification for MITC in various plant matrices (Benz and Mackenroth, 2003)

Matrix	Compound	Detection method	Fortification level [mg/kg]	n	Recovery rates [%]	
					Range	Mean values $\pm$ RSD
Oilseed rape seed	MITC	GC-MSD	0.01*	5	66 – 84	72.5 $\pm$ 10.9
			0.1	5	69 – 75	72.2 $\pm$ 3.5
Strawberry fruit	MITC	GC-MSD	0.01*	5	72 – 81	78.4 $\pm$ 5.1
			0.1	5	87 – 99	92.2 $\pm$ 4.7
Tomato fruit	MITC	GC-MSD	0.01*	5	78 – 89	85.2 $\pm$ 5.1
			0.1	5	88 – 92	88.4 $\pm$ 2.5
Lettuce	MITC	GC-MSD	0.01*	5	79 – 84	81.4 $\pm$ 2.6
			0.1	5	74 – 91	84.4 $\pm$ 7.4
Lemon fruit	MITC	GC-MSD	0.01*	5	73 – 88	81.0 $\pm$ 7.9
			0.1	5	81 – 103	87.1 $\pm$ 10.5
Wheat grain	MITC	GC-MSD	0.01*	5	52 – 63	57.5 $\pm$ 8.8
			0.1	5	57 – 69	61.3 $\pm$ 7.3

* Limit of quantification

Table B.5.2.1-2: Independent laboratory validation data of BASF method 234/2 for the determination of MITC in lettuce and lemons (Wormald, 2005)

Matrix	Compound	Detection method	Fortification level [mg/kg]	n	Recovery rates [%]	
					Range	Mean values $\pm$ RSD
Lettuce	MITC	GC-MSD	0.01*	5	81 – 87	84.6 $\pm$ 3.0
			0.1	5	53 – 85	71.3 $\pm$ 16.2
Lemon fruit	MITC	GC-MSD	0.01*	5	73 – 80	76.8 $\pm$ 3.4
			0.1	5	67 – 74	70.5 $\pm$ 3.7

* Limit of quantification

Conclusion:

The GC-MS method 234/2 is successfully validated (incl. ILV) for the determination of MITC in food commodities of plant origin with high water content and with high acid content. The LOQ of the method is 0.01 mg/kg.

Besides, it should be noted that the method appears not suitable for the determination of MITC in dry crops, e.g. wheat grain (poor recoveries). For food matrices with high fat content (e.g. oilseed rape seed), recoveries were also relatively low and moreover, no ILV was provided for this matrix.

Added in August 2010:

Additional validation experiments were performed using the 'BASF method no. 234/2' for determination of MITC in pepper, carrot, corn salad, cucumber, eggplant and grape. The additional validation data were provided in support of the additional residue trials (cf. Vol.3, B.7.6.1) and are therefore presented in Appendix 1.

B.5.2.1.1 Methods that do not meet the requirements

- References:

- Determination of Methyl Isothiocyanate (MITC) in crops (Meyers, 1988 – Report No. WRC 88-43)
- MITC: Independent Laboratory Confirmation of an analytical method for the determination of residues of the metabolite methyl isothiocyanate (MITC) in crops (Wheals, 1993 – Report No. RJ1508B)

GLP:

GLP-compliance stated for Wheals, 1993

Principle of the method: **WRC 88-43**

Chopped crop samples are extracted with ethyl acetate in the presence of water (or 7% aq. nitric acid for cabbage and broccoli) and sodium chloride. The resulting ethyl acetate extract is analyzed for methyl isothiocyanate using GC-NPD (DB-17 or DB-1 column; 30mx0.25mm, 0.25µm film thickness)

Findings:

Specificity – interferences: Assignment of identity by retention time match between sample and reference standard chromatogram;
No chromatographic interferences; Blank values < 30% of LOQ (demonstrated by representative chromatograms)
No confirmatory method provided

Linearity: Primary validation: Linear range: 0.01 – 1 µg/mL (corresponding to 0.01 – 1 ppm residue in sample); *no calibration graph or correlation coefficient provided*

ILV: Linear range: 0.01 – 1 µg/mL; *no calibration graph or correlation coefficient provided*

Recovery – precision: Primary validation: see Table B.5.2.1-3;

Overall average recovery (RSD) at lowest fortification level: 98.9% (7.4%); Overall average recovery (RSD) at highest fortification level: 96.7% (6.3%)

ILV: Recoveries on cabbage ranged from 89% to 100% at levels 0.028 ppm and 0.1 ppm (*only 2 determinations at each fortification level*)

Validation by an independent laboratory Primary validation by Zeneca AG Products, Western Research Center (USA);

ILV by Zeneca Agrochemicals, Jealott's Hill Research Station (UK): Same methodology as the primary validation

Limit of quantification (LOQ): 0.028 mg/kg MITC

Table B.5.2.1-3: Validation data for analytical method (GC-NPD) for determination of MITC in crops (Meyers, 1988)

Matrix	Fortification level (mg/kg) MITC*	Recovery range		RSD	
		(mean)	(range)	[%]	n
Basil (spice) (fresh)	0.028	103.3	96-112	6.6	6
	0.100	98.2	98-98	6.3	4
Bean (succulent) (seed and pot)	0.028	93.4	89-97	3.6	6
	0.100	91.7	86-97	4.8	4
Bean (succulent) (vine)	0.028	90.5	85-93	3.4	6
	0.100	94.9	93-97	1.9	4
Bean (succulent) (hay)	0.028	94.9	92-100	2.9	6
	0.100	92.0	90-96	2.9	4
Broccoli (fresh)	0.028	100.4	97-103	2.2	6
	0.100	99.8	98-103	2.4	4
Cabbage (W Wrapper)	0.028	100.3	96-106	3.4	6
	0.100	96.7	93-99	2.8	4
Cantaloup (fruit)	0.028	101.0	93-114	8.8	6
	0.100	106.2	96-115	9.5	4
Corn, sweet (sweet corn)	0.028	82.6	80-88	4.5	6
	0.100	82.8	72-88	8.7	4
Corn, sweet (forage)	0.028	97.7	96-109	1.8	6
	0.100	97.6	96-99	1.4	4
Cucumber (fruit)	0.028	106.2	103-116	4.8	6
	0.100	101.6	98-111	6.0	4
Garlic (bulb)	0.028	97.8	91-104	4.9	6
	0.100	93.3	90-96	2.5	4
Lettuce (Head) (W Wrapper)	0.028	115.5	105-124	6.3	5
Lettuce (Head) (W/O Wrapper)	0.028	109.9	106-122	6.7	5
Lettuce (Leaf) (W Wrapper)	0.028	106.7	96-118	8.8	5
Lettuce (Leaf) (W/O Wrapper)	0.028	98.6	96-104	3.2	5
Mint (hay)	0.028	99.3	97-101	1.5	6
	0.100	98.0	96-99	1.4	4
Mustard (greens)	0.028	87.6	81-92	4.8	6
	0.100	87.1	82-92	5.0	4
Onion, bulb (bulb)	0.028	91.0	88-95	2.9	6
	0.100	89.5	92-95	5.9	4
Onion, green (whole plant)	0.028	100.0	93-106	4.2	6
	0.100	105.7	99-111	5.2	4
Potato (tuber)	0.028	109.7	98-112	5.3	6
	0.100	105.2	102-108	2.4	4
Radish (fresh)	0.028	98.3	98-100	0.9	6
	0.100	99.1	97-100	1.7	4
Spinach (fresh)	0.028	91.7	89-95	3.0	6
	0.100	92.2	91-94	1.4	4
Strawberry (fresh)	0.028	95.8	93-98	1.9	6
	0.100	101.6	97-104	3.3	4
Tomato (fruit)	0.028	99.9	96-103	2.7	6
	0.100	99.1	98-100	0.8	4
Turnip (roots)	0.028	100.7	98-104	2.6	6
	0.100	99.4	96-105	4.0	4

Conclusion:

The proposed methodology for residue determination in plants does not meet the requirements of SANCO/825/00 rev. 7 (no confirmatory method provided; unclear what GC-column was used for validation;

ILV performed for only one matrix (cabbage) with only 2 determinations at each fortification level; linearity not fully addressed, etc.) and can hence not be recommended for enforcement purposes.

