ZIRAM

Addendum to the monograph

September 2000

B.2 Physical and chemical properties

B.2.1 Physical and chemical properties of the active substance (Annex IIA 2)

Purity of test substances used : purified a.s. : 99% pure (Batch number HRC 9.8.95/11)
technical a.s. : 98% pure (Batch number HRC 9.8.95/12)

Table B.2.1-1 : Physical and chemical properties of Ziram

Study	Guidelines and GLP	Findings	Evaluation and conclusions	References
B.2.1.1 Melting point, freezing point or solidification point (IIA 2.1.1)	- EEC-method A1 (metal block method) - GLP-compliance stated	purified a.s. : melting point range : 251.0 to 252.5 °C with colour change beginning at 238°C	- Acceptable	Flack, 1996
B.2.1.4 Relative density (IIA 2.2)	- EEC-method A3 (pycnometer method) - GLP-compliance stated	purified a.s. : relative density at 20 °C = 1.7097	- Acceptable	Flack, 1996
B.2.1.5 Vapour pressure (IIA 2.3.1)	- EEC-method A4 (vapour pressure balance method) - GLP-compliance stated	purified a.s. : vapour pressure at 25°C = 1.8×10^{-5} Pa (max. value estimated by imposing a shallow slope of -2000 for the the vapour pressure/temp. relationship)	- Acceptable - Ziram is “very slightly volatile”	Flack, 1996
B.2.1.6 Volatility, Henry's law constant (IIA 2.3.2)	- Calculation - GLP-compliance stated	purified a.s. : H (unitless) at 25°C = 2.3×10^{-6}	- Acceptable (corresponds with 5.7×10^{-3} $Pa.m^3.mol^{-1}$) - Ziram is “very slightly volatile”	Flack, 1996

Study	Guidelines and GLP	Findings	Evaluation and conclusions	References
B.2.1.7 Physical state (IIA 2.4.1)	- Subjective assessment - GLP-compliance stated	purified a.s. and technical a.s.: powder	- Acceptable	Flack, 1996
B.2.1.8 Colour (IIA 2.4.1)	- ASTM D 1535-80 (Munsell color system) - GLP-compliance stated	purified a.s. and technical a.s.: white	- Acceptable	Flack, 1996
B.2.1.9 Odour (IIA 2.4.2)	- Subjective assessment - GLP-compliance stated	purified a.s. and technical a.s.: sweet, musty odour	- Acceptable	Flack, 1996
B.2.1.10		purified a.s. :	- Acceptable	Flack, 1996

Study	Guidelines and GLP	Findings	Evaluation and conclusions	References
Spectra of the active substance (IIA 2.5.1)	- GLP-compliance stated	*Following spectra were provided :* **UV/VIS** (methanol/water 10/90; spectra measured between 1) 190-900 nm and 2) 190-400 nm) **MS** (fast atom bombardment - positive ion) **IR** (KBr; spectrum measured over range 500 - 4500 cm^{-1}) **^{1}H-NMR** (deuterated chloroform) The different spectra were found to be consistent with the structure of Ziram *UV/VIS absorption characteristics :* λ_{max} (nm) / ε ($L.mol^{-1}.cm^{-1}$) neutral : 270 / 31586 251 / 39989 basic : 279 / 54478 251 / 52739 acidic : 206 (below UV cut off) No absorption between 400 - 900 nm		
B.2.1.11 Spectra for impurities (IIA 2.5.2)			- Not applicable (TMTU is not considered to be of toxicological significance)	
B.2.1.12 Solubility in water (IIA 2.6)	- EEC-method A6 (flask method+ HPLC) - GLP-compliance stated	purified a.s. : solubility in distilled water at 20 °C = 9.67 x 10^{-4} g/L (pH range 7.22 - 7.38) test was performed in distilled water only, as Ziram is not capable of reversible protonation	- Acceptable	Flack, 1996

Study	Guidelines and GLP	Findings	Evaluation and conclusions	References
B.2.1.13 Solubility in organic solvents (IIA 2.7)	- In-house method (based on EEC A6 - modified flask method) - GLP-compliance stated	purified a.s. : solubility at 20 °C in n-heptane : 5.86×10^{-3} g/L xylene : 0.90 g/L 1,2-dichloroethane : 5.84 g/L methanol : 0.11 g/L acetone : 2.30 g/L ethyl acetate : 1.01 g/L	- Acceptable	Flack, 1996
B.2.1.14 Partition coefficient n-octanol/water (IIA 2.8)	- EEC-method A8 (shake-flask method) - GLP-compliance stated	purified a.s.: at 20 °C : log P_{ow} = 1.65 (pH range aqueous phase : 7.36 - 8.56) test was performed unbuffered, as Ziram is not capable of reversible protonation	- Acceptable	Flack, 1996
B.2.1.17 Quantum yield of direct phototransformation (IIA 2.9.3)	- US EPA N-161-2 (Heraeus Suntest apparatus : Xenon arc light source with UV filter for $\lambda < 290$ nm) - GLP-compliance stated	a.s. ^{14}C-labelled - radiochemical purity : 97.0% at 25°C (sterile conditions) in pH 9 buffered solution (borate buffer) under continuous irradiation : 1) quantum yield Φ : 2.35×10^{-2} 2) environmental lifetime : calculated using GC-SOLAR program lifetime (days) in river water at 40° N latitude : Depth (cm) / Spring / Summer / Fall / Winter 0 / 0.31 / 0.26 / 0.53 / 0.89 10 / 0.35 / 0.29 / 0.60 / 1.01 20 / 0.39 / 0.32 / 0.68 / 1.13 30 / 0.44 / 0.36 / 0.76 / 1.27	- Final report remains to be submitted, but results are acceptable based on the draft report which is currently available	Burgener, 2000

Study	Guidelines and GLP	Findings	Evaluation and conclusions	References
B.2.1.18 Dissociation in water of purified active substance (IIA 2.9.4)	- OECD-guideline 112 (Spectrophotometric method) - GLP-compliance stated	purified a.s. : Ziram has bee shown to decompose under acidic conditions and undergo a non-protonation form of dissociation under basic conditions	- Acceptable	Flack, 1996
B.2.1.19 Estimated photo chemical oxidative degradation (IIA 2.10)	- Atkinson calculation method - GLP-compliance stated	Atkinson method involves the input of data into a computer program (“Atmospheric Oxidation Program”) using SMILES notation. However, as SMILES notation does not allow for the input of metal ions and Ziram contains metal in the form of zinc, calculation by Atkinson method is not possible	- Acceptable	Flack, 1996
B.2.1.20 Flammability (IIA 2.11.1)	- EEC-method A10 - GLP-compliance stated	technical a.s. : preliminary screening test : no propagation of combustion Þ Ziram is not highly flammable	- Acceptable	Flack, 1996
B.2.1.21 Auto-flammability (IIA 2.11.2)	- EEC-method A16 - GLP-compliance stated	technical a.s. : no self-ignition Þ Ziram is not autoflammable	- Acceptable	Flack, 1996
B.2.1.23	- EEC-method A14	technical a.s. :	- Acceptable	Flack, 1996

Study	Guidelines and GLP	Findings	Evaluation and conclusions	References
Explosive properties (IIA 2.13)	- GLP-compliance stated	· thermal sensitivity (effect of flame) : no explosions or deformation of tubes · mechanical sensitivity (shock and friction) : no visible or audible reaction Þ Ziram has no explosive properties		
B.2.1.24 Surface tension (IIA 2.14)	- EEC-method A5 (OECD harmonized ring-method) - GLP-compliance stated	technical a.s. : 90% saturated aqueous solution of Ziram : σ = 73.0 mN/m at 16°C	- Acceptable	Flack, 1996
B.2.1.25 Oxidizing properties (IIA 2.15)	- Statement (according to EEC-method A17) - GLP-compliance stated	"Examination of the structural formula of Ziram shows the absence of oxygen. The material is therefore unable to react exothermically with a combustible material and the test need not be performed" Þ Ziram has no oxidizing properties	- Acceptable	Flack, 1996

B.2.2 Physical and chemical properties of the plant protection products (Annex IIIA 2)

The Annex III dossier submitted by the Task Force concerns a water dispersible granule containing 76% Ziram.

