ANNEX B

Ziram

Addendum to the monograph

January 2003

B.5 Methods of analysis

B.5.1.2 Analytical methods for the determination of isomers, impurities and additives in the active substance as manufactured (Annex IIA 4.1.2)

UCB

- 5-batch analysis of Ziram (Biedermann, 2001)

GLP :
GLP-compliance stated

Principle of the methods :
1) impurity 1 : an accurately-weighed sample of Ziram technical is dissolved in dimethylformamide (DMF) after which the solution is analysed by HPLC (Luna C8 (2), 100 x 4.6 mm, 3 µm; gradient elution : 0.4% formic acid in water/0.4% formic acid in acetonitrile) with UV detection at 254 nm. Quantification by external standardisation.
2) impurity 2 : see validated Elf Atochem (Cerexagri) method for impurity 4 (addendum dd. September 2000)
3) impurity 3 and 4 : see validated Elf Atochem (Cerexagri) method for impurity 2 and 3 (addendum dd. September 2000)
4) impurity 5 : see validated Elf Atochem (Cerexagri) method for impurity 1 (addendum dd. September 2000)
5) additive 6 : an accurately-weighed sample of Ziram technical (ca. 5 g) is extracted with bidistilled water for at least 30 minutes using ultrasound, after which the extract is centrifuged (5 minutes at 4000 U/min). An aliquot (4 mL) of the clear liquid is diluted to 20 mL with bidistilled water, after which 2 mL is filtrated and the absorbance of the filtrate is measured at 274 nm (pure Ziram is used as background reference). Quantification by external calibration.

Findings :
see Table B.5.1.2-1

Table B.5.1.2-1 : Validation of methods for determination of impurities in technical Ziram - UCB (Biedermann, 2001)

Impurity	**Specificity/interferences**	**Linearity**	**Accuracy**		**Repeatability (% RSD)**	**LOQ**
			spiking level	**recovery (%)**		
1	demonstrated by confirmatory method; no interferences detected	r^2 = 0.997 (n=5)	0.2% 0.4%	100.9 113.0	21.8 (n=6; mean conc. 0.066%)*	0.026%
2	see validated Elf Atochem (Cerexagri) methods for impurities 1, 2, 3 and 4 (Table B.4.1.2-1 in addendum dd. September 2000)					
3						
4						
5						
6	not addressed	r^2 = 1.000 (n=4x3)	0.3% 0.6%	79 89.8	6.6 (n=5; mean conc. 0.448%)	0.1%
7	no appropriate method available					

* high RSD is due to the low concentration and the influence of the high Ziram concentration on the stability of impurity 1 in solvents

Conclusions :
Suitable methods are available for the determination of all significant impurities in technical Ziram. As far as the additives are concerned, no appropriate method is currently available for determination of additive 7, while specificity of the method for additive 6 remains to be addressed. However, as the additives are not of any toxicological concern and the quantities in which they are added are known, analysis of these additives is considered to be of minor importance and the required methodology can be dealt with at member state level.

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

Method KP-028-00

- Validation of analytical method of Ziram in stonefruit (Li & Flannery, 2001a)

GLP :
GLP-compliance stated
Principle of the method :
To 5 g of fruit sample 0.05 g L-cysteine is added, followed by 16 mL of 0.25 M EDTA-solution (to decouple the metal ion in Ziram) and 20 mL of methanol, after which the resulting acid (DMDC = dimethyldithiocarbamate) is immediately derivatized with iodomethane at 70°C to form dimethyldithiocarbamate methyl ester (DMDC-Me).
The methylated product is separated from the matrix by centrifugation, after which the sample is extracted once more with methanol and the 2 extracts are combined.
The derivative DMDC-Me is determined by RP-HPLC (Nova-pak C18, 150 x 3.9 mm, 4µ; gradient elution : water/methanol/acetonitrile) with UV-detection at 270 nm. Quantification by external standard calibration.
Findings :
Specificity – interferences : - confirmatory method : not addressed
- no significant matrix interferences (control values < 30% of LOQ)

Linearity : response of HPLC-UV system to DMDC-Me (peak area vs. conc.) was demonstrated to be linear within a concentration range from 0.005 to 0.5 µg/mL; e.g. $r^2 = 0.99997$ (n=7; 5 conc. levels)
Recovery – precision : see Table B.5.2-1
Validation by an independent laboratory (ILV) : not addressed
Limit of determination (LOQ) : 0.05 mg/kg
Conclusions :
Method KP-028-00 appears suitable for the determination of Ziram residues in stone fruit with a LOQ of 0.05 mg/kg, but the validation data package is incomplete : confirmatory technique and Independent Lab Validation are not addressed.