- **Reference:** Determination of metam-sodium in crops (McKay, 1990 – Report No. WRC 89-47)

GLP:

No GLP-compliance stated

Principle of the method:

A chopped crop sample is treated in an EDTA (ethylenediamine tetraacetate acid) solution with hydrochloric acid-stannous chloride reagent in a sealed flask. Carbon disulfide formed by decomposition of Metam-sodium is quantified by using a GC equipped with a flame photometric detector (FPD) with a bandpass filter selective for sulfur emission.

Justification:

Linearity and specificity of this method were not addressed; No ILV. Method is not specific (other dithiocarbamates like maneb, zineb, etc. are also detected by this method, which is based on decomposition into CS₂); Due to decomposition procedure, varying backgrounds of CS₂ in certain crops (e.g. cabbage, broccoli, onions, garlic), thus raising the lower detection limit. Hence, this method cannot be accepted for enforcement purposes (cfr. SANCO/825/00 rev. 7).

Added in June 2008 (< addendum dated June 2008) – peer reviewed in June 2008:

Taminco NV has obtained access rights co-ownership from with Kanesho Soil Treatment sprl/bvba to of the following analytical method for MITC in crops.

- **Reference:** Method 234: Methyl mustard oil (MITC) – Gas chromatographical determination – Soil and tomatoes (Mayer, 1985 – Report No. 1985/1000325)

GLP:

No GLP-compliance stated

Principle of the method: 234

After a steam distillation of the sample the methyl isothiocyanate (MITC) in the aqueous phase is adsorbed on a C₁₈ SPE and eluted with ethyl acetate/methyl tert-butylether (MTBE). The final determination was performed by gas chromatography with capillary column (GC-PND or GC-S-FPD).

Justification:

No linearity data (calibration plot, equation, correlation coefficient), no confirmatory method, no ILV, only 4 samples per fortification level, control values not reported.

B.5.2.2 Animal origin

Analytical methods are not required, as no MRL's are proposed.

B.5.3 Analytical methods (residue) for soil, water, air (Annex IIA 4.2.2 to 4.2.4; Annex IIIA 5.2.2 to 5.2.4)**B.5.3.1 Soil**

- **Reference:** Validation of an analytical method for the determination of methyl isothiocyanate (MITC) from air monitoring tubes and soil (Rooseboom-Reimers, 2002 – Report No. V4211) + amendments 1 to 3

GLP:

GLP-compliance stated

Principle of the method:

The analytes in soil samples were extracted with water/ethyl acetate (containing eptam as internal standard). After settling and phase separation an aliquot of ethyl acetate was transferred into a vial and analyzed by GC-NPD. The extracts were analyzed at two columns: one column used for quantification (RTX-200, 30mx0.25mm, 0.25µm film) and the other for confirmation of compound identity (CP Sil 5CB, 25mx0.25mm, 0.4µm film).

Soil matrices used for recovery tests:

- Sand (Drenthe, Grolloo 2 – Tolner): 4.5% organic matter, pH 4.8, 14.4% moisture;
- Sand (Beuling, Eerste Exlörmond 1): 7.3% organic matter, pH 4.9, 20.7% moisture;
- Sand (Roosjen, Eeserveen 1): 4.1% organic matter, pH 5.6, 13.0% moisture;

Findings:

Specificity – interferences : Confirmation of identity by using second GC column of different polarity (CP Sil 5CB) (validation data: see Table B.5.3.1-1); No chromatographic interferences; Blank values < 30% of LOQ (demonstrated by representative chromatograms)

Linearity : Linear range 0.008 – 1.0 mg/L (corresponding to 0.004 – 0.5 ppm residu in soil sample); $r^2 > 0.999$ (n=7); Calibration graphs provided

Recovery – precision : see Table B.5.3.1-1

Limit of quantification (LOQ) : LOQ = 0.02 mg/kg MITC

Table B.5.3.1-1: Validation data for analytical method for the determination of residues of MITC in soil (Rooseboom-Reimers, 2002 – V4211)

Analyte	Matrix	Method	Fortification level (mg/kg)	Recovery range [%]		RSD	
				mean	range	[%]	n
MITC	Soil	GC-NPD					
		RTX-200	0.02*	102	98.4 – 104.3	2.4	5
		CP Sil 5CB	0.02*	101	97.0 – 105.6	3.4	5
		GC-NPD					
		RTX-200	0.5	97	95.3 – 99.4	1.7	4 ^(*)
		CP Sil 5CB	0.5	100	97.6 – 102.7	2.3	4 ^(*)

* Limit of quantification

(*) The recovery of the fifth sample is a statistical outlier identified by Dixon's. Without discarding this sample, the average recovery was 93.6% with RSD of 8.8%, which is still in accordance with SANCO/825/00 rev. 7.

(*) The recovery of the fifth sample is a statistical outlier identified by Dixon's. Without discarding this sample, the average recovery was 95 % with RSD of 11.9%, which is still in accordance with SANCO/825/00 rev. 7.

Conclusions:

The GC-NPD method is sufficiently validated according to SANCO/825/00 rev. 7 and appears suitable for the determination of residues of MITC in soil. The LOQ of the method (i.e. 0.02 mg/kg) is considered sufficiently low (< general upper limit 0.05 mg/kg) and the method can thus be accepted as enforcement method.

B.5.3.1.1 Methods that do not meet the requirements

- **Reference:** Determination of Metam-sodium in soil (Meyers, 1986 – Report No. RRC 86-06)

GLP:

No GLP-compliance stated

Principle of the method:

The method is based upon the rapid conversion of Metam-sodium in soil to MITC. Frozen soil samples are extracted with ethyl acetate in the presence sodium chloride. The extracts are analyzed for MITC by capillary GC using NPD detector. The residues of Metam-sodium found are expressed as equivalent concentrations of MITC.

Justification:

Validation data are not in accordance with SANCO/825/00 rev. 7.: linearity not addressed, no confirmatory technique, samples spiked with metam-sodium although analysed for MITC, etc. Hence, this method cannot be accepted for enforcement purposes.