Table B.2.2-1 : Physical and chemical properties of ZIRAM 76 WG (water dispersible granule : Ziram)

Study	Guidelines and GLP	Findings	Evaluation and conclusion	References
B.2.2.4 Explosive properties (IIIA 2.2.1)	- Applicant's statement	Study on explosive properties of the active substance demonstrates that ziram is not explosive. The MSDS of the additives do not reveal explosive properties ÞZIRAM 76 WG is not explosive	- Acceptable	
B.2.2.5 Oxidizing properties (IIIA 2.2.2)	- Applicant's statement	Ziram does not have oxidizing properties The MSDS of the formulants do not indicate any danger for oxidation reactions, nor do they mention any chemical incompatibility with other compounds. ÞZIRAM 76 WG is not oxidizing	- Acceptable	
B.2.2.7 Flammability (IIIA 2.3)	- EEC-method A10 - GLP-compliance stated	Preliminary screening test : no propagation of combustion Þ ZIRAM 76 WG is not highly flammable	- Acceptable	Tremain, 2000
B.2.2.8	- EEC-method A16	No sign of energy release between room temperature	- Study was not	Danschutter, 1999

Study	Guidelines and GLP	Findings	Evaluation and conclusion	References
Auto-flammability (IIIA 2.3)	- No GLP-compliance stated	and melting process Þ ZIRAM 76 WG is not autoflammable	performed in GLP-lab. However, taking into account the GLP-study on auto-flammability of the a.s., the data are considered to be acceptable.	
B.2.2.15 Bulk or tap density (IIIA 2.6.2)	- CIPAC MT 169.2	5 batches produced between Jan 1997 and Feb 1998 : tap density (range) : 0.563 g/ml to 0.621 g/ml	- Acceptable	Van Hecke, 1998a
B.2.2.16 Stability after storage for 14 days at 54 °C (IIIA 2.7.1)	- CIPAC MT 46	after storage <u>for 14 days at 54 °C</u> : 1) a.s. content : 75.7% (initial : 75.6%) 2) suspensibility : 97% (initial : 86.4%) 3) wet sieve residue on 75 µm : 0.10% (initial : 0.13%) 4) pH : 6.2 (initial : 6.1) 5) dispersibility : 96% (initial : 97%) Þ accelerated storage has no influence on relevant physico-chemical properties	- Acceptable	Van De Putte, 1996 Janssens, 2000
B.2.2.20	- No guideline specified	after storage <u>for 54 months</u> in polyethylene jars under	- Acceptable	Dossche et al., 1998

Study	Guidelines and GLP	Findings	Evaluation and conclusion	References
Shelf life (IIIA 2.7.3)		warehouse conditions (storage temperature range 5 - 30°C) : 1) a.s. content :75.1% (initial : 77%) 2) water content : 2.3% (initial : 1.29%) 3) suspensibility : 100% (after 30 months : 97.7%) 4) dustiness : 7.3 mg (after 30 months : 0.94 mg) 5) flowability : 99.5% (after 30 months : 100%) 6) packaging : no signs of corrosion, of decomposition; no change in colour, no cracking ÞZIRAM 76 WG still complies with FAO-specification		Dossche et al., 2000 (addendum)
B.2.2.21 Wettability (IIIA 2.8.1)	- CIPAC MT 53.3	3 batches produced between Oct 1997 and Feb 1998 : wetting time (range) : 21 to 70 s Þ complies with FAO-specification	- Acceptable	Van Hecke, 1998b
B.2.2.22 Persistent foaming (IIIA 2.8.2)	- CIPAC MT 47	5 batches produced between Jan 1997 and Feb 1998 : foam after 1 min (range) : 2 to 3 ml Þ complies with FAO-specification	- Acceptable	Janssens, 1998a
B.2.2.23 Suspensibility (IIIA 2.8.3)	- In-house procedure, based on CIPAC MT 168	5 batches produced between Jan 1997 and Feb 1998 : 1% suspension in CIPAC water D : suspensibility (range) : 92% to 98% Þ complies with FAO-specification	- Acceptable	Van Hecke & Janssens, 1998
B.2.2.24 Spontaneity of dispersion (IIIA 2.8.3)	- CIPAC MT 174	5 batches produced between Jan 1997 and Feb 1998 : dispersibility (range) : 95% to 99%	- Acceptable	Janssens, 1998b
B.2.2.26 Dry sieve test and wet sieve test (IIIA 2.8.5)	- CIPAC MT 167	5 batches produced between Jan 1997 and Feb 1998 : wet sieve residue (range) : 0.002% to 0.40% retained on 75 µm sieve Þ complies with FAO-specification	-Acceptable	Van Hecke, 1998c

Study	Guidelines and GLP	Findings	Evaluation and conclusion	References
B.2.2.27 Size distribution of particles - Nominal size range of particles (IIIA 2.8.6.1)	- CIPAC MT 170 - GLP-compliance stated	range : x1 = 125 µm where Rx ³ 90% x2 = 425 µm where Rx £ 10%	- Acceptable	O'Connor & Mullee, 2000
B.2.2.28 Dust content and particle size of dust (IIIA 2.8.6.2)	- CIPAC MT 171 - GLP-compliance stated	collected dust : 0.9 mg ® "nearly dust-free" Þ complies with FAO-specification	- Acceptable	O'Connor & Mullee, 2000
B.2.2.29 Friability and attrition characteristics of granules (IIIA 2.8.6.3)	- CIPAC MT 178 - GLP-compliance stated	attrition resistance : 96.2%	- Acceptable	O'Connor & Mullee, 2000
B.2.2.32 Flowability (IIIA 2.8.8.1)	- CIPAC MT 172	5 batches produced between Jan 1997 and Feb 1998 : flowability (range) : 99.8% to 100% passes through 5 mm sieve after 20 drops Þ complies with FAO-specification	- Acceptable	Van Hecke, 1998d

ZIRAM

Addendum to the monograph

September 2000

B.4 Methods of analysis

B.4.1 Analytical methods for formulation analysis

B.4.1.1 Analytical methods for the determination of pure active substance in the active substance as manufactured (annex II 4.1.1)

Elf Atochem

- Ziram technical Analytical Method Validation : Ziram active ingredient (Beckwith, 2000a)
GLP :
GLP-compliance stated.
Principle of the method : based on CIPAC method 31/TC/M/3
The sample is digested by reflux in dilute sulfuric acid to release carbon disulfide. The latter is aspirated through 2 absorbers containing lead acetate solution to remove hydrogen sulfide formed during the decomposition of the sample. The carbon disulfide is then absorbed in methanolic potassium hydroxide, to form potassium methylxanthate. This xanthate solution is neutralized with dilute acetic acid and titrated with 0.1 N iodine solution on starch indicator.
Findings :
Specificity-interferences : the method is officialy accepted for dithiocarbamates
Linearity : not applicable
Accuracy : checked by using a secondary reference standard (sodium diethyldithiocarbamate trihydrate)
average recovery = 99.5% (RSD = 0.39%)
Repeatability : RSD = 0.20% (n = 2 x 5)
Conclusions :
The applicability of officially accepted CIPAC method 31/TC/M/3 is demonstrated.

- Ziram technical Analytical Method Validation : Zinc (Beckwith, 2000b)
GLP :
GLP-compliance stated.
Principle of the method : SOP DLA-006.1
The sample is decomposed in boiling hydrochloric acid, followed by titration with EDTA
Findings :
Specificity-interferences : the method is generally accepted for zinc determination in dithiocarbamates
Linearity : not applicable
Accuracy : recovery = 101%
Repeatability : RSD = 0.29% (n = 2 x 5)
Conclusions :
The method is suitable for determination of zinc in Ziram technical.

FMC Foret

- Ziram (technical) : Five-batch analysis (Comb, 1997)
GLP :
GLP-compliance stated
Principle of the method :
The sample of Ziram technical is dissolved in and diluted to volume with acetonitrile, after which the final solution is analyzed by HPLC (Hypersil ODS; 150 x 4.6 mm; isocratic elution) with UV detection at 257 nm. Quantification by external standardization.
Findings :
Specificity-interferences : no interferences are mentioned
Linearity : the response of the HPLC-UV system (= peak area) to ziram was found to be linear
concentration range : 40 - 200 mg/L (n = 5)
y = 38.0 x + 50.6
r = 0.9998
Accuracy : demonstrated by linearity of assay system
sample prepared at 75% of nominal sample weight : 104.4% of reference value
sample prepared at 125% of nominal sample weight : 99.7% of reference value
Repeatability : RSD = 0.44% (n = 10)
Conclusions :

The HPLC-method is suitable for the determination of Ziram in Ziram technical.