Table B.5.2-1 : Validation of HPLC-UV method KP-028-00 (Li & Flannery, 2001a)

Matrix	**Analyte** *fortification (determination)*	**Fortification level (mg/kg commodity)**	**Recovery**			
			Number of samples	**Range (%)**	**Mean (%)**	**RSD (%)**
nectarines	Ziram (determined as DMDC-Me)	0.05	5	95 – 102	99	2.6
		0.5	5	77 – 82	80	2.5
apricots		0.05	5	76 – 80	77	2.2
		0.5	5	65 – 85	74	9.7

Method KP-028-03

- Validation of LC/MS/MS analytical method of Ziram in apples (Li, 2001a)
- Residue analysis of Ziram in fruits by LC-MS/MS : Independent laboratory validation (Klumpp, 2001)

GLP :
GLP-compliance stated
Principle of the method :
To 5 g of frozen apple sample 0.05 g L-cysteine is added, followed by 16 mL of 0.25 M EDTA-solution (to decouple the metal ion in Ziram) and 4 mL of methanol, after which the resulting ions (DMDC = dimethyldithiocarbamate) are immediately derivatized with iodomethane to form dimethyldithiocarbamate methyl ester (DMDC-Me). The extraction and derivatization procedure is performed as quickly as possible, in order to minimize degradation of Ziram during sample work up.
The methylated product is separated from the matrix by centrifugation, after which the sample is extracted once more with methanol and the 2 extracts are combined. SPE on reversed phase cartridge is used as an optional step for further clean-up or concentration (elution with acetonitrile).

The derivative DMDC-Me is determined by LC-MS/MS (RP-HPLC on Zorbax SB-C3, 150 x 3.0 mm, 5µ; gradient elution : 0.1% HCOOH in water/0.1% HCOOH in methanol) with APCI, operating in positive MRM mode (m/z 136 → 88). Quantification by external standard calibration.

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 DMDC-Me (peak area vs. conc.) was demonstrated to be linear within a concentration range from 0.5 to 10 µg/L; e.g. r = 0.9996 (n=5)
(ILV : linear within range from 10 to 300 µg/L (n=7); r^2 = 0.9998)

Recovery – precision : see Table B.5.2-2

Validation by an independent laboratory (ILV) : first validation by Cerexagri (developer of method); second validation by GAB-IFU

Limit of determination (LOQ) : 0.01 mg/kg

Conclusions :

Method KP-028-03 is suitable for the determination of Ziram residues in fruit with a LOQ of 0.01 mg/kg. Independent lab validation was addressed in an acceptable manner.

Table B.5.2-2 : Validation of LC-MS/MS method KP-028-03

Matrix	**Analyte** *fortification (determination)*	**Fortification level (mg/kg commodity)**	**Recovery**			
			Number of samples	**Range (%)**	**Mean (%)**	**RSD (%)**
First validation (Li, 2001a)						
apples	Ziram (determined as DMDC-Me)	0.01[1]	5	74 – 81	77	3.5
		0.1[1]	5	73 – 77	75	2.0
		0.01[2]	5	81 – 104	89	11.3
		0.1[2]	5	72 – 82	76	5.0
Independent lab validation (ILV) (Klumpp, 2001)						
apples	Ziram (determined as DMDC-Me)	0.01[2]	5	83 – 95	87	6
		0.1[2]	5	76 – 89	82	6

[1] : without SPE
[2] : with SPE clean-up

Overall conclusion

Fully validated LC-MS/MS method KP-028-03 allows determination of Ziram residues in fruit with a LOQ of 0.01 mg/kg and thus enables enforcement of the proposed MRL's (5 mg/kg for cherries, peach/apricot and pome fruit; 2 mg/kg for plums).

