ANNEX B

Thiram

Addendum to the monograph

November 2002

B.5 Methods of analysis

B.5.2 Analytical methods (residue) for food and feed (Annex IIA 4.2.1; Annex IIIA 5.2.1)

As the evaluation of new information regarding the presence of metabolite M1 in the metabolism of the rat has led to confirmation of the provisional residue definition (= Thiram), a specific method to determine M1 is not necessary and the HPLC-method for determination of Thiram that was included in the monograph, is acceptable and sufficient.

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 (Annex IIA 4.2.2)

- Analytical method for determining Thiram® in soil (Blaszczynski, 2001)

GLP :
GLP-compliance stated
Principle of the method :
Soil samples are extracted with methylene chloride followed by a mixture of acetonitrile and methylene chloride. The extract containing Thiram is reduced in volume on a rotary evaporator, transferred to a graduated centrifuge tube and centrifuged to separate suspended impurities from the solution.
Residues of Thiram are determined by HPLC (Phenomenex Luna ODS (2), 250 x 4.6 mm, 5µ; isocratic elution : 0.01M ammonium acetate (pH 3.5)/methanol 50:50) with an electrochemical detector; quantification by external standard method. Confirmatory analysis by LC/MS (Phenomenex Ultracarb 5 ODS 30, 150 x 2 mm; gradient elution : 0.05M formic acid/methanol) with MS operated in positive ion mode under MRM conditions (m/z 241 → m/z 88 and m/z 120).
Findings :
Specificity – interferences : - specificity is demonstrated by LC-MS confirmatory method
- no significant matrix interferences (control values < 30% of LOQ)

Linearity : response of LC-ECD system to Thiram (peak area vs. conc.) was demonstrated to be linear within a concentration range of resp. 160 to 240 µg/L (n=3x2) and 400 to 600 µg/L (n=3x2); r = 0.994 – 0.997 and 0.993 – 0.998 resp.
Recovery – precision : see Table B.5.3.1-1
Limit of determination (LOQ) : 0.05 mg/kg
Conclusions :
Method is suitable for the determination of Thiram residues in soil with a LOQ of 0.05 mg/kg.

Table B.5.3.1-1 : Validation of method for soil (Blaszczynski, 2001)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
soil (sandy loam)	thiram	0.05[1]	5	69 – 83	79	7.1
		0.05[2]	5	78 – 88	84	4.3
		0.5[1]	5	91 – 102	98	4.4
		0.5[2]	5	89 – 94	91	2.2

[1] : day 1
[2] : day 2

B.5.3.2 Water (Annex IIA 4.2.3)

- Analytical method for determination of Thiram in pond water (Blaszczynski, 2000)

GLP :
GLP-compliance stated
Principle of the method :
Water samples are passed through a SPE C-18 cartridge, under vacuum. Thiram is retained on the cartridge and eluted with acetonitrile.
Residues of Thiram are determined by HPLC (Phenomenex Luna ODS (2), 250 x 4.6 mm, 5µ; isocratic elution : acetonitrile/0.01M ammonium acetate (pH 3.5) 50:50) with an electrochemical detector; quantification by external standard method. Confirmatory analysis by LC/MS (Phenomenex Ultracarb 5 ODS 30, 150 x 2 mm; gradient elution : 0.05M formic acid/methanol) with Selected Ion Monitoring (m/z 241).
Findings :
Specificity – interferences : - specificity is demonstrated by LC-MS confirmatory method
- no significant matrix interferences (control values < 30% of LOQ)

Linearity : response of LC-ECD system to Thiram (peak area vs. conc.) was demonstrated to be linear within a concentration range of resp. 16 to 24 µg/L (n=3x2) and 32 to 48 µg/L (n=3x2); r = 0.992 – 0.991 and 0.997 – 0.992 resp.

Recovery – precision : see Table B.5.3.2-1
Note : No fortification experiments were performed with drinking water, but as the method permits the reliable determination of Thiram residues in *surface water* at the level of 0.1 µg/L → no problem to obtain level of 0.1 µg/L in *drinking water*

Limit of determination (LOQ) : 0.1 µg/L
Conclusions :
Method is suitable for the determination of Thiram residues in water (surface water as well as drinking water) with a LOQ of 0.1 µg/L.