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

Elf Atochem

- Ziram Technical Analytical Method Validation : impurities (Beckwith, 2000c; Beckwith, 2000d; Beckwith, 2000 e; Beckwith, 2000f; Beckwith, 2000g; Beckwith, 2000i)

GLP :

GLP-compliance stated.

Principle of the methods :

1) impurity 1 : an accurately-weighed sample of Ziram technical and DI water are placed into a Qorpak bottle, that is placed on a reciprocating shaker for 30 min at ambient temperature. The extract is filtered and analyzed by IC (Dionex AS4A-SC (4 mm) with AG4A-SC) with conductivity detection. Quantification by external standardization.

2) impurity 2 and 3 : an accurately-weighed sample of Ziram technical and a known volume of deionized water are placed into a Qorpak bottle, that is placed on a reciprocating shaker for 30 min at ambient temperature. The extract is filtered and analyzed by ICP-AES (213.9 nm for impurity 2 and 589.6 nm for impurity 3). Quantification by external standardization.

3) impurity 4 : an accurately-weighed sample of Ziram technical is placed in a dessicator over magnesium perchlorate at ambient temperature for 120 h. The difference in weight is recorded.

4) impurity 5 : an accurately-weighed sample of Ziram technical (ca. 0.5 g) is dissolved in 20 mL acetonitrile. The extract is filtered and analyzed by HPLC (LiChroCART 125-4 Superspher 100 RP-18) with UV detection at 270 nm. Quantification by external standardization. (Method is based on SOP DLA-227.2)

5) impurity 6 : an accurately-weighed sample of Ziram technical is decomposed in the presence of nitric and sulfuric acids (according to CIPAC method 31/TC/M/5 - part A). The digest is analyzed by ICP-AES. Quantification by external standardization.

6) additive 7 : an accurately-weighed sample of Ziram technical (ca. 4 g) and HPLC-grade water are placed into a Qorpak bottle and the sample is stirred magnetically for 15 min. The extract is filtered and analyzed by HPLC (TSK Gel G3000SW) with UV detection at 280 nm. Quantification by external standardization. (Method is based on SOP DLA-227.1)

Findings :

see Table B.4.1.2-1

Table B.4.1.2-1 : Validation of methods for determination of impurities in Technical Ziram - Elf Atochem (Beckwith, 2000c; Beckwith, 2000d; Beckwith, 2000 e; Beckwith, 2000f; Beckwith, 2000g; Beckwith, 2000i)

Impurity	Specificity/interferences	Repeatability (% RSD)	Spike recovery (%)	Linearity
1	sample blank : low-level contamination at retention time of analyte Þ blank correction	3.2 (n = 2 x 5)	88	n = 5 (4.0 - 59.7 mg/L) y = 2.326 10^6x - 8.147 r = 0.9997
2	meets requirements	13 (n = 2 x 5)	108	n = 4 (0 - 21.02 mg/L) y = 141042.9 x + 30228.1 r = 0.9997
3	meets requirements	0.82 (n = 2 x 5)	106	n = 4 (0 - 20.93 mg/L) y = 138638.0 x - 45310.7 r = 0.9993
4	NA	44* (n = 2 x 5)	NA	NA
5	meets requirements	16	97	n = 5 (0.11 - 10.5 mg/L) y = 39764.9 x

Impurity	Specificity/interferences	Repeatability (% RSD)	Spike recovery (%)	Linearity
		(n = 2 x 3)		r = 0.9999
6	meets requirements	-**	109	n = 5 (0 - 41.68 mg/L) y = 7218.0 x + 3385.7 r = 0.9993
7	meets requirements	1.3 (n = 2 x 3)	71	n = 5 (41 - 306 mg/L) y = 70.898 x + 811.18 r = 0.9996

NA : not applicable
* : High value is likely due to fact that < 5 mg of weight was lost
** : Repeatability could not be calculated since concentration levels were below the detection limit (0.001%) for all preparations (n = 2 x 5) of the test substance

Conclusions :
Suitable methods are available for the determination of all significant impurities and additives in the technical a.s.. However, according to the FAO-specification for Ziram, the preferred method for impurity 4 is the Dean and Stark method

UCB

- Composition of Ziram Phyto (Van De Putte, 1998a)
- ZIRAM 76 WG : impurity 6 content (Van De Putte, 1998b)
- Analysis of Thiram 80 WG, Thiram technical, Ziram 76 WG, Ziram technical for FAO specifications (Van De Putte, 1995)
- Determination of impurities in Ziram Phyto (No author specified, 1994)

GLP :
No GLP-compliance stated.
Principle of the methods :
1) impurity 1 : a sample of Ziram technical is dissolved in dimethylformamide and diluted with water. Impurity 1 is determined by HPLC (Adsorbosphere C18, 10 µm particles, 250 x 4.6 mm; mobile phase : 70% methanol + 30% acetate buffer) with UV detection at 254 nm. Quantification by external standard calibration.
2) impurity 2 : a sample of Ziram technical is dried by means of an infra-red lamp and the loss of weight is recorded
3) impurity 3, 4 and 5 : a sample of Ziram technical is dissolved in chloroform (5 min in ultrasonic bath), after which the solution is filtered on a preweighed P5 glass filter. After rinsing with chloroform, the filtrate is discarded and the filter is dried, cooled and weighed to determine the insolubles in chloroform.
Afterwards, water is added to the filter and the filtrate collected. Impurity 3 and 4 are determined in the filtrate using Atomic Absorption Spectrometry (AAS). The sulfate content is checked by an appropriate anion chromatography method.
Finally, 1% HNO_3 is added to the filter and the filtrate collected. Impurity 5 is determined in the filtrate using AAS.
4) impurity 6 : a sample of Ziram technical is dissolved in dimethylformamide and the solution is analyzed by HPLC (Purospher RP18 - endcapped - 5 µm, 250 x 4 mm; mobile phase : micellar aqueous medium) with UV detection at 254 nm. Quantification by external standard calibration.
5) impurity 7 : a sample of Ziram technical is decomposed in the presence of strong acids (according to CIPAC method 31/TC/M/5 - part A). The digest is analyzed by AAS using the hydride system. Quantification by external standardization.
6) additive 9 : a sample of Ziram technical is dissolved in water, after which the solution is filtered on a glass fiber filter and the filtrate collected. The aqueous extract is subjected to UV-spectroscopic analysis at 274 nm. Quantification by external standard calibration.
Findings :
The accuracy of method 3 was stated to have been tested by adding impurity 3, 4 and 5 in a 0.1% concentration to pure Ziram. This standard addition procedure was carried out 3 times and recoveries between 75 and 110%

were found.
Method 4 : linearity range is stated to be 5 - 100 mg/l and limit of detection 0.05 mg/l, without providing any supporting data. Chromatograms of a standard and a sample solution are shown without further details, indicating that the method is suitable to separate impurity 6 from the a.s. and from impurity 1
Conclusions :
Analytical methods are available for the determination of all significant and relevant impurities in the technical a.s.. The submitted validation data package is however insufficient.

FMC Foret

- Ziram (technical) : Five-batch analysis (Comb, 1997)
- Ziram (technical) : Five-batch analysis - Additional report : Determination of impurity 3 in technical grade Ziram (Artigas, 1998a)
- Ziram (technical) : Five-batch analysis - Additional report : Determination of impurity 4 in technical grade Ziram (Artigas, 1998b)

GLP :
GLP-compliance stated for study by Comb (1997)
Principle of the methods :
1) impurity 1 : a sample of Ziram technical is dissolved in and diluted to volume with acetonitrile, after which the final solution is analyzed by HPLC (Hypersil ODS; 150 x 4.6 mm; isocratic elution) with UV detection at 257 nm. Quantification by external standardization.
2) impurity 2 : a sample of Ziram technical is accurately weighed into a dish, which is subsequently placed in a vacuum oven set at 40°C. The dish is periodically weighed until the content has dried to a constant weight.
3) impurity 3 : a sample of Ziram technical is dissolved with sulfuric and nitric acids. After reduction to the trivalent state, arsenic is determined using colorimetry at 540 nm based on the reaction of arsine with silver diethyldithiocarbamate in pyridine to form a soluble red complex. Quantification by external standard calibration.
4) impurity 4 : a sample of Ziram technical is dissolved in distilled water, after which the slurry is filtered. Impurity 4 is determined in the leachate using spectrophotometry, based on the formation of a chromogenic complex by reaction between impurity 4 and sodium nitroprussiate. Absorbance difference is measured between 665 nm and 500 nm. Quantification by standard addition