With respect to its specificity, the following should be noted. As the method is based on the conversion of Ziram to DMDC-Me which is subsequently quantified, it is much more specific than the methods based on CS_2 detection. Whereas the CS_2 methods determine all dithiocarbamates, this method eliminates contributions from dithiocarbamates that don't contain the DMDC-moiety. According to a statement provided by the notifier, crop matrix fortification tests have shown that Thiram is also detected by this method, but the recoveries were only about 50%, which is to be expected taking into account the relative stability of the S-S bond. Possible co-analysis of Thiram is however not considered to be too problematic, as the available specific method for Thiram can be used for confirmation of identity.

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)

Method KP-028-00

- Validation of analytical method of Ziram in soil (Li & Flannery, 2001b)

GLP :
GLP-compliance stated
Principle of the method :
To 5 g of soil sample 0.05 g L-cysteine is added, followed by 16 mL of 0.25 M EDTA-solution (to decouple the metal ion in Ziram) and 20 mL of methanol, after which the resulting acid (DMDC = dimethyldithiocarbamate) is immediately derivatized with iodomethane at 70°C to form dimethyldithiocarbamate methyl ester (DMDC-Me).
The methylated product is separated from the matrix by centrifugation, after which the sample is extracted once more with methanol and the 2 extracts are combined. The combined extract is then extracted on an Oasis HBL SPE cartridge (elution with acetonitrile), after which the eluant is concentrated on a nitrogen-evaporator and diluted to 4 mL with water.
The derivative DMDC-Me is determined by RP-HPLC (Nova-pak C18, 150 x 3.9 mm, 4µ; gradient elution : water/methanol/acetonitrile) with UV-detection at 270 nm. Quantification by external standard calibration.
Findings :
Specificity – interferences : - confirmatory method : not addressed
- no significant matrix interferences (control values < 30% of LOQ)
Linearity : response of HPLC-UV system to DMDC-Me (peak area vs. conc.) was demonstrated to be linear within a concentration range from 0.005 to 0.5 µg/mL; e.g. $r^2 = 0.99995$ (n=8; 5 conc. levels)
Recovery – precision : see Table B.5.3.1-1
Limit of determination (LOQ) : 0.01 mg/kg
Conclusions :
Method KP-028-00 appears suitable for the determination of Ziram residues in soil with a LOQ of 0.01 mg/kg, but the validation data package is incomplete : confirmatory technique is not addressed.

Table B.5.3.1-1 : Validation of HPLC-UV method KP-028-00 (Li & Flannery, 2001b)

Matrix	**Analyte** *fortification (determination)*	**Fortification level (mg/kg commodity)**	**Recovery**			
			Number of samples	**Range (%)**	**Mean (%)**	**RSD (%)**
soil	Ziram (determined as DMDC-Me)	0.01 0.1	5 5	85 – 112 73 – 96	99 87	11.0 10.2

Method KP-029-01

- Validation of LC/MS/MS analytical method of Ziram in soil (Li, 2001b)

GLP :
GLP-compliance stated
Principle of the method :
To 5 g of frozen soil sample 0.05 g L-cysteine is added, followed by 16 mL of 0.25 M EDTA-solution (to decouple the metal ion in Ziram) and 4 mL of methanol, after which the resulting ions (DMDC = dimethyldithiocarbamate) are immediately derivatized with iodomethane to form dimethyldithiocarbamate methyl ester (DMDC-Me). The extraction and derivatization procedure is performed as quickly as possible, in order to minimize degradation of Ziram during sample work up.
The methylated product is separated from the matrix by centrifugation, after which the sample is extracted once more with methanol and the 2 extracts are combined.

The derivative DMDC-Me is determined by LC-MS/MS (RP-HPLC on Zorbax SB-C3, 150 x 3.0 mm, 5µ; gradient elution : 0.1% HCOOH in water/0.1% HCOOH in methanol) with APCI, operating in positive MRM mode (m/z 136 → 88). Quantification by external standard calibration.