B.5.3.2 Water

- **Reference:** Validation of an analytical method for the determination of methyl isothiocyanate in water, according to SANCO/825/00 (Rooseboom-Reimers, 2002 – Report No. V4440) + amendment 01

GLP:

GLP-compliance stated

Test material:

- Surface water: from Amsterdam-Rhine Channel
→ *Characteristics:* pH = 7.99; TOC = 10 mg/L; silt content 500 mg/L; total hardness = 11 °D
- Drinking water: tap water
- Ground water: from Linschoten (TNO code 069904/0241)

Principle of the method:

The analytes in water samples (surface water, drinking water, groundwater) were extracted with diethyl ether. The organic layer was collected and dried over an anhydrous sodium sulphate filter. The extraction procedure was repeated twice. After the last fraction the sodium sulphate filter was washed using diethyl ether. The combined organic extracts were evaporated to somewhere below 5 ml and after addition of internal standard solution (eptam) the final extract volume was adjusted to a known volume using diethyl ether. The extracts were transferred into a GC-vial and analyzed for MITC content by GC-NPD. The extracts were analyzed at two columns: one column used for quantification (RTX-200, 30mx0.25mm, 0.25µm film) and the other for confirmation of compound identity (CP Sil 5CB, 25mx0.25mm, 0.4µm film).

Findings:

Specificity – interferences: Confirmation of identity by using second GC column of different polarity (CP Sil 5CB) (validation data: see Table B.5.3.2-1); No chromatographic interferences (demonstrated by representative chromatograms); Blank values were up to 30% of LOQ (blank correction carried out).

Linearity: Linear range 0.005 – 1.0 mg/L (corresponding to 0.05 – 10 µg/L residu in water sample); $r^2 > 0.999$ (n=6); Calibration graph provided

Lower response for matrix-matched standards (in water matrix) compared to standards in diethylether (this matrix effect was around 30% for surface water and around 10% for ground and drinking water). Matrix-matched calibration was used for validation in surface water.

Recovery – precision: see Table B.5.3.2-1

Limit of quantification (LOQ): 0.1 µg/L MITC

Table B.5.3.2-1: Validation data for analytical method for the determination of residues of MITC in water (Rooseboom-Reimers, 2002 – V4440)

Analyte	Matrix	Method	Fortification level (µg/L)	Recovery range [%]		RSD	
				mean	range	[%]	n
MITC	Surface water	GC-NPD					
		RTX-200	0.1*	83	68-99	15	5
		CP Sil 5 CB	0.1*	83	70-97	12.5	5
		GC-NPD					
		RTX-200	1	87	76-94	8	5
		CP Sil 5 CB	1	93	85-99	5.8	5
MITC	Drinking water	GC-NPD					
		RTX-200	0.1*	70	69,70	n.a.	2
		CP Sil 5 CB	0.1*	71	69,72	n.a.	2
MITC	Ground water	GC-NPD					
		RTX-200	0.1*	70	75,64	n.a.	2
		CP Sil 5 CB	0.1*	66	70,61	n.a.	2

* Limit of quantification; n.a.: not applicable

The GC-NPD method for the determination of MITC in water was fully validated in surface water (which is the most complex matrix), using matrix-matched calibration. In addition, it was demonstrated by duplicate recovery determinations at LOQ level that the method is also suitable for other water types (i.e. drinking water and ground water). The low recoveries found in drinking and ground water are probably due to the matrix effect. Better recovery would be expected, if matrix-matched calibration was applied.

Conclusions:

The method is successfully validated and appears suitable for the determination of MITC in drinking, ground and surface water. The LOQ of the method (i.e. 0.1 µg/L) is sufficiently low, since all relevant concentrations that have an impact on non-target organisms were found to be above that level (cfr. B.9.2.15). Moreover, the LOQ equals the EU drinking water limit of 0.1 µg/L. The method can thus be accepted as enforcement method in water.

B.5.3.2.1 Methods that do not meet the requirements

- **Reference:** Potassium N-methyldithiocarbamate (PNMDC): Analytical method validation in freshwater (Miles, 1992 – Report No. J9110001a)

GLP:

GLP-compliance stated

Test material:Filtered (5µm) freshwater (pH 8.3; hardness: 94 mg/L as CaCO₃)Principle of the method:

Quantitation of PNMDC (i.e. metam-potassium) was performed by HPLC (Supelco LC-18, 15 cm x 4.6 mm) using a UV-VIS detector (247 nm) and the external standard technique. The method was validated by fortifying aliquots of freshwater with PNMDC at the upper (150 µg/mL) and lower ends (0.2 µg/mL) of the expected concentration range to be used in the aquatic toxicity tests.

Justification:

Validation data (see Table B.5.3.2.1-1) are not in accordance with SANCO/825/00 rev. 7.: fortification levels are chosen inappropriately (should be LOQ and 10 x LOQ; not low enough), only 3 determinations at each fortification level, no confirmatory technique, Hence, this method cannot be accepted for enforcement purposes.

- **Reference:** Potassium N-methyldithiocarbamate (PNMDC): Analytical method validation in filtered saltwater (Miles, 1992 – Report No. J9201013a)

GLP :

GLP-compliance stated

Principle of the method :

Quantitation of PNMDC (i.e. metam-potassium) was performed by HPLC (Supelco LC-18, 15 cm x 4.6 mm) using a UV-VIS detector (247 nm) and the external standard technique. The method was validated by fortifying aliquots of filtered saltwater with PNMDC at the upper (150 µg/mL) and lower ends (1 µg/mL) of the expected concentration range to be used in the aquatic toxicity tests.

Justification:

Validation data (see Table B.5.3.2.1-1) are not in accordance with SANCO/825/00 rev. 7.: fortification levels are chosen inappropriately (should be LOQ and 10 x LOQ; not low enough), only 3 determinations at each fortification level, RSD > 20%, high recovery (110%), no confirmatory technique, Hence, this method cannot be accepted for enforcement purposes.

- **Reference:** Validation of method to determine the concentrations of PNMDC Technical in unfiltered saltwater by liquid chromatography (Miles, 1993 – Report No. J9201013b)

GLP :

GLP-compliance stated

Principle of the method :

Quantitation of PNMDC Technical was performed by HPLC (Zorbax ODS, 15cm x 4.6 mm) using a UV/VIS detector (220 nm) and the external standard technique. The method was validated by fortifying the unfiltered saltwater with PNMDC Technical at levels encompassing the upper (100 ppm) and lower ends (2.51 ppm) of the expected range of concentrations to be utilized in toxicity tests.

Justification:

Validation data (see Table B.5.3.2.1-1) are not in accordance with SANCO/825/00 rev. 7.: fortification levels are chosen inappropriately (should be LOQ and 10 x LOQ; not low enough), only 3 determinations at each fortification level, RSD > 20%, no confirmatory technique, Hence, this method cannot be accepted for enforcement purposes.

Table B.5.3.2.1-1: Validation data for analytical methods for the determination of metam-potassium in water

Reference	Analyte	Method	Matrix	Fortification level (µg/mL)	Recovery range [%]		RSD	
					mean	range	[%]	n
Miles, 1992 - J9110001a	Metam- potassium	HPLC-UV	fresh water	0.2	109	104-113	5	3
				150	106	106-107	1	3
Miles, 1992 - J9201013a	Metam- potassium	HPLC-UV	filtered saltwater	1	110	85-124	21	3
				150	84	81-88	4	3
Miles, 1993 - J9201013b	Metam- potassium	HPLC-UV	unfiltered saltwater	2.51	104	97-111	7	3
				100	110	110	0	3

B.5.3.3 Air

- Reference: Validation of an analytical method for the determination of MITC of coconut charcoal air sampling tubes (Schouten, 2007 – TNO Report No. V7111)

GLP:

GLP-compliance stated

Principle of the method:

After suction of air under prescribed conditions, coconut charcoal tubes are extracted with ethyl acetate with 5% carbon disulfide. The extracts are analysed by GC-NPD on two columns: one column (A) used for quantification (RTX-200, 30mx0.25mm, 0.25µm film) and the other (B) for confirmation of compound identity (CP Sil 5CB, 25mx0.25mm, 0.4µm film). An internal standard EPTC (5-ethyl-N,N-dipropyl thiocarbamate) was added and peak ratio was used for quantification.