Findings :
see Table B.4.1.2-2

Table B.4.1.2-2 : Validation of methods for determination of impurities in Technical Ziram - FMC Foret (Comb, 1997; Artigas, 1998a; Artigas, 1998b)

Impurity	Specificity/interferences	Repeatability (% RSD)	Mean recovery (%)	Linearity
1	no interferences are mentioned	1.63 (n = 5)	78.9 (0.1% w/w level; n = 3) 101.3 (0.6% w/w level; n = 3)	n = 5 (0.5 - 2.6 mg/L) y = 21.8 x + 0.652 r = 0.9997 LOD = 0.06 mg/L
2	generally accepted method			
3	under similar conditions, SbH_3 yields a red colour with max. absorption at 540 nm and thus interferes	5.2 (n = 3)	97.5 (2.5 ppm level; n = 2) 101.5 (5 ppm level; n = 2)	n = 5 (0.02 - 0.4 mg/L) y = 0.972 x - 0.015 r = 0.9995

Impurity	Specificity/interferences	Repeatability (% RSD)	Mean recovery (%)	Linearity
4	no interferences are mentioned	12.3 - 24.7 (n = 5)	recovery range (n = 5) : 93 - 107 (2 - 8 ppm level)	example of standard addition line (n = 5) : y = 0.00800 x + 0.0167 r = 0.9992

Conclusions :
Suitable methods are available for the determination of all significant impurities in the technical a.s.. However, according to the FAO-specification for Ziram, the preferred method for impurity 2 is the Dean and Stark method

B.4.2 Analytical methods (residue) for food and feed (Annex IIA 4.2.1; Annex IIIA 5.2.1)

Validation of a specific method for determination of ziram residues on plants and plant products is currently ongoing. Results are expected for November 2000.

B.4.3 Analytical methods (residue) in soil, water, air (Annex IIA 4.2.2 to 4.2.4; Annex IIIA 5.2.2 to 5.2.4)

B.4.3.1 Analytical methods for soil (Annex IIA 4.2.2; Annex IIIA 5.2.2)

Validation of a specific method for determination of ziram residues in soil is planned. Results are expected beginning 2001.

B.4.3.2 Analytical methods for water (Annex IIA 4.2.3; Annex IIIA 5.2.3)

- Determination of Ziram in water (Castro et al., 2000)
GLP :
GLP-compliance stated.
Principle of the method :
To a 5 ml water sample, 0.25 ml of a 0.25M EDTA-solution is added to decouple the metal atom in Ziram, after which the pH of the sample is adjusted to a value in between 8-10.5. The resulting acid is then methylated with iodomethane (ca. 40 min at 65-70°C) to form dimethyldithiocarbamate methyl ester (DMDC-Me).
The methylated species is analyzed using LC/MS/MS (RP-HPLC on Alltech Alltima C18, 5 µ, 100 x 4.6 mm; isocratic elution) operating in Multiple Reaction Monitoring (MRM; m/z 136>88) scanning mode.
Quantification by external standard calibration.
Findings :
Specificity - interferences : According to the representative chromatograms of calibration standards, control and recovery samples, the method is suitable to determine Ziram as its DMDC-Me derivative. Unfortified control samples exhibit no detectable interferences at the retention time of DMDC-Me.
Linearity : The response of the LC/MS/MS system (= peak area) to DMDC-Me was demonstrated to be linear over a concentration range from 0.05 µg/L to 1.0 µg/L.
Recovery - precision : see Table B.4.3.2-1
Limit of determination (LOQ) : 0.1 µg/L

Table B.4.3.2-1 : Validation of method for residues in potable water (Castro et al., 2000)

Matrix	Analyte	Fortification level (µg/L commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
well water	Ziram (as	0.1	5	58 - 73	65.6	8.5

Matrix	Analyte	Fortification level (µg/L commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
	DMDC-Me)	1.0	5	69 - 88	76.2	9.6
		0.1 - 1.0	10	58 - 88	70.9	11.7
spring water		0.1	5	77 - 96	85.0	8.1
		1.0	5	73 - 78	76.0	2.8
		0.1 - 1.0	10	73 - 96	80.5	8.4
overall	Ziram (as DMDC-Me)	0.1	10	58 - 96	75.3	15.7
		1.0	10	69 - 88	76.1	6.7
		0.1 - 1.0	20	58 - 96	75.7	11.7

Conclusions :
The method is suitable for determination of Ziram residues in potable water with a LOQ of 0.1 µg/l.

B.4.3.3 Analytical methods for air (Annex IIA 4.2.4; Annex IIIA 5.2.4)

- Analysis of air samples for the fungicides Ziram and Mancozeb and the breakdown product Ethylenethiourea (Hsieh et al., 1994)

GLP :
No GLP-compliance stated.

Principle of the method :
Ziram residues in air are trapped on glass fiber filters (GFF) using air sampling at rates of ca. 15 L/min for periods of time up to 24 h. After sampling, the filters are sealed in screw-cap glass jars and eventuelly stored at -15°C to -20°C.
The glass fiber filters containing residues are placed in 22 mL glass headspace vials, after which 2 mL iso-octane and 5-10 mL of a reagent containing 3% $SnCl_2$ in 37% HCl, is added to each vial to convert the dithiocarbamates to CS_2. The vials are sealed, placed in an oven at 80 °C for 1 h and afterwards allowed to cool to room temperature. During cooling, the vials are shaken to help partition the CS_2 into the iso-octane layer, which is subsequently washed with water.
CS_2 is determined by gas chromatography (DB-1 megabore fused silica column, 30 m x 0.53 mm) with flame photometric detector (FPD) in the sulfur mode; quantification by external standardization.

Findings :
Specificity - interferences : the method determines all dithiocarbamate residues and thus is not specific for Ziram.
Calibration : standard curve is prepared by spiking clean GFF's with ziram standard solutions (range 0.48 - 17.3 µg per GFF) and is described by a 3rd-order polynomial (r^2 = 1.000)
Freezer stability : ziram is stable in cold storage at -15°C to -20°C for periods up to 2 months
2.6 µg/GFF spike : average recovery 92.4% (n = 5; RSD 9.1%)
1.04 µg/GFF spike : average recovery 97.3% (n = 3; RSD 3.0%)
Air sampling stability :7 - 10 µg /GFF spike : average recovery 88.2% (n = 6; RSD 22.0%)
after 15 L/min flow for 24 h (23°C)
Limit of determination (LOQ) : limit of detection is stated to be about 0.3 µg/GFF ziram (equivalent to 14 ng/m^3 at 15 L/min flow rate for 24 h sampling), but the recovery obtained at this concentration level was not mentioned in the report.

Conclusions :
The method allows no selective determination of Ziram since all dithiocarbamate residues are measured.
The available validation data do not allow to establish the LOQ of the method unequivocally.

B.4.4 Analytical methods (residue) wildlife and for use in support of diagnostic and therapeutic regimes (Annex IIA 4.2.5; Annex IIIA 5.2.5)

A method of analysis for blood (toluene-3,4-dithiol method) and a method of analysis for urine (TTCA or 2-thiothiazolidine-4-carboxylic acid method) were submitted on September 5th 2000. Evaluation of these studies is ongoing.

ZIRAM

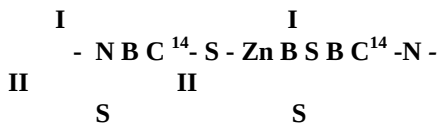
Addendum to the monograph

September 2000

B.5 Toxicology and metabolism

B.5.1 Absorption, distribution, excretion and metabolism in rats (toxicokinetics) (Annex IIA 5.1)

Preliminary results from the ADME in rats:



(^{14}C)-Ziram: ADME in the rat following single oral administration (Covance study nE 514/40, Study status report, 2000).

Findings:
According to the results reported in table B.5.1. -1, absorption rate of ziram is low: peak plasma concentrations were attained after 6.8-10 h for the low dose reaching 24 h after high dose.
Increasing the dose by a factor of 10, increased Cmax plasma 17 times and Cmax blood 30 times; simultaneously, the half-life of elimination increased with the dose, all together suggesting increased saturation of excretion.
Elimination half-live is higher than 24 h suggesting that accumulation occur. Binding to red blood cells is suggested.