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 DMDC-Me (peak area vs. conc.) was demonstrated to be linear within a concentration range from 0.5 to 10 µg/L (n=5); r = 0.9993

Recovery – precision : see Table B.5.3.1-2

Limit of determination (LOQ) : 0.01 mg/kg

Conclusions :

Method KP-029-01 is suitable for the determination of Ziram residues in soil with a LOQ of 0.01 mg/kg. It can also serve as confirmatory method to abovementioned HPLC-UV method KP-028-00.

Table B.5.3.1-2 : Validation of LC-MS/MS method KP-029-01 (Li, 2001b)

Matrix	**Analyte** *fortification (determination)*	**Fortification level (mg/kg commodity)**	**Recovery**			
			Number of samples	**Range (%)**	**Mean (%)**	**RSD (%)**
soil	Ziram (determined as DMDC-Me)	0.01	5	77 – 88	82	5.4
		0.1	5	74 – 81	76	3.5

Overall conclusion

Fully validated methods KP-028-00 (HPLC-UV) and KP-029-01 (LC-MS/MS) allow determination of Ziram residues in soil with a LOQ of 0.01 mg/kg and thus enable enforcement of the relevant residue limit (0.05 mg/kg = general upper limit).

As both methods are also based on the conversion of Ziram to DMDC-Me, the comments on specificity stated under the overall conclusions of B.5.2 also apply here.

B.5.3.2 Water (Annex IIA 4.2.3)

With regard to the residue analytical method for water that was evaluated in the first addendum to the monograph dd. September 2000, the notifier submitted in April 2001 the final version of the report in question (Castro et al., 2000 – study N° KP-2000-13), as well as an amendment to this report. In this amendment, additional information is given concerning the water sources used for validation of the method. In the report itself, these two types of water were described as resp. "well water" and "spring water", which led to some confusion as these are colloquial terms.

Both types of water were taken from West Chester, Chester County, Pennsylvania. The "well water" samples were obtained from a domestic well used for drinking water. This type of water is typical of the *groundwater* in this region. The "spring water" was taken from Broad Run Creek, a rapidly-flowing stream which traverses both domestic and agricultural land and which is part of the Brandywine River watershed. The "spring water" used to validate the method can thus be correctly described as *surface water*, which implies that validation data for surface water were already included in the report and that further validation of the method is thus not necessary. For the sake of completeness, the surface water data are repeated in Table B.5.3.2-1

Conclusions :

The LC-MS/MS method allows determination of Ziram residues in water (surface water as well as drinking water) with a LOQ of 0.1 µg/L and thus enables enforcement of the relevant residue limits for drinking water (0.1 µg/L = EU drinking water limit) and for surface water (0.97 µg/L = LC_{50} of *Lepomis macrochirus*).

As this method is also based on the conversion of Ziram to DMDC-Me, the comments on specificity stated under the overall conclusions of B.5.2 also apply here.

Table B.5.3.2-1 : Surface water validation data (Castro et al., 2000)

Matrix	Analyte *fortification (determination)*	Fortification level (µg/L commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
surface water	Ziram (determined as DMDC-Me)	0.1	5	76 – 95	84	8.2
		1.0	5	73 – 77	75	2.4
		0.1 – 1.0	10	73 – 95	80	8.4

B.5.3.3 Air (Annex IIA 4.2.4)

- Analytical method for the determination of Ziram in air (Bacher, 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. 1.5 L/min during a period of 6 hrs (air sampled : ca. 0.55 m^3), after which the 2 portions of the adsorption material are extracted separately with dichloromethane. The extract is concentrated and transferred into methanol. By addition of 0.25M aqueous EDTA solution (for decoupling of zinc ion from Ziram), L-cysteine and methyl iodide (for methylation of the resulting anion), Ziram is derivatized to form dimethyldithiocarbamic acid, methyl ester (DMDC-Me).