Findings:

Specificity – interferences: No chromatographic interferences; Blank values < 30% of LOQ (demonstrated by representative chromatograms); Confirmation of identity by using second GC column of different polarity (CP Sil 5CB)

Linearity: Linear range 1.7 – 163 µg/L (corresponding to approximately 10 – 978 ng/tube); $r^2 > 0.999$ ($n \geq 7$); Calibration graphs provided.

Recovery – precision:

- Desorption efficiency: average 86.5% (RSD=3-7%);
- Storage stability: fortified gas adsorption tubes stored at <-18°C are stable for at least 2 months. Acceptable recovery was demonstrated: after 14 days storage mean recoveries: 80-104%; after 62 days storage mean recoveries: 91-102%.
- Back section of the fortified tubes was analysed after air passage. No significant breakthrough was observed.
- Validation recovery data for MITC (corrected for desorption efficiency and small blank values): see Table B.5.3.3-1

Limit of quantification (LOQ): MITC: 0.5 µg/m³ air (4 h worst case sampling); 0.25 µg/m³ air (8 h ambient sampling)

Table B.5.3.3-1: Validation data for GC-NPD method for the determination of MITC in air (Schouten, 2007 – Report No. V7111)

Matrix / sampling conditions	Analyte	Method	Fortification level [ng/charcoal tube]	GC-column ^a	Recovery range [%]		RSD	
					mean	range	[%]	n
Air (worst case conditions: 35°C, 80% RH, 4 hrs at 250 mL/min)	MITC	GC-NPD	30*	A	60	55-67	8	5
				B	68	64-72	5	5
			225	A	65	63-68	3	5
				B	73	72-76	2	5
			2254	A	79	76-83	3	5
				B	81	80-82	1	5
Air (ambient conditions: 19°C, 44% RH, 8 hrs at 250 mL/min)	MITC	GC-NPD	30*	A	89	82-94	5	5
				B	89	83-96	6	5
			225	A	97	89-104	7	5
				B	95	93-97	2	5
			2254	A	97	91-105	6	5
				B	99	97-101	2	5

*Limit of quantification: 30 ng/tube (= 0.5 µg/m³ for 4h (worst case) sampling ; = 0.25 µg/m³ for 8h (ambient) sampling);

^a Column A: RTX-200; Column B: CP Sil 5 CB

Conclusion:

The GC-NPD method appears suitable for the determination of MITC in air with a LOQ of 30 ng/tube. It is noted that the sampling time of the experiment for the worst-case conditions (35°C, 80% RH) was 4 hours, which is not fully in accordance with SANCO 825/00 rev. 7. However, this deviation is deemed to be of minor significance and is therefore considered to be acceptable. It is also noted that at worst-case conditions (4 h, 35°C, 80% RH, 250 mL/min), recoveries at lowest spiking level were below 70% and thus not fully in accordance with SANCO 825/00 rev. 7. However, mean recovery obtained with GC-column B was only slightly below 70% (68%) at the LOQ level. In addition, good recovery and repeatability was demonstrated in the recovery tests at ambient temperature and normal R.H. (19°C, 44% RH, 8 h, 250 mL/min). Therefore, the method is considered acceptable for enforcement purposes.

B.5.3.3.1 Methods that do not meet the requirements**- References:**

- Validation of an analytical method for the determination of methyl isothiocyanate (MITC) from air monitoring tubes and soil (Rooseboom-Reimers, 2002 – Report No. V4211) + amendments 1 to 3
- Analysis of study samples for MITC by GC-NPD from HLB study 101 and 102 (Rooseboom-Reimers, 2002 – Report No. V4294)

GLP:

GLP-compliance stated

Principle of the method:

Air monitoring tubes were emptied in a vial. To the charcoal 4 mL ethyl acetate (with 0.1% CS₂) containing eptam as internal standard was added. After clarifying at -10°C an aliquot was analyzed by GC-NPD. The extracts were analyzed at two columns: one column used for quantification (RTX-200, 30mx0.25mm, 0.25µm film) and the other for confirmation of compound identity (CP Sil 5CB, 25mx0.25mm, 0.4µm film). Recovery experiments were carried out with 80 ng and 40 ng per tube.

Justification:

Provided validation data are not fully in accordance with SANCO/825/00 rev. 7.: Temperature and relative humidity of air are not specified, nor is the exact volume of sampled air (required to establish exact LOQ of method). The study also mentions a breakthrough test, but it does not provide any details on how this was done. Extraction efficiency and storage stability are not addressed.

- Reference: Methyl isothiocyanate from metham-sodium - determination in air (Leung, 1982 – Report No. RRC-82-35)

GLP:

No GLP-compliance stated

Principle of the method:

A known volume of air is drawn through a charcoal tube via a battery-operated sampling pump. The methyl isothiocyanate present in the air is quantitatively adsorbed on the charcoal. The charcoal is then desorbed with carbon disulfide; the extract is analyzed for methyl isothiocyanate by gas chromatography with nitrogen-phosphorus alkali flame ionization detection.

Justification:

Validation data are not in accordance with SANCO/825/00 rev. 7.: calibration/linearity insufficiently addressed, no confirmatory technique, no breakthrough data, only 4 recovery determinations at each spiking level, lowest spiking level (i.e. 12.5 µg MITC/m³ air) not low enough etc. Hence, this method cannot be accepted for enforcement purposes.

- Reference: Het ontwikkelen van een bepalingmethode voor de grondontsmettingsmiddelen 1,3-dichloorpropeen en methylothiocyanat (Tuinstra *et al.*, 1986 – Report No. 86.02)

GLP:

No GLP-compliance stated

Principle of the method:

The method is based on the adsorption of MITC at charcoal followed by liquid desorption (with acetone) and GC-NPD analysis.

Justification:

The method does not comply with the provisions described in SANCO/825/00 rev. 7.: recovery insufficiently addressed, reported recovery too low (50-60%), no confirmatory technique, no breakthrough data, etc. Hence, this method cannot be accepted for enforcement purposes.

B.5.4 Analytical methods (residue) for body fluids and tissues (Annex IIA 4.2.5; Annex IIIA 5.2.5)

Notifier: “The main metabolite of Metam, Methylisothiocyanate (MITC), is the predominantly active substance against the target organisms in soil. MITC is considered to be toxic. During the rat metabolism studies with MITC, MITC was not found in measurable amounts in urine and blood. The acetyl cysteine conjugate of MITC, *N*-acetyl-S-[(methylamino)carbothioyl]cysteine, was identified as major metabolite in all rat metabolites studies and therefore an analytical method for the determination of this metabolite in **body fluids** was developed.”