Table B.5.1-1: kinetic parameters of ziram after single low and high dose in rat.

	15 mg/kg bw				**150 mg/kg bw**			
	–		–		–		–	
	blood	**plasma**	**blood**	**plasma**	**blood**	**plasma**	**blood**	**plasma**
C_{max}(ìg equiv./g)	1.286	0.859	1.304	0.804	31.31	13.68	36.06	17.98
T_{max}(h)	14.4	6.8	21.6	10.4	43.2	24	48	24
$T_{1/2el}$ (h)	170.5	33.44	196.2	38.9	234.1	36.1	247.9	38.22
$AUC_{(0-t)}$(ìg equi.h/g)	157.0	21.97	194.2	22.24	4721	659.2	5498	818.2
$AUC_{(0-_)}$(ìg equi.h/g)	273.8	29.95	357.5	31.31	11064	809.4	13181	994.5

Tissue distribution:

Tissue distribution was large:
2 hr after single oral low dose, high radioactivity was detected in carcass and fat. Excretory organs such as liver, kidney and lung reached a maximum at 8 hr while thyroid contained the highest radioactivity at 24 h.
2 h after single oral high dose, highest levels of radioactivity were observed in carcass(male and females), lung (females)and thyroid (females); at 8 hr, radioactivity residue was the highest in female pituitary gland while liver and kidney and other tissues contained the highest residue at 24h. At that time, blood and plasma residues were also the highest. Female presented higher levels of radioactivity in tissues than males.
At 96 h, radioactivity was still present in all tissues except brain, fat and pituitary for males and fat for females. Highest residues were observed in thyroid, liver, kidney and blood. It was not possible to estimate from these results how many percent or ìg of the initial administered dose were recovered in the organs (Table B.5.1-2).

Table B.5.1. -2: Tissue distribution in rats after low and high dose of ziram

	ìg equivalents/g (^{14}C)-ziram															
	2 h				**8 h**				**24 h**				**96 h**			
	15 mg		150mg		15 mg		150mg		15 mg		150mg		15 mg		150mg	
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
car cass	6.7	7.2	**130**	**134**	2.9	2.0	81	95	.4	.3	10	21	.14	.17	1.5	2.0
bone	.15	.15	1.6	1.1	.55	.33	3.2	2.6	.20	.15	4.9	5.6	.15	.15	2.0	2.2
lung	.93	1.14	5.8	**51**	1.6	1.7	9.2	6.5	.84	.87	9.5	20	.39	.51	3.0	4.9
liver	3.1	2.4	17	13	4.6	4.2	11	13	2.4	2.2	**14**	**21**	.84	.98	4.5	6.0
kidney	2.3	1.5	7.4	5.9	3.8	2.7	9.8	9.1	1.4	1.2	**19**	**38**	0.6	0.7	4.0	6.9
brain	.43	.34	1.8	2.6	.70	.69	2.8	3.3	.21	.21	4.5	**9.8**	.1	.12	-	.98
fat	2.4	2.1	10	10	1.9	2.0	19	12	.54	.26	11	**39**	.35	.34	-	-
Spleen	1.2	1.1	3.8	9.3	1.4	1.4	5.6	4.6	0.7	0.6	**8.8**	**18**	.37	.43	2.1	4.2
pituitary	.25	.11	.51	-	.80	-	2.7	**1020**	.48	.21	2.8	26	.19	.23	-	1.7
Testes/ ovary	.43	.48	1.7	1.8	.80	.15	2.9	3.8	.25	.03	**5.3**	12	.09	.01	.54	2.2
thyroid	.37	1.1	3.2	**62**	1.8	3.6	7.5	2.8	2.5	4.7	21	24	.63	-	4.8	9.4
adrenal	.95	.85	2.8	5.6	2.7	1.9	5.7	7.2	1.2	1.3	**9.1**	**17**	.49	.73	2.2	4.5
muscle	.35	.31	2.0	1.0	.66	.54	2.3	1.9	.22	.19	3.9	**8.3**	.11	.12	.99	.46
heart	.63	.49	2.2	3.4	1.2	1.1	3.9	4.3	.75	.73	8	**15**	.52	.56	3.2	5.5
Blood	.55	.50	1.8	1.5	1.6	1.5	5	6.2	1.0	1.0	9	**16**	.69	.89	2.9	7.8
plasma	.68	.57	2.1	1.7	1.23	1.08	4.2	4.1	.34	.31	5.6	**10**	.08	.13	1.2	1.5
total	**21.4**	**20.3**	**193.7**	**300**	**28.2**	**24.9**	**175.8**	**1196**	**13.4**	**14.3**	**146.4**	**300.7**	**5.74**	**6.08**	**32.9**	**60.2**

- : not detected

Conclusion: from the preliminary results here reported, it can be concluded that absorption rate and extent are low; elimination rate is very low suggesting accumulation of ziram or its metabolites. Tissue distribution is large and comparable in male and female rats after low dose. After high dose, in female rats, higher levels of radioactivity were observed in pituitary (at 8hr)and thyroid (at 2h).
Maximal tissue residue is observed at 8 h after low dose and at 24h after high dose.
At 96-h post dosing, radioactivity was still present in the majority of organs.

In the monograph, it was concluded that the oral absorbed dose reached 58-61% after low dose and a correction factor of 50% was applied. Therefore, based on the new data, no further correction is necessary.
In the monograph, it was suggested that the compound did not accumulate, however, based on the kinetic parameters from the new study, we can expect that ziram accumulates.
No further information on excretion.
We are still expecting metabolite identification as well as a metabolism scheme.

Guidelines: up to now, it is not possible to conclude if the experimental protocol of the study is in compliance with the test method B dir.87/302/EEC. Results are described as a preliminary report.

In this study, the applicant explained that they had problems for achieving a mass balance and the excretion study was therefore, delayed. In addition, a number of pilot studies have been conducted both to assist with the study design and also to solve problems associated with the excretion balance studies.
Originally the low and high dose levels were set at 15 and 352 mg/kg bw respectively, however during the early stages of the study, rats showed toxic effects at the high dose level. The high dose level was therefore lowered to 150 mg/kg bw and the high dose pharmacokinetic study were repeated. There were also problems for obtaining a homogenous suspension and the formulation method will be used.

Material and methods: 5 Sprague Dawley rats/sex/dose received a single oral dose of 15 or 150 mg/kg bw ziram (100 iCi/kg; radiochemical purity > 98%; non-radiolabelled ziram, batch nE. ; >98%) in 0.3% (w/v) aqueous carboxymethyl cellulose, for the 168 h toxicokinetic study. At 168-h post dose, animals were killed and blood and tissues were collected.
Bile duct of 4 rats/sex/dose was cannulated.
Information on the extent of metabolism will be undertaken from a pilot study. Samples from volatile traps may also be taken to identify volatile metabolites.

B.5.3.3.1 28 day inhalation toxicity in the rat (Annex IIA 5.3.3)

Range finding study in rats: 5 day repeated dose snout only inhalation toxicity (Brooker, 2000, Task force study)

Findings:
At the top dose, animals were sacrificed day 4 after exposure due to proliferative changes observed in larynx.

Body weight gain was decreased at all tested doses.

Histopathology of larynx: changes were seen at all dose levels. In the high dose group, the epithelial changes were generally both ulcerative/inflammatory and proliferative (hyperplasia/squamous metaplasia) in character, whereas in the low and intermediate dose groups the changes were predominantly proliferative (hyperplasia/squamous metaplasia).
In lungs, lesions were detected in all treated groups and demonstrated a dose-relationship. In the airways, epithelial hyperplasia was detected in the bronchioles and terminal bronchioles with prominent goblet cells seen in the major airways. In the alveolar ducts, granulomatous inflammation with increased mural fibrous tissue was detected. In addition, extravasation of eosinophils was detected.
Nasal turbinate: changes were variable, generally more common in females and confined to the high dose group. The lesions were detected at a low incidence in olfactory, respiratory and transitional epithelium and involved both degeneration and proliferative responses.