The derivative DMDC-Me is determined by LC-MS/MS (Zorbax SB-C3, 150 x 3 mm, 5µm; gradient elution : 0.1% formic acid in methanol/ 0.1% formic acid in water) with APCI, monitoring characteristic parent-daughter ion fragmentation (m/z 136 → 88). 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 < 15% of LOQ)

Linearity : response of LC-MS/MS system to DMDC-Me (peak area vs. conc.) was demonstrated to be linear within a concentration range from 20 to 5000 µg/L (n=15; 7 conc. levels); r^2 = 0.9972

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

Storage stability : demonstrated for overnight storage in a freezer at ≤ -21°C

Limit of determination (LOQ) : ≈ 2 µg/m^3 (experiments during method development showed that a LOQ of 0.2 µg/m³ was not feasible, due to limitations in selectivity and sensitivity of the method)

Conclusions :

The fully validated LC-MS/MS method allows determination of Ziram residues in air with a LOQ of ≈ 2 µg/m^3 and thus enables enforcement of the relevant residue limit (C = 4.8 µg/m³, based on proposed $AOEL_{syst}$ of 0.016 mg/kg b.w./d).

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

Matrix	Analyte *fortification (determination)*	Fortification level (µg/m^3 commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
ambient air (26°C, 27% RH)	Ziram (determined as DMDC-Me)	1.84	8	73 – 120	99	17
		18.4	5	71 – 84	77	7
warm, humid air (36°C, 100% RH)		1.83	5	62 – 94	80	15
		18.5	5	78 – 109	96	16
breakthrough (i.e. recovery of a.s. in back portion of adsorption tubes) : < 5% of fortified amount at higher fortification level						

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

Toluene-3,4-dithiol method

- Toluene-3,4-dithiol analysis of blood for assessing carbon disulfide exposure (Valentine et al., 1999)

GLP :
No GLP-compliance stated (published article)

Principle of the method :
The method consists of cleaving the thiocarbonyl function of free CS_2 or certain CS_2-generated modifications on proteins (e.g. dithiocarbamate moieties on protein lysyl amino groups), using toluene-3,4-dithiol. The resulting toluene trithiocarbonate product is then quantified using RP-HPLC (Lichrospher 100, 5 µm; isocratic elution) with UV detection at 370 nm. Quantification by external standardization.

Findings :
Sensitivity, dose response and kinetics of this biomarker in blood were examined by administering rats CS_2 by inhalation, intraperitoneal (ip) injection or gavage. To evaluate the relative specificity of toluene-3,4-dithiol analysis, blood was also examined following administration of captan, a compound that produces TTCA but does not liberate CS_2.

Dose response : - following ip exposure : linear over exposure range 0.04 – 5.0 mmol/kg
- following inhalation exposure : linear over exposure range 0-800 ppm

Kinetics : - single ip injection : levels peak within resp. 1 h (hemolysate) and 3 h (plasma);
elimination of reactive groups resp. first order (plasma; DT_{50} = 11.9 h) and biphasic (hemolysate; DT_{50} = 1 and 75 h)
- repeated subacute ip dosing (at 24 h intervals for 5-day period) :
levels resp. plateau after 3rd dose (plasma) and increase over entire period (hemolysate);
elimination of reactive groups : first order with DT50 resp. 20.5 (plasma) and 129.6 h (hemolysate)
- repeated subchronic po dosing (once a day, 5 days/week (weekdays only) for 5-week period) :
cummulative dose response in the Friday samples, but no sequential increases reflected in the Monday morning values
elimination of reactive groups after cessation of exposure : DT_{50} resp. 19.3 (plasma) and 693.0 h (hemolysate)

Relative specificity : after captan administration : no trithiocarbonate generated from blood samples;
mean 24-h urinary excretion of TTCA : 660 nmol

Conclusions :
The toluene-3,4-dithiol method appears useful for assessing internal exposure to CS_2 over acute to subchronic periods. Validation of the analytical method itself is not addressed here, but is covered in the report by Valentine (2001).