Table B.5.3.2-1 : Validation of method for water (Blaszczynski, 2000)

Matrix	Analyte	Fortification level (µg/L commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
pond water	Thiram	0.1[1]	5	89 – 92	90	1.2
		0.1[2]	5	74 – 87	80	6.1
		1[1]	5	85 – 109	100	9.3
		1[2]	5	80 – 85	83	2.4

[1] : day 1
[2] : day 2

B.5.3.3 Air (Annex IIA 4.2.4)

- Development and validation of an analytical method for the determination of Thiram in air (Class, 2001)

GLP :
GLP-compliance stated
Principle of the method :
Air is sucked through XAD® adsorption tubes (filled with 2 portions of XAD-2 porous polymer, separated from each other by a plug of glass wool) at a flow rate of approx. 1.6 L/min during a period of 6 hrs (air sampled : ca. 0.6 m^3), after which the 2 portions of the adsorption material are extracted separately with acetonitrile.
Thiram residues in the extract are determined by LC-MS/MS (Phenomenex Luna RP-C_{18}, 250 x 3 mm, 5µm; gradient elution : methanol/ 0.1% formic acid in water) with APCI, monitoring characteristic parent-daughter ion fragmentation (m/z 241 → 196). Quantification by external standard method.

Findings :

Specificity – interferences : - LC-MS/MS is highly specific → no need for additional confirmatory method
- no significant matrix interferences (control values < 30% of LOQ)

Linearity : response of LC-MS/MS system to Thiram (peak area vs. conc.) was demonstrated to be linear within a concentration range of 0.015 to 1.5 mg/L (n=8; 5 conc. levels); $r^2 = 0.9997$

Recovery – precision : see Table B.5.3.3-1 (dynamic retention efficiency)

Storage stability : demonstrated for at least 5 days in a freezer at < -23°C

Limit of determination (LOQ) : 2.6 µg/m^3

Conclusions :

Method is suitable for the determination of Thiram residues in air with a LOQ of 2.6 µg/m^3.

Table B.5.3.3-1 : Validation of method for air (Class, 2001)

Matrix	**Analyte**	**Fortification level (µg/m^3 commodity)**	**Recovery**			
			Number of samples	**Range (%)**	**Mean (%)**	**RSD (%)**
ambient air (24°C, 22% RH)	Thiram	2.5	5	89 – 104	95	7
		27	5	96 – 108	104	5
warm, humid air (36°C, 86% RH)		2.6	5	80 – 95	88	8
		27	5	82 – 89	87	3
breakthrough (i.e. recovery of a.s. in back portion of adsorption tubes fortified at 27 µg/m^3) : < 2%						

B.5.6 References relied on

Annex point	Author(s)	Year	Title, Generated by (company or organisation), Submitted by (company(ies) or organisation), Report/File N° of submitting company, Date of report	GLP GEP	Published Protected
IIA 4.2.2	Blaszczynski E.	2001	Analytical method for determining Thiram® in soil Generated by : UCB S.A., Uniroyal Chemical Limited Submitted by : UCB S.A., Uniroyal Chemical Limited (on 26/12/2001) Study N° : 2000-187 Date of report : 23 February 2001	yes	unpublished protected
IIA 4.2.3	Blaszczynski E.	2000	Analytical method for determination of Thiram in pond water Generated by : UCB S.A., Uniroyal Chemical Limited Submitted by : UCB S.A., Uniroyal Chemical Limited (on 17/11/2000) Study N° : 99188 Date of report : 27 March 2000	yes	unpublished protected
IIA 4.2.4/02	Class T.	2001	Development and validation of an analytical method for the determination of Thiram in air Generated by : UCB S.A., Uniroyal Chemical Limited Submitted by : UCB S.A., Uniroyal Chemical Limited (on 7/9/2001) Study N° : 2001-040 – Report N° : B 472 G Date of report : 16 May 2001	yes	unpublished protected