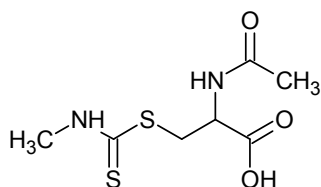
- **Reference:** Validation of BASF method 557/0: The determination of Reg. No. 5076613 (Metabolite of Dazomet) in body fluids (Grosshans, 2004 – Report No. 2004/1013738)

GLP:

GLP-compliance stated

Principle of the method : **BASF method 557/0**

A 1mL sample (blood plasma or urine) is diluted with 1mL water and applied onto an Amino SPE-cartridge. After washing, the column is combined with am Phenyl SPE-cartridge. The analyte is eluted from the Amino through the Phenyl column using a water/acetonitrile/formic acid mixture. The eluate is adjusted to the final volume and the analyte is determined by HPLC-MSD (Phenomenex Columbus C18, 250x4.6mm, 5µm; negative ionization mode, $m/z = 235$). For confirmatory purposes, LC-MS-MS is used (Phenomenex Phenyl-Hexyl, 250x4.6mm, 5µm; positive ionization mode, transitions $m/z\ 237 \rightarrow 122$ and $m/z\ 237 \rightarrow 130$). The chemical structure, IUPAC-name and molecular weight of the analyte (i.e. metabolite of MITC) are shown below:



N-acetyl-S-[(methylamino)carbothioyl]cysteine (Reg.No. 5076613)
→ MW= 236.31 g/mol

Findings :

Specificity – interferences:

- HPLC-MSD (neg. ionization mode): $m/z = 235$;
- Confirmation by HPLC-MS-MS (pos. ionization mode): transitions $m/z\ 237 \rightarrow 122$ and $m/z\ 237 \rightarrow 130$.
- no significant matrix interferences (control values < 30% of LOQ; demonstrated by representative chromatograms)

Linearity : Linear range: 2.5 ng/mL (corresponds to an analyte conc. of 0.01 mg/L body fluid) – 20 ng/mL;
 $r^2 > 0.99$ ($n = 4 \times 2$); Representative calibration graphs provided

Recovery – precision:

- HPLC-MSD: see Table B.5.4-1
- Confirmatory method (HPLC-MS-MS): For both fortification levels (0.05 mg/L and 0.5 mg/L) and for both transitions, average recoveries were found to be in range of 82 to 91%. RSD values were in range 4.5 to 8.8%.

Limit of quantification (LOQ) : 0.05 mg/L (*N*-acetyl-S-[(methylamino)carbothioyl]cysteine)

Table B.5.4-1: Validation of BASF method 557/0 for determination of acetyl cysteine conjugate of MITC in body fluids (Grosshans, 2004)

Matrix	Fortification level [mg/L]	n	Recovery rates [%]		
			Range	Mean values $\pm$ RSD	Overall recovery $\pm$ RSD
Blood plasma	0.05*	5	90 – 95	90.9 $\pm$ 5.1	84.8 $\pm$ 9.5
	0.50	5	70 – 84	78.7 $\pm$ 7.1	
Urine	0.05*	5	96 – 101	98.6 $\pm$ 2.0	95.4 $\pm$ 4.9
	0.50	5	88 – 99	92.3 $\pm$ 5.0	

* Limit of quantification

Conclusions :

BASF method 557/0 is successfully validated and considered to be suitable as enforcement method for the determination of N-acetyl-S-[(methylamino)carbothioyl]cysteine in body fluids (blood plasma and urine) down to a level of 0.05 mg/L.

- **Reference:** Development and validation of a residue analytical method for methylisothiocyanate in liver (Wolf, 2006 – RCC Study No. A68872)

GLP:

GLP-compliance stated

Principle of the method :

An amount of 10 g of the liver is weighed into a glass flask. Hexane and celite are added and the specimen is homogenised for approximately 1 minute using a high speed homogenizer. After settling, an aliquot of the extract is filtered (0.45 μ m PTFE filter) and subsequently analysed by GC-NPD (DB Wax, 100% polyethylene glycol, 40m x 0.32 mm x 0.5 μ m film).

Findings :

Specificity – interferences :

- Confirmation of identity by using second GC column of different polarity (RTX 1701, ((14%-cyanopropyl)phenyl)-methylpolysiloxane, 30 m x 0.25 mm x 0.1 μ m film)
- no significant matrix interferences (demonstrated by representative chromatograms)

Linearity : Linear range: 0.01 μ g/mL (corresponds to an analyte conc. of 0.05 mg MITC/kg liver) – 1 μ g/mL; $r^2 > 0.99$ (n = 7); Analysis of spiked control extract showed that there is no significant matrix influence; Representative calibration graphs provided

Recovery – precision: see Table B.5.4-2

Limit of quantification (LOQ): 0.1 mg/kg (MITC)

Table B.5.4-2: Validation data for analytical method for the determination of MITC in liver (Wolf, 2006)

Analyte	Matrix	Method	Fortification level (mg/kg)	Recovery range [%]		RSD	
				mean	range	[%]	n
MITC	Liver	GC-NPD					
		DB Wax	0.1*	80	70 – 89	9	5
		RTX 1701	0.1*	98	96 – 100	2	5
		GC-NPD					
		DB Wax	1.0	95	91 – 100	4	5
		RTX 1701	1.0	95	94 – 99	2	5

* Limit of quantification

Conclusion:

The method is sufficiently validated and appears suitable for the determination of MITC in liver with a LOQ of 0.1 mg/kg.

B.5.5 Evaluation and assessment**B.5.5.1 Analytical methods for technical active substance and formulation analysis**

The submitted methods allow determination of the a.i. content and the content of significant and relevant impurities in the aqueous metam-sodium solution (TK) and in the aqueous metam-potassium solution (TK).

Table B.5.5.1-1 : Summary of analytical methods for technical active substance and formulation analysis

Matrix	Analyte	Type of method	Validation ^a	References
Technical active substance (metam-sodium or metam-potassium)	Metam-sodium	CS ₂ -evolution method (CIPAC method 20/13/M/1.3)	Full validation	UCB 09E3411 (Blom & Demuynck, 2002)
	Metam-potassium			UCB 09E3D1
	MITC and Impurity 1 (DMTU)	HPLC-UV	Full validation	(Schouten, 2002a/b)
	Impurity 2	Titration	Full validation	UCB 09E3415 / UCB 09E3414 (Schouten, 2002a) UCB 09E3D5 / UCB 09E3D4 (Schouten, 2002b)
	Impurity 3a/b	Ion chromatography	Full validation	(Schouten, 2002a/b)
Formulations METAM-SODIUM 510 g/L METAM-POTASSIUM 690 g/L	Metam-sodium	CS ₂ -evolution method (CIPAC method 20/13/M/1.3)	Full validation	UCB 09E3411 (Blom & Demuynck, 2002)
	Metam-potassium			
	Impurities MITC and DMTU	HPLC-UV	Full validation	(Schouten, 2002a/b)

^a "Full validation" means that the data package is fully in accordance with SANCO/3030/99 rev. 4, or that deviations are minor and considered to be acceptable.

CIPAC methods

CIPAC method 20/13/M/1.3 (revision 1967; Book 1) and CIPAC method 20/SL/M/3 (revision 1981; Book E) are available for the determination of metam-sodium content in metam-sodium aqueous solutions.

Taken into account the non-selective principle of the CS₂-evolution method, the CIPAC method can also be applied for the determination of metam-potassium in metam-potassium aqueous solutions.

B.5.5.2 Analytical methods (residue) for food and feed

An enforcement method is required for the determination of MITC in *food of plant origin*. MRL will be set at LOQ of the method. ~~Fully validated enforcement methodology was not provided and thus remains to be submitted.~~

Added in June 2008 (< addendum dated June 2008) – peer reviewed in June 2008:

Taminco NV has obtained ~~access rights~~ **co-ownership from** with Kanesho Soil Treatment sprl/bvba ~~to~~ of the following analytical method for MITC in crops.