- Validation of toluene-3,4-dithiol analysis for carbon disulfide in plasma and hemolysate (Valentine, 2001)

GLP :
No GLP-compliance stated

Principle of the method : see above
50 µL plasma (resp. hemolysate) is mixed with 100 µL water, 150 µL 0.2 M phosphate buffer (pH 8.5), 270 µl (resp. 250 µL) methanol and 30 µL (resp. 50 µL) of 100 mM toluene-3,4-dithiol solution in methanol. The mixture is incubated in a water bath at 65°C for 1 hr with occasional mixing and subsequently cooled to room temperature. 200µL ethyl acetate is added after which the solution is mixed vigorously and stored at –20°C overnight. Finally, the test mixture is centrifuged at 10000 g for 10 min.
The resulting toluene trithiocarbonate (TTC) in the supernatant is determined by RP-HPLC (Lichrospher® 100, 125 x 4 mm, 5 µm; isocratic elution : methanol/water 80/20) with UV detection at 370 nm. Quantification by external standardization.

Findings :
Specificity – interferences :
- specificity demonstrated by HPLC-DAD (UV absorption spectra of TTC peak in plasma (resp. hemolysate) sample and TTC standard peak are shown)
- no significant matrix interferences (control values < 30% of LOQ)

Linearity : response of the HPLC-UV system to CS_2 (TTC peak area vs. conc.) is demonstrated to be linear :
- for plasma : within a concentration range from 0 to 3.76 mg/L (n=5x2); r = 0.994
- for hemolysate : within a concentration range from 0 to 15.2 mg/L (n=6x2); r = 0.9999

Recovery – precision : see Table B.5.4-1
Limit of determination (LOQ) : 0.15 mg/L
Conclusions :
The toluene-3,4-dithiol method is suitable for determination of CS_2 in blood with a LOQ of 0.15 mg/L. Although the LOQ exceeds the general limit for blood specified in SANCO/825/00 rev. 6 (i.e. 0.05 mg/L), the rapporteur considers that the method is acceptable : this method with an LOQ of 0.15 mg/l allows to determine residue level in the blood which is relevant for possible acute exposure (i.e. exposure equivalent to LD50 (rat)/100)

Table B.5.4 -1 : Validation of toluene-3,4-dithiol method (Valentine, 2001)

Matrix	Analyte	Fortification level (mg/L commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
plasma	CS_2	0.15	5	87 – 113	103	10
		1.52	5	94 – 101	98	3
hemolysate		0.15	5	95 – 103	100	3
		1.52	5	79 – 97	87	9

TTCA method

- 2-Thioxothiazolidine-4-carboxylic acid (TTCA) : Determination in urine (Eben et al., 1990)
- Identification and determination of 2-thiothiazolidine-4-carboxylic acid in urine of workers exposed to carbon disulfide (Van Doorn et al., 1981)

GLP :
No GLP-compliance stated (published articles)
Principle of the method :
TTCA is a metabolite excreted in the urine of persons exposed to CS_2.
After acidification of and addition of sodium chloride to the urine, TTCA is extracted with diethyl ether. The ether phase is evaporated and the residue is dissolved in methanol. TTCA is determined by HPLC (Hypersil ODS, 5 µm; gradient elution) with UV detection at 273 nm. Quantification by external standardization.
Findings :
Specificity – interferences : - unfortified control urine samples generally exhibit no interfering peak at the retention time of TTCA; representative chromatograms are shown
- method is stated to be diagnostically specific i.e. no TTCA is found in urine of persons who have not been exposed to CS_2. However, the article by Valentine et al. (1999) demonstrates that TTCA is also detected in urine after exposure to captan.

Linearity : the response of the HPLC-UV system (= peak area) to TTCA is stated to be linear over a concentration range up to 20 mg/L
Recovery – precision : see Table B.5.4-2 (only summary data available)
Limit of determination (LOQ) : the detection limit is stated to be 0.2 mg/L, but the mean recovery and RSD obtained at this concentration level were not given. Based on the summary recovery results that were submitted, a LOQ of 1.0 mg/L seems appropriate.
Conclusions :
Confirmatory method was not addressed. Usefulness of TTCA as a marker for CS_2-exposure is questionable, taking into account the fact that it is also detected in urine after exposure to captan.