Exposure level	**0**		**0.002 mg/l = 0.540 mg/kg bw/d**		**0.005 mg/l= 1.35 mg/kg bw/d**		**0.015 mg/l = 4.05 mg/kg bw/d**	
	–	–	–	–	–	–	–	–
Body weight							$ 12%	$ 11%
Body weight **gain**			$ 10%	$29%	$36%	$74%	Killed day 4	Killed day 4
Food consump.			$ 8 %	$5%	$ 6%	$2%		
Water cons.			$ 2 %	$52%	$13%	$21%		
Organ weight :								
Lung,bronchi							$28%	$33%
spleen							$ 17 %	$ 25%
Histopathological findings : 5 rats/sex examined								
larynx								
Ventrolateral-epithelial ulceration	0	0	0	0	0	0	2	1

Ventrolateral squamous metaplasia	0	0	5*	5*	5*	4**	4**	4**
Ventral-epithelial hyperplasia	0	0	5*	5*	5*	5*	4**	3
Arytenoid-epithelial hyperplasia	0	0	2	3	3	2	5**	3
Ventral-epithelial ulceration	0	0	0	0	0	0	3	1
Lungs:								
Alveolar ducts-granulomatous inflamm./fibrous tissue:								
	0	0	1	1	4*	3	5**	4*
Terminal bronchioles-epithelial hyperplasia:								
	0	0	0	0	4*	2	5**	3
Bronchiolar epithelium-prominent goblet cells:								
	0	0	4*	1	3	4*	5**	2
Extravasation of eosinophils:								
	0	0	3	2	5**	4*	4*	3
Bronchiolar epithelium hyperplasia:								
	0	0	0	0	0	0	5**	2
Nasal turbinate:								
Olfactory epithelium atrophy	1	0	0	1	2	0	2	4**
Tracheal and tracheal bifurcation:								
Epithelial hyperplasia	0	0	1	0	1	0	4**	4**
Epithelial hyperplasia bifurcation	0	0	3	0	3	3	5**	5**

* P<0.05; ** p < 0.01 with Fisher >s exact test

Conclusion: evidence of inflammatory, degenerative and proliferative changes was detected throughout the respiratory tract at all dose levels.
In this study, no NOAEL can be proposed.

LOAEL= 0.540 mg/kg bw/d

Guidelines: Experimental protocol in compliance with the test method B.8 of dir.96/54/EEC.
GLP status: yes (no attest of competent authority)
Material and methods:
Preliminary study: 5 rats/sex/dose were exposed by snout to ziram (batch nE V755/G8803961: 99.4 %) for 5 days, 6 hour/day at 0, 0.002, 0.005 and 0.015 mg/l. Animals were sacrificed 2 days after last exposure.
Conversion of mg/l in mg/kg bw/d: dose (mg/l) x 270

B.5.4 Genotoxicity (Annex IIA 5.4)

B.5.4.1 In vitro Genotoxicity testing (Annex IIA 5.4.1)

B.5.4.1.2 Gene mutation test in mammalian cells (Annex IIA 5.4.1)

- Mammalian cell mutation assay with ziram technical: forward mutations (TK) in mouse lymphoma cells L5178Y (Ransome, 1999, Task Force study) was provided to the RMS in October-November 1999.

Findings:
Cytotoxicity was determined in the preliminary experiment by measuring relative survival (Table B.5.4.1.2-1).
Mutation tests: 2 tests were performed: T1 with 3 hour treatment (Table B.5.4.1.2-2) and T2 with 24 h treatment (Table B.5.4.1.2-3).
- Without S9 mix, a statistically significant increase in mutant frequency, which was outside the historical control range, was observed at 2 ìg/ml in test 1. In test 2, no statistically significant increases in mutant frequency were observed even at levels of extreme toxicity.
- With S9 mix, no statistically significant increases in mutant frequency, which were outside the historical control range, were observed in test 1. In test 2, a statistically significant increase, outside the historical control, was observed at 3 ìg/ml, but the D0 relative survival (4%) at this level was below the acceptable limit of toxicity.

Conclusion: no biologically significant increase in mutant frequency was observed at any of the tested concentrations. Ziram can be considered as non-mutagenic with or without metabolic activation when tested at concentrations extending into toxic doses.

Table B.5.4.1.2-1: preliminary cytotoxicity test.

Conc. Ziram technical (ìg/ml)	**% relative survival**		
	W/o S9 mix		**With S9 mix**
	3 h	**24 h**	**3 h**
0	100	100	100
0.01		113	
0.05		7	
0.1		0	
0.25		0	
0.5		0	
1		0	
1.96	26	0	51
2.5		0	
3.91	2		3
	0		0

7.82			
15.63	0		0
31.25	0		0
62.50	0		0
93.75	0		0
125	0		0

Table B.5.4.1.2-2: Mutation tests Test 1:

Conc. Ziram technical (ìg/ml)	% relative survival	Mean relative %cloning efficiency	Mean mutant frequency	% relative survival	Mean relative %cloning efficiency	Mean mutant frequency
	W/o S9 mix			With S9 mix		
	D0	D2		D0	D2	
0	100	100	0.000179	100	100	0.000217
0.005	74			100		
0.01	109			111		
0.05	106			91		
0.1	78	93	0.000203	84	88	0.000219
0.25	100	70	0.000259	103	76	0.000252
0.5	90	82	0.000301	106	84	0.000230
1	71	68	0.000299	118	86	0.000222
2.00	26	58	0.000377**	52	82	0.000302
3.00	0			0		
MC 2.5ìg/ml				97	73	0.001327**
MMS 10ìg/ml	47	56	0.001356**			

** Statistically significant increase outside historical control range

Table B.5.4.1.2-3: Mutation tests Test 2.

Conc. Ziram technical	% relative survival	Mean relative %cloning efficiency	Mean mutant frequency	% relative survival	Mean relative %cloning efficiency	Mean mutant frequency

(ìg/ml)						
	w/o S9mix			With S9mix		
	D0	D2		D0	D2	
0	100	100	0.000153	100	100	0.000129
0.0001	50					
0.0005	109					
0.001	97	78	0.000173			
0.0025	57	86	0.000159			
0.005	71	70	0.000188			
0.01	58	69	0.000206			
0.025	4	58	0.000171			
0.05	0			81		
0.1	0			60		
0.25				80		
0.5				81		
1				83	82	0.000145
1.5				70	97	0.000119
2.00				40	80	0.000131
2.5				11	66	0.000316
3.00				4	57	0.000383
MC 2.5ìg/ml				74	78	0.000601**
MMS 5ìg/ml	60	57	0.001505 **			

** Statistically significant increase outside historical control range.

Guidelines:
Experimental protocol in compliance with test method B.17 of directive 96/54/EEC and with TG OECD 476 (1997).
GLP status: the study is GLP.
Material and methods:
Mouse lymphoma L5178Y/TK+/- cell suspensions were exposed to Ziram (UCB 99.4 %, lot nE. V755/G8803961) with or without S9 (rat liver treated with Aroclor 1254). A pretest and a main test were performed.
Preliminary toxicity test:
3 h treatment (+/- S9), doses: 1.96, 3.91, 7.82, 15.63, 31.25, 62.5, 93.75, 125 ìg/ml
24 h treatment (-S9), doses: 0.01, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 ìg/ml

Mutation tests:
-S9: test 1 (3 h treat.): 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 1, 2, 3 ìg/ml
Test 2 (24 h treat.): 0.0001, 0.0005, 0.001, 0.0025, 0.005, 0.01, 0.025, 0.05, 0.1 ìg/ml
+ S9: test 1 (3-h treat.) : 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 1, 2, 3 ìg/ml
Test 2 (3-h treat.) : 0.05, 0.1, 0.25, 0.5, 1, 1.5, 2, 2.5, 3 ìg/ml
Positive control: methyl methanesulphonate (-S9), 3-methylcholanthrene with S9.Solvent was DMSO.

B.5.5.1 Long-term (2 years) oral toxicity in the rat (Annex IIA 5.5)

A long-term rat study of ziram was published in the open literature by Enomoto et al., 1989 and Maita et al., 1997 and was summarised in the monograph.
The full report of the study was made available to the RMS on April 1999 by the applicant and was re-evaluated on this basis.