The GC-MS method 234/2 has been sufficiently validated (incl. ILV) for the determination of MITC in food commodities with high water content and with high acid content. The LOQ of the method is 0.01 mg/kg (MITC).

Table B.5.5.2-1 : Summary of analytical methods (residue) for food

Matrix	Analyte	Type of method	Method range (mg/kg commodity)	Validation ^b	References
Plant origin					
Foodstuff with high water content (strawberry, tomato, lettuce)	MITC	GC-MS (conf. method: comparison of ion ratio)	0.01* – 0.1	Full validation → enforcement	BASF method 234/2 (Benz & Mackenroth, 2003)
Foodstuff with high acid content (lemon)					ILV (Wormald, 2005)

* LOQ = limit of quantification

^b “Full validation” means that the data package is fully in accordance with SANCO/825/00 rev. 7, or that deviations are minor and considered to be acceptable

No MRL's are proposed for food of *animal origin* and therefore, no enforcement method is required.

B.5.5.3 Analytical methods (residue) for soil, water and air

Table B.5.5.3-1: Summary of analytical methods (residue) for soil, water and air

Matrix	Analyte	Type of method	Method range (mg/kg commodity)	Validation ^b	References
Soil	MITC	GC-NPD (conf. technique: column of different polarity)	0.02* – 0.5	Full validation → enforcement	(Rooseboom- Reimers, 2002 – Report No. V4211)
Surface water Drinking water Ground water	MITC	GC-NPD (conf. technique: column of different polarity)	0.1* – 1.0 µg/L	Full validation → enforcement	(Rooseboom- Reimers, 2002 – Report No. V4440)
Air 35°C, 80%RH (4h, 250 mL/min) 19°C, 44% RH (8h, 250 mL/min)	MITC	GC-NPD (conf. technique: column of different polarity)	30* – 2254 ng/tube 0.5* – 38 µg/m ³ 0.25* – 19 µg/m ³	Full validation → enforcement	(Schouten, 2007 – Report No. V7111)

^b “Full validation” means that the data package is fully in accordance with SANCO/825/00 rev. 7, or that deviations are minor and considered to be acceptable.

* LOQ = limit of quantification

The LOQ of the GC-NPD method (Rooseboom-Reimers, 2002 – Report No. V4211), i.e. 0.02 mg/kg, is sufficiently low for enforcement of the general upper limit of 0.05 mg/kg for **MITC** in **soil**.

The LOQ of the GC-NPD method (Rooseboom-Reimers, 2002 – Report No. V4440), i.e. 0.1 µg/L, is sufficiently low for enforcement of the (drinking) water limit of 0.1 µg/L for **MITC** in **water**. Furthermore, all relevant concentrations that have an impact on non-target organisms were found to be above the LOQ.

Fully validated GC-NPD method (Schouten, 2007) allows determination of **MITC** in **air** with a LOQ of 0.5 µg/m³ and thus enables enforcement of the relevant residue limit (C = 1.2 µg/m³, based on proposed AOEL_{systemic} of 0.004 mg/kg b.w./day). It is noted that the sampling time of the experiment for the worst-case conditions (35°C, 80% RH) was 4 hours, which is not fully in accordance with SANCO/825/00 rev. 7. However, this deviation is deemed to be of minor significance and is therefore considered to be acceptable.

B.5.5.4 Analytical methods (residue) for body fluids and tissues

Table B.5.5.4-1 : Summary of analytical methods (residue) for body fluids

Matrix	Analyte	Type of method	Method range (mg/kg commodity)	Validation ^b	References
Body fluids (blood plasma, urine)	acetyl cysteine conjugate of MITC: N-acetyl-S-[(methylamino)carbothioid]cysteine	HPLC-MS (conf. technique: HPLC-MS-MS)	0.05* – 0.5 mg/L	Full validation	BASF method 557/0 (Grosshans, 2004)
Body tissues (liver)	MITC	GC-NPD (conf. technique: column of different polarity)	0.1* – 1.0 mg/kg	Full validation	(Wolf, 2006)

* LOQ = limit of quantification

^b “Full validation” means that the data package is fully in accordance with SANCO/825/00 rev. 7, or that deviations are minor and considered to be acceptable.

The submitted HPLC-MS method 557/0 allows determining the **acetyl cysteine conjugate of MITC** (i.e. N-acetyl-S-[(methylamino) carbothioid]cysteine) in **body fluids** (blood plasma and urine) at a LOQ of 0.05 mg/L.

The fully validated GC-NPD method (Wolf, 2006) allows determining **MITC** in **liver** at a LOQ of 0.1 mg/kg.

B.5.6 References relied on**B.5.6.1 Analytical methods for the active substance**

Annex point / reference number	Author(s)	Date	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 4.1.1 /01	Blom P, Demuyneck M	2002-08	Metamsodium- All Qualities. Active ingredient based on CS2 content UCB, 09E3411 Not GLP Not published	Y	Taminco
IIA 4.1.1 /02	CIPAC	1967	CIPAC method 20/13/M/1.3 Metam-sodium aqueous solutions CIPAC, 20/13/M/1 Not GLP published	N	public
II A 4.2.1 /05 [added in June 2008 – peer reviewed in June 2008]	Benz A., Mackenroth C.	2003	Validation of the analytical method 234/2: Determination of Methyl iso-thiocyanate (MITC, Reg.No. 250 285) in various plant matrices and soil BASF AG, Agrarzentrum Limburgerhof; Limburgerhof, Germany Fed.Rep. 2002/1004108 Yes unpublished	Y	KST Taminco (access)
II A 4.2.1 /06 [added in June 2008 – peer reviewed in June 2008]	Wormald, S.	2005	Independent laboratory validation of analytical methodology for the determination of methyl iso-thiocyanate in lettuce and lemons. Central Science Laboratory (CSL), Sand Hutton, York, United Kingdom CSL Study No. PGD-206 KST 2005/001 Yes unpublished	Y	KST Taminco (access)
IIA 4.2.2 /01	Rooseboom- Reimers A	2002-06-27	Validation of an analytical method for the determination of methyl isothiocyanate (MITC) from air monitoring tubes and soil + amendments 1 to 3 TNO Nutrition and Food Research, TNO V4211 GLP Not published	Y	Taminco, FMC Foret, Lainco
IIA 4.2.3 /01	Rooseboom- Reimers A	2002-10-09	Validation of an analytical method for the determination of methyl isothiocyanate in water, according to SANCO/825/00 + amendment 01 TNO Nutrition and Food Research, V4440 GLP Not published	Y	Taminco; FMC Foret, Lainco

Annex point / reference number	Author(s)	Date	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 4.2.4 /05	Schouten, A.	2007-05-22	Validation of an analytical method for the determination of MITC on coconut charcoal air sampling tubes TNO Report V7111 GLP Not published	Y	Taminco
IIA 4.2.5 /01	Grosshans F	2004-05-19	Validation of BASF Method 557/0: The determination of Reg.No. 507 6613 (Metabolite of Dazomet) in body fluids BASF AG, 2004/1013758 GLP Not published	Y	KST, Taminco
IIA 4.2.5 /02	Wolf, S.	2006-10-27	Development and validation of a residue analytical method for methylisothiocyanate in liver RCC Study number A68872 GLP Not published	Y	Taminco

B.5.6.2 Analytical methods for the formulation METAM-SODIUM 510 g/L

None

Appendix 1:

Analytical methods (residue) in support of pre-registration data requirements

Added in August 2010:

Additional validation experiments were performed using the 'BASF method no. 234/2' (cf. B.5.2.1) for determination of MITC in pepper, carrot, corn salad, cucumber, eggplant and grape in support of the additional residue trials (cf. Vol.3, B.7.6.1).