Table B.5.4 -2 : Validation of TTCA-method (Eben et al., 1990)

Matrix	Analyte	Fortification level (mg/L commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
urine	TTCA	1.0	10	-	95	2.6
		5.0	10	-	91	1.7

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 4.1.2	Biedermann K.	2001	5-batch analysis of Ziram Generated by : UCB S.A. Submitted by : UCB S.A. (on 26/12/01) Report/File N° : 825557 Date of report : 18 December 2001	yes	unpublished protected
IIA 4.2.1/03	Li F. Flannery M.	2001a	Validation of analytical method of Ziram in stonefruit Generated by : Elf Atochem Agri s.a. Submitted by : Elf Atochem Agri s.a., UCB (on 13/04/2001) Report/File N° : KP-2000-24 Date of report : 10 April 2001	yes	unpublished protected
IIA 4.2.1/05	Li F.	2001a	Validation of LC/MS/MS analytical method of Ziram in apples Generated by : Ziram Task Force Submitted by : Ziram Task Force (on 26/12/01) Report/File N° : KP-2001-17 Date of report : 20 December 2001	yes	unpublished protected
IIA 4.2.1/06	Klumpp M.	2001	Residue analysis of Ziram in fruits by LC/MS/MS : Independent Laboratory Validation Generated by : Ziram Task Force Submitted by : Ziram Task Force (on 26/12/01) Report/File N° : 20011069/01-RVP Date of report : 12 December 2001	yes	unpublished protected
IIA 4.2.2/01	Li F. Flannery M.	2001b	Validation of analytical method of Ziram in soil Generated by : Elf Atochem Agri s.a. Submitted by : Elf Atochem Agri s.a., UCB (on 13/04/2001) Report/File N° : KP-2000-25 Date of report : 10 April 2001	yes	unpublished protected
IIA 4.2.2/02	Li F.	2001b	Validation of LC/MS/MS analytical method of Ziram in soil Generated by : Ziram Task Force Submitted by : Ziram Task Force (on 26/12/01) Report/File N° : KP-2001-18	yes	unpublished protected

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
			Date of report : 10 December 2001		
IIA 4.2.3/04	Castro L. Ampofo S. Li F.	2000	Determination of Ziram in potable water using LC/MS/MS Generated by : Elf Atochem Agri s.a. Submitted by : Elf Atochem Agri s.a., UCB (on 13/04/2001) Report/File N° : KP-2000-13 + amendment 1 dd. 24/01/2001 Date of report : 17 October 2000	yes	unpublished protected
IIA 4.2.4	Bacher R.	2001	Analytical method for the determination of Ziram in air Generated by : Ziram Task Force Submitted by : Ziram Task Force (on 26/12/01) Report/File N° : P/B 481 G Date of report : 13 December 2001	yes	unpublished protected
IIA 4.2.5/03	Valentine W. M.	1999	Toluene-3,4-dithiol analysis of blood for assessing carbon disulfide exposure Source : Toxicological Sciences, 50, 155-163 (1999) Submitted by : Ziram Task Force (on 05/09/00)	no	published unprotected
IIA 4.2.5/04	Eben A. Freudlsperger FP. Angerer J.	1990	2-Thioxothiazolidine-4-carboxylic acid (TTCA) Source : Analyses of haz. sub. in bio mat., Vol 4 XXII+265P. VCH, 207-221 Submitted by : Ziram Task Force (on 05/09/00)	no	published unprotected
IIA 4.2.5/06	Van Doorn R. Delbressine L.P.C. Leijdekkers Ch.-M. Vertin P.G. Henderson P. Th.	1981	Identification and determination of 2-Thiothiazolidine-4-carboxylic acid in urine of workers exposed to carbon disulfide Source : Arch Toxicol, 47, 51-58 (1981) Submitted by : Ziram Task Force (on 05/09/00)	no	published unprotected
IIA 4.2.5/07	Valentine W. M.	2001	Validation of Toluene-3,4-dithiol analysis for carbon disulfide in plasma and hemolysate Generated by : Ziram Task Force Submitted by : Ziram Task Force (on 26/12/01) Report/File N° : - Date of report : 12 October 2001	no	unpublished protected