Twenty-four-month dose chronic toxicity and carcinogenicity study of ziram in rats (Harada et al., 1983)

Findings:
Main findings are described in table B.5.5.1-4. The MTD was reached.
Mortality: a slight increase in cumulative death rate in male top dose group was observed and was mainly due to foreign body pneumonia and rhinitis. No such an effect was observed in females. These effects were considered not treatment-related, since the same respiratory lesions were also observed in the control group.
Clinical signs: abnormal changes in the hindlimbs were observed in 3 males of the top dose group at the terminal stage of the study. Lose of vigour in hindlimbs from the knee joint to the crural part and paralytic-like signs were observed. These changes were considered to be due to tibia abnormalities observed in macroscopic, X-ray and histological examination.
Haematology: in the 2000 ppm group, slight but significant decrease in hematocrit, haemoglobin and RBC count were observed in males in week 78 and anaemic tendency. In females, significant decrease in hematocrit, haemoglobin and RBC count were observed in week 52 and 78 and mean corpuscular volume, mean corpuscular haemoglobin and reticulocyte count also increased. These changes recovered almost completely in week 104 where only decreased RBC count and increased corpuscular volume were still observed.
Blood chemistry: at 2000 ppm, from week 52 for males, and week 26 for females, glucose, and total cholesterol were low, reflecting the low nutrition state of the animals. Significant decrease in calcium is probably related to the thyroid and bone pathology and strongly suspected as a compound-related effect.
Organ weight: decreases in absolute and relative weights of the crural muscle were observed in males at 200 ppm and 2000 ppm from week 52 and in females from week 78 of administration.
Spleen increased weight was considered to be a secondary change in response to anaemia observed in haematology. Increased liver weight was not associated with abnormal histology.
Necropsy findings: at the top dose, hypertrophy of thyroid and atrophy of the crural muscle were observed with a high frequency in males and females at killing schedule in week 52 and 104 of administration. In males of the same group, proximal arcuation of the tibia and extension deficiency of the knee joint were observed sporadically in some animals at the end of examination. In the soft X-ray examination of the diseased limbs of animals with severe changes, clear extension of the tibia, proximal arcuation of the knee joint, and dilatation of the epiphysis were observed. Such changes were not observed in animals given 200 ppm or less.
Histopathology of non neoplastic lesions:
At 2000ppm:
Follicular hypertrophy of the thyroid was observed (sometimes at 200 ppm). This effect was considered to be related to treatment, but there were no proliferative changes of increase in frequency of tumours and the change was considered to be rather the regressive lesion.
In the stomach, increased mucosal cornification of anterior stomach was increased from week 26 and was considered to be a local response to the direct stimulation in the gastric wall by ziram.
In bone marrow, hematogenesis was increased from week 79 in males and in females, extramedullary hematopoieisis of the spleen was increased in some periods.
In the liver, small granulomas were observed in males, but there were no noticeable enzymatic or histological changes.
In males, dilatation of the oesophageal cavity was considered to be related to onset of foreign body nasal catarrh and pneumonia.
In addition, increases in inner bronchial groove remnant of the thyroid, sinus dilatation of the adrenals, foreign body pneumonia and rhinitis, and dilatation of oesophageal cavity which were observed in the treated groups were considered to be probably due to incidental increase in background lesions and not directly related to ziram.
In the knee-joint, excessive remnant of chondrial plate of the epiphysis (delay in closure plate of epiphysis) which is usually closed with ageing were observed in 22 of the 77 males and in 13 of the 73 females. The same lesions were observed mainly in the tibia of males and in both tibia and femur of females. Furthermore, chondrial plate of epiphysis of the tibia was hypertrophied in part of the males because of hyperplasia of cartilage cells and hyperplasia of the neo-bone trabecula. These changes were not due to direct action of ziram on the cartilage cells, but rather the

decrease in calcium observed in the blood biochemical examination and the relationship to the above-mentioned thyroidal lesions suspected.
In the crural muscle, focal muscular atrophy was observed from week 52 of administration and in sciatic nerve, nerve fibre degeneration was observed in males and females with a high frequency in week 104 of administration. This lesion was clearly due to ziram, but since atrophy of the muscle fibre was localised, neurogenicity was suspected, and the relationship to nerve fibre degeneration of the sciatic nerve, which increased in males and females of 2000-ppm group was spotlighted.
At 200 ppm: total frequency of follicular hypertrophy of the thyroid in females and increased cornification of anterior stomach and atrophy of the crural muscle increased significantly from week 78.
At 20 ppm: there were no differences with the control (Table B. 5.5.1-5).

In neoplastic lesions, the kind and onset time and frequency of the lesions developed in each treated group were similar to those in the control group, and there were no marked differences between the results of the present study and those in the control group of the other toxicity studies performed in the laboratory (Table B. 5.5.1-6).

Table B. 5.5.1-4: 104-week toxicity study in rats.

Endpoints/dose	**0 ppm**		**20 ppm**		**200 ppm**		**2000 ppm**	
	–	–	–	–	–	–	–	–
Mortality								
after 12 month	0/56	0/56	1/56	2/56	1/56	4/56	0/56	2/56
after 26month	15/56	21/56	12/56	17/56	15/56	23/55	22/56	22/56
Clinical signs			see text					
Body weight changes							20 %	27 %
Food consumption							(11%)	(13%)
Water consumption							817% week1880	89 % week14848
Urinalysis: specific gravity					0.5 %		2 %week 528104	2 %week 26, 52
Haematology:								
MCV							3 %*	85%*
MCH							4%*	
RBC								23%**
Clinical chemistry								
glucose							12 %**	7 %*
cholesterol							44 %***	
Ca^{2+}							5 %***	7%***
bilirubine								37%*
Rel. organ weight								
liver							815%***	819%***
spleen							824%*	
testes							882%***	
crural muscle				4%*	25%***	22%***	27%***	15%***
thyroid								878%***
Necropsy findings								
thyroid swelling, hypertrophy	5/80	1/80	4/80	4/80	12/80	4/80	29/80	15/80
crural muscle atrophy	0/80	1/80	0/80	0/80	4/80	2/80	34/80	23/80

*, **; *** Statistically significantly different from control at 5, 1 or 0.1% level of probability.

() : not statistically significant

Table B. 5.5.1-5 : 104 week toxicity study in rats : non-neoplastic lesions.

Endpoints/dose	0 ppm		20 ppm		200 ppm		2000 ppm	
	–	–	–	–	–	–	–	–
nE. animal examined	80	80	80	80	80	80	80	80
Spleen:increased brown pigmentation	4	21	4	26	7	34	10	31
congestion	3	6	6	2	4	7	10	6
extramedullary hematopoiesis		13		16		14		34***
Liver: small granuloma	5		10		10		15*	
thymus: lymphocyte hyperplasia	1		2		2		5	2
Oesophagus cavity dilatation	3	14	3	8	5	22	21**	15
Stomach mucosal cornification	5	1	1	6	13	11**	46	34**
Lung: foreign body pneumonia	3	12	2	8	4	16	17**	15
Pancreas fibrosis	5	2	5	1	1	2	10	8
Thyroid: acinous hypertrophy	2	1	4	1	3	9**	64***	66***
inner bronchial groove remnant	1		3	1	4	4	17***	29***
Adrenal :								
sinus dilatation	3	7	4	4	6	10	13**	37***
medullar hyperplasia	2		3		4		6	
Sciatic nerve: nerve fibre degeneration			2	2			30***	26***
Bone marrow: increased hematogenesis	10	7	10	4	4	3	21*	23**
Bone and knee joint: delay in closure of chondrial plate of tibia epiphysis		1				5	22***	13***
chondrial cell hyperplasia of tibia epiphysis							6	
Crural muscle atrophy	1	2	4		49***	25***	68***	46***
	7	10	6	7	7	21*	37***	32***

Nasal cavity rhinitis:								
Auditory canal otitis:	4	12	3	10	5	25	13	17

*,**,*** : statistically significant at p<0.05, <0.01, <0.001 : level of significance by Fisher=s exact probability test.

Table B. 5.5.1-6 : 104 week toxicity study in rats : neoplastic lesions.

Endpoints/dose	0 ppm		20 ppm		200 ppm		2000 ppm	
	–	–	–	–	–	–	–	–
nE. animal examined	80	80	80	80	80	80	80	80
Liver: nodular cell hyperplastic foci		2	1			1	3	3
Perputal adenoma	3		3		4		8	
Thyroid:								
C- cell adenoma	11	6	11	6	15	6	7	1
C- cell adenocarcinoma			1	1	3	2	1	1
follicular adenoma	1						1	
follicular adenocarcinoma			1				1	
Total benign tumours	105	51	102	46	106	33	100	23
Total malignant tumours	18	14	13	16	17	6	13	10
Total benign and malignant tumours	123	65	115	62	123	39	113	33

Conclusion: ziram is not carcinogenic in this study.

NOAEL = 20 ppm = 0.56 mg/kg bw/d.