- Validation of analytical methodology for the determination of methyl isothiocyanate in carrot and corn salad (Wormald, 2009 – Study No. FSGD-052)

- Validation of analytical methodology for the determination of methyl isothiocyanate in cucumber, egg plant and grape (Wormald, 2010 – Study No. FSGD-082)

- Residue of Methyl Isothiocyanate (MITC) in lettuce, pepper, tomato at harvest in protected crop following one application of Tamisol 510 in pre-planting Italy, 2008 (Miserocchi, 2008 – Study No. 08083RMC – Analytical phase report)

GLP:

GLP-compliance stated

Principle of the method: BASF method 234/2

Methyl isothiocyanate (MITC) is distilled from aqueous solution into a cooled reservoir containing a known amount of ethyl acetate. The aqueous distillate collected is saturated with sodium chloride and the analyte extracted into the ethyl acetate layer. The final determination of MITC is performed by GC-MS (DB-17, 30m, 0.32mm i.d., 0.25µm film; electron ionisation (EI), monitoring ions with m/z: 73 (for quantitation), 72 and 70).

Findings:

Specificity – interferences:

- No significant chromatographic interferences were observed for the quantifier ion m/z = 73, i.e. blank values were < 30% of LOQ (demonstrated by representative chromatograms).
- GC-MS is a highly specific technique. However, due to MW of MITC, use of 3 fragment ions with m/z > 100 is not possible.
- Confirmation of residue (identity) can be done by comparison of ion ratios (m/z 73/72 and 70/72) in samples with those in calibration standards. However, due to a significantly interfering peak observed for ion m/z = 70 in the control samples of corn salad, cucumber, eggplant and grape, confirmation of MITC residues in these matrices appears to be not possible using this technique.

Linearity: Linear range: 0.01 µg/mL (corresponds to residue conc. 0.0005 mg/kg) – 2.5 µg/mL; $r^2 = 0.9988$ (n=8); series of MITC solvent standards

Recovery – precision: see Table 1

Limit of quantification (LOQ): 0.01 mg/kg (MITC)

Table 1: Recovery rates and limits of quantification for MITC in various plant matrices (Wormald, 2009; Wormald, 2010)

Matrix	Compound	Type of method	Fortification level [mg/kg]	n	Recovery rates [%]	
					Range	Mean values $\pm$ RSD
Carrot	MITC	GC-MSD	0.01*	5	71.1 – 83.7	76.5 $\pm$ 6.3
			0.1	5	55.2 – 77.5	71.8 $\pm$ 13
Corn salad	MITC	GC-MSD	0.01*	5	65.3 – 83.6	73.5 $\pm$ 11.7
			0.1	5	74.1 – 78.9	76.7 $\pm$ 2.9
Cucumber	MITC	GC-MSD	0.01*	5	80.9 – 87.1	83.2 $\pm$ 3.7
			0.1	5	63.4 – 77.1	71.6 $\pm$ 7.4
Eggplant	MITC	GC-MSD	0.01*	5	84.8 – 88.1	86.1 $\pm$ 1.5
			0.1	5	75.0 – 83.0	80.5 $\pm$ 4.1
Grape	MITC	GC-MSD	0.01*	5	72.8 – 82.9	78.4 $\pm$ 5.4
			0.1	5	77.5 – 85.1	82.1 $\pm$ 3.8
Pepper	MITC	GC-MSD	0.01*	5	80.1 – 86.0	83.5 $\pm$ 2.6
			0.1	5	86.8 – 109.0	93.2 $\pm$ 9.8

* Limit of quantification

Conclusion:

The validation data meet the requirements of SANCO/3029/99 rev. 4 and show that the GC-MS method 234/2 is suitable for the quantitative determination of MITC residues in carrot, corn salad, cucumber, eggplant, grape and pepper with a LOQ of 0.01 mg/kg. It is noted that the method does not allow an unequivocal positive confirmation of MITC residues in corn salad, cucumber, eggplant and grape due to the presence of a significant chromatographic interference in these matrices. However, the method is considered sufficiently validated for the use in residue trials.

Added in August 2010:

A new method was developed and validated for the determination of DMTU residues in various crops. This method was used in the new residue trials provided in the resubmission dossier (cf. Vol.3, B.7.6.1). The method was also validated for the determination of DMTU in soil.

- Validation of analytical methodology for the determination of N,N'-dimethylthiourea in carrot, corn salad, tomato and soil (Wormald, 2009 – Study No. FSGD-051)

- Validation of analytical methodology for the determination of N,N'-dimethylthiourea in cucumber, eggplant, pepper, strawberry, potato and grape (Wormald, 2010 – Study No. FSGD-081)

GLP:

GLP-compliance stated

Principle of the method:

N,N'-dimethylthiourea (DMTU) is extracted from homogenised samples of the commodity (carrot, corn salad, tomato, cucumber, eggplant, pepper, strawberry, potato, grape and soil) using water (with 5% w/w Na₂CO₃ for soil, carrot, cucumber, eggplant, pepper, strawberry, potato and grape). Following centrifugation, the extracts are filtered, collected and diluted with water. Analysis is performed by means of LC-MS/MS (Waters Atlantis dC18, 3 μ m, 50 x 2.1 mm; isocratic elution with 10 mM ammonium acetate / methanol (95/5); Atmospheric Pressure Chemical Ionisation (APCI); ion transition monitored: m/z 105 $\rightarrow$ 74). Quantification is based on matrix-matched calibration (for matrices soil, carrot, corn salad, tomato, cucumber and strawberry) or on internal standard calibration with ETU d4 (ethylenethiourea; C₃H₂N₂SD₄) as internal standard (for matrices eggplant, pepper, potato and grape).

Findings :**Specificity – interferences :**

- LC-MS/MS is a highly specific technique. Beside the transition monitored for quantification (m/z 105 $\rightarrow$ 74), an additional transition is suggested for confirmatory purposes (m/z 105 $\rightarrow$ 59), but data demonstrating the suitability of this second mass transition were not presented in the report.
- No significant chromatographic interferences were observed, i.e. blank values were < 30% of LOQ (demonstrated by representative chromatograms).

Linearity : Series of matrix-matched calibration standards showed a linear response within the concentration range 0.00015 $\mu\text{g/mL}$ – 0.01 $\mu\text{g/mL}$ (corresponding to residue conc. 0.003 – 0.2 mg/kg); $r^2 \geq 0.99$ (n=3 to 5; duplicate determinations);
Exception was soil, where linearity was only demonstrated in the range 0.00015 – 0.0015 $\mu\text{g/mL}$ (corresponding to residue conc. 0.003 – 0.03 mg/kg);
Where internal standard (IS) was used, peak area ratio (DMTU vs. IS) showed a linear correlation with the concentration within at least the range 0.00015 to 0.005 $\mu\text{g/mL}$; $r^2 \geq 0.9869$ (n=4x2)

Recovery – precision : see Table 2

Limit of quantification (LOQ): 0.01 mg/kg (DMTU)

Table 2: Recovery rates and limits of quantification for DMTU in various plant matrices (Wormald, 2009; Wormald, 2010)

Matrix	Compound	Type of method	Fortification level [mg/kg]	n	Recovery rates [%]	
					Range	Mean values $\pm$ RSD
Soil	DMTU	LC-MS/MS	0.01*	5	79.3 – 84.9	81.0 $\pm$ 2.8
			0.1	5	87.6 – 134.9	105.0 $\pm$ 16.9
Carrot	DMTU	LC-MS/MS	0.01*	5	91.2 – 100.1	97.1 $\pm$ 4.0
			0.1	5	95.4 – 110.4	104.6 $\pm$ 6.3
Corn salad	DMTU	LC-MS/MS	0.01*	5	67.3 – 84.1	75.2 $\pm$ 8.1
			0.1	5	76.5 – 89.0	84.5 $\pm$ 6.0
Tomato	DMTU	LC-MS/MS	0.01*	5	98.8 – 103.6	101.7 $\pm$ 2.0
			0.1	5	104.0 – 107.0	105.0 $\pm$ 1.1
Cucumber	DMTU	LC-MS/MS	0.01*	5	82.8 – 89.8	86.6 $\pm$ 3.3
			0.1	5	82.6 – 92.8	88.1 $\pm$ 5.1
Eggplant	DMTU	LC-MS/MS	0.01*	5	64.4 – 95.0	78.4 $\pm$ 17.9
			0.1	5	64.7 – 80.0	70.8 $\pm$ 8.1
Pepper	DMTU	LC-MS/MS	0.01*	5	70.6 – 102.4	81.5 $\pm$ 16.1
			0.1	5	81.4 – 95.4	88.6 $\pm$ 6.2
Strawberry	DMTU	LC-MS/MS	0.01*	5	81.0 – 97.8	91.6 $\pm$ 7.3
			0.1	5	85.2 – 110.0	93.4 $\pm$ 10.9
Potato	DMTU	LC-MS/MS	0.01*	5	73.4 – 81.6	77.1 $\pm$ 4.0
			0.1	5	87.5 – 100.8	92.5 $\pm$ 5.7
Grape	DMTU	LC-MS/MS	0.01*	5	70.8 – 78.2	72.7 $\pm$ 4.3
			0.1	5	86.9 – 96.6	92.9 $\pm$ 4.3

* Limit of quantification

Conclusion:

The validation data meet the requirements of SANCO/3029/99 rev. 4 and show that the LC-MS/MS method is suitable for the quantitative determination of DMTU residues in carrot, corn salad, tomato, cucumber, eggplant, pepper, strawberry, potato and grape with a LOQ of 0.01 mg/kg. The method is considered sufficiently validated for the use in residue trials. Acceptable validation data were also presented for the method for determining DMTU in soil, although it is to be noted that the demonstrated linearity of the detector response did not cover the full fortification range (highest fortification level not covered).

Added in August 2010:

A method based on BASF method no. 234/2, which was used for determining MITC residues in various plant matrices (cf. B.5.2.1 and appendix 1), was validated for the determination of MITC in **arthropods**. This method was used in a study on the effects of secondary poisoning (Annex point IIIA 10.1.4), in which the level of MITC in arthropods sampled in a field treated with metam-sodium was determined. Reference is made to Vol.3, B.9.1.7.

- Validation of analytical methodology for the determination of methyl isothiocyanate in arthropods (Wormald, 2009 – Study No. FSGD-053)

Principle of the method: based on BASF method 234/2

Methyl isothiocyanate (MITC) is distilled from aqueous solution into a cooled reservoir containing a known amount of ethyl acetate. The aqueous distillate collected is saturated with sodium chloride and the analyte extracted into the ethyl acetate layer. The final determination of MITC is performed by GC-MS (DB-17, 30m, 0.32mm i.d., 0.25µm film; monitoring ions with m/z: 73 (for quantitation), 72 and 70).

Findings:**Specificity – interferences:**

- No significant chromatographic interferences were observed, i.e. blank values were < 30% of LOQ (demonstrated by representative chromatograms).
- GC-MS is a highly specific technique. However, due to MW of MITC, use of fragment ions with m/z > 100 is not possible.
- Confirmation of residue (identity) can be done by comparison of ion ratios (m/z 73/72 and 70/72) in samples with those in calibration standards. This was demonstrated for the fortified samples.

Linearity: Linear range: 0.01 µg/mL – 1 µg/mL (corresponds to residue conc. 0.01 – 1 mg/kg); $r^2 = 0.9978$ (n=7); series of MITC solvent standards

Recovery – precision: see Table 3

Limit of quantification (LOQ): 0.05 mg/kg (MITC)

Table 3: Recovery rates and limits of quantification for MITC in arthropods (Wormald, 2009)

Matrix	Compound	Type of method	Fortification level [mg/kg]	n	Recovery rates [%]	
					Range	Mean values ± RSD
Arthropods (beetles)	MITC	GC-MS	0.05*	5 ^a	73.3 – 76.9	75.0 ± 2.1
			0.5	5	58.9 – 77.6	71.6 ± 10.2

* Limit of quantification; ^a recovery figures based on n=4 (1 outlier identified by Dixon's test)

Conclusion:

The validation data meet the general requirements of SANCO/3029/99 rev. 4 and show that the GC-MS method 234/2 is suitable for the quantitative determination of MITC residues in arthropods (validated with beetles) with a LOQ of 0.05 mg/kg. The method also allows a positive confirmation of MITC residues in beetles by means of ion ratio comparison.

References Appendix 1

Annex point / reference number	Author(s)	Date	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 4.2.1/07	Wormald S.	2009-07-17	Validation of Analytical Methodology for the Determination of Methyl Iso-Thiocyanate in Carrot and Corn salad. FERA, York, U.K. Report No. FSGD-052 GLP: yes Not published	Y	Taminco
IIA 4.2.1/08	Wormald S.	2010-02-03	Validation of Analytical Methodology for the Determination of Methyl Iso-Thiocyanate in Cucumber, Egg Plant and Grape FERA, York, U.K. Report No. FSGD-082 GLP: yes Not published	Y	Taminco
IIA 6.3/22	Miserocchi G.	2008-10-14	Residue of methyl isothiocyanate (MITC) in lettuce, pepper and tomato at harvest in protected crop following one application of Tamisol 510 in pre-planting, Italy, 2008 S.P.F. GAB Italia s.r.l., Ferrara, Italy Report No. 08083RMC - I132 GLP: yes Not published	Y	Taminco
IIA 4.2.1/09	Wormald S.	2009-09-17	Validation of analytical methodology for the determination of N,N'-dimethylthiourea in carrot, corn salad, tomato and soil FERA, York, UK Report No. FSGD-051 GLP: yes Not published	Y	Taminco
IIA 4.2.1/10	Wormald S.	2010-02-03	Validation of analytical methodology for the determination of N,N'-dimethylthiourea in Cucumber, Egg Plant, Pepper, Strawberry, Potato and Grape FERA, York, UK Report No. FSGD-081 GLP: yes Not published	Y	Taminco
IIA 4.2.2/03	Wormald S.	2009-09-17	Validation of analytical methodology for the determination of N,N'-dimethylthiourea in carrot, corn salad, tomato and soil FERA, York, UK Report No. FSGD-051 GLP: yes Not published	Y	Taminco

Metam
Belgium

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Annex point / reference number	Author(s)	Date	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
	Wormald S.	2009-12-03	Validation of analytical methodology for the determination of Methyl Iso-Thiocyanate in arthropods FERA, York, UK Report No. FSGD-053 GLP: yes Not published	Y	Taminco

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