Guidelines : Experimental protocol not fully in compliance with Dir. 87/302/EEC annex V. B or OCDE 453 (1981).
Deviation from official protocol : haematology not performed at 3 month. Urinary volume not measured and sediment not examined.
GLP status : no attest of study director, attest of QAU, no attest of competent authority.
Material and methods:
80 Fischer rats (F344/DuCrj strain, SPF)/sex/dose received ziram (97.5%) in the diet at 0, 20, 200 or 2000 ppm for 24 months. 8 animals /sex/dose were sacrificed at week 26, 52 or 78 .
Stability of ziram in diet : The dietary admixture was prepared 3 times a week. The results of stability of ziram in diet indicate that integrity of ziram in test diets could be preserved 80% or more in the 20 and 200-ppm diet. At 2000 ppm 90% of ziram was preserved.
Taking the aspect of unstability of ziram in diet into account , at doses of 20 to 200 ppm, it is necessary to apply a correction factor of 20% and of 10% for the 2000-ppm estimated dietary intake.
Converted dose intake : for male : 0.56 ; 5.52 or 66.6 mg/kg bw/d
For females :0.664; 6.8; 81.9 mg/kg bw/d
The study is accepted.

Establishment of an Acceptable Daily Intake (ADI)

An ADI is proposed on the basis of the long-term toxicity study in rats in which a NOAEL of 0.56 mg/kg bw/d is acceptable. An assessment factor of 100 is proposed.

ADI = 0.006 mg/kg bw/d

Establishment of an Acceptable Operator Exposure Level (AOEL)

In the monograph, a systemic AOEL short-term was calculated on the basis of a NOAEL from the 90 day rat study , satellite study of 104 week rat study, of 3.2 mg/kg bw/d. A correction factor of 50% was applied for oral absorption and an assessment factor of 100 was used for extrapolation to human, giving a

AOEL systemic = 0.016 mg/kg bw/d.

In the monograph, a NOAEL for dermal exposure was also proposed which was based on a 21 dermal rabbit study.

AOELdermal = 1 mg/kg bw/d.

During the ECCO 78, it was concluded that a repeat dose inhalation study was necessary.

The results of the range finding study reported in this addendum , point B.5.3.3.1, suggest that at the tested doses, no NOAEL can de derived as at the lowest tested dose of 0.540-mg/kg bw/d , signs of respiratory tract diseases were reported.
A provisional AOELinhalation is proposed using the LOAEL from the 5day range-finding study, and applying an assessment factor of 1000:

Provisional AOELinhalation = 0.00054 mg/kg bw/d.

Due to the fact that inhalation of ziram induced target organ toxicity at lower doses than those observed after oral ingestion, we considered that it was necessary to make a first approach of operator exposure using a dermal AOEL as defined in the monograph as well as the provisional AOEL obtained from the 5 day preliminary range finding inhalation study reported in this addendum. , waiting the results of the main study (October 2000)

B.5.14 Exposure data (Annex IIIA 7.2)

B.5.14.1 Estimation of operator exposure (annex IIIA 7.2.1.1)

Ziram WG formulations are mainly applied in orchards (pomefruit, stonefruit, and almond). The application rates are in the range of 1.18 to 3.04 kg a.s. /ha. Spray concentrations are in the range 75-304-532 g a.s. /hl. At maximum, 4 to 6 applications per season can be applied with an interval of 7-14 days. Main application occurs through atomisers or sprayers between 1000 and 2000-l water per ha but can change depending on, the area, the type of material used and the size of the trees. The UK POEM and the German model for the following typical scenario estimated the operator exposure:

Table B.5.14.1-1 : Orchard treatment (cherry, plum, peach), 3.04 kg/ha, 304 g a.s. /hl.

	UK POEM model	**German model**
	Use rate : 3.04 kg a.s./ha	
Number ha treated/day	30 ha/day	8 ha/day
Method of use:	vehicle air assisted broadcast > 400 l/ha	high crop tractor mounted
AOEL dermal	= 1 mg/kg bw/d	
AOELinhalation	= 0.000540 mg/kg bw/d (based on the LOAEL = 0.540 mg/kg bw/d; AF= 1000)	
Body weight	60 kg	70 kg
Dermal absorption :	5% during M/L 30% during A	

In a first approach, exposure estimation were made using the UK POEM and the German model.
As shown in table Table B.5.14.1-2, when no protections are used, operator exposure is above the AOELdermal and AOELinhalation. Operatore exposure was then estimated using protective equipment such as gloves for the UK data and gloves as well as inhalation protection in the German model. In both cases, operator exposure was higher than the respective AOELs.
In the monograph, a study was reported in which measurement of operator exposure to ziram was described. The data reported in the study were compared with the respective AOELs and from the bottom of the table B.5.14.1-2, it appears that dermal absorbed dose is acceptable but inhalation exposure is too high.

Table B.5.14.1-2: Operator exposure as calculated according to the UK POEM and the German model and comparison of data from measurement of operator exposure with AOELdermal and AOELinhalation.

UK model	**German model**
Absorbed dose (mg/kg bw/d) and % of respective AOEL	
No protection :	

Total	3.37 mg/kg bw/d = 21062% AOELsyst	1.226 mg/kg bw/d = 7662 % AOELsyst
Dermal	3.36 mg/kg bw/d = 336% of AOEL	1.217 mg/kg bw/d = 121% of AOEL
Inhalation	0.015 mg/kg bw/d = 2777 % of AOEL	0.0089 mg/kg bw/d = 1648% of AOEL
With gloves:		
Total	1.325 mg/kg bw/d = 8281% AOELsyst	
Dermal	1.310 mg/kg bw/d = 131% of AOEL	
Inhalation	0.015 mg/kg bw/d = 2778% of AOEL	
With gloves and RPE-FF2-SL-P2 for inhalation during M/L		
Dermal	-	1.183 mg/kg bw/d = 118% of AOEL
Inhalation	-	**0.0063 mg/kg bw/d = 1166% of AOEL**

Measurement of exposure : performed w/o cabs or open cabs		
	Absorbed dose (mg/kg bw/d) and % of respective AOEL	
	3.04kg/ha, 30 ha/day	3.04 kg/ha, 8 ha/day
Dermal route	0.079 mg/kg bw/d = 7.9% of AOEL	0.021 mg/kg bw/d = 2.1 % of AOEL
Inhalation	0.0228 mg/kg bw/d = 4222% of AOEL	0.006 mg/kg bw/d = 1111 % of AOEL

Conclusion: from this preliminary assessment of operator exposure, it can be anticipated that, even if the NOAELinhalation from the expected 28 day rat inhalation toxicity study is 10 times lower than that reported in the range-finding study, inhalation exposure will be to high giving rise to an unacceptable inhalation risk for operators.

- Spontaneous tumours in F344/DuCrj rats from 12 control groups of chronic and oncogenicity studies from the same laboratory as which one performed the above mentioned carcinogenicity study in rat (Maita et al., 1987).

Results from studies carried out during a 1978-1983 period in 960 males and 959 female rats used as controls in 12 chronic and oncogenicity studies.

Findings:
C-cell adenoma of the thyroid usually occurred unilaterally but was not rare to arise bilaterally. The criteria to define between hyperplasia and adenoma are much debatable. Maita Criterion for C-cell adenoma was based on size of the lesion, and more than one follicular size was diagnosed as adenoma. When the tumour cells showed a proliferation beyond the capsule, or metastasis to the neighbouring lymphnode or lung, adenocarcinoma was given as a diagnosis. C-cell adenoma was seen predominantly in males. These results suggest that thyroid tumours have a high rate of spontaneous occurrence.

1. Age-related occurrence of tumours in **male F344/DuCrj rats** at more than 5% incidence and variability in incidence among 12 control groups:

Histological types of tumours	**Overall incidence of tumours and variability in incidence**	**Age-related occurrence of tumours nbre affected (%)**			
		0-58 wk.	**59-84**	**85-97**	**98-110**
NE. of animals examined during each interval of age	960	198	144	57	591

Leukaemia (mononuclear cell)	7.1 (3.8-12.5)	3 (1.5%)	6 (5.3)	12 (21.1)	47 (8)
Testis : interstitial cell tumour	74.6 (63.8-82.5)	4 (2)	88 (77.2)	41(71.9)	583(98.6)
Preputial gland : adenoma	5.3 (1.3-12.5)	1 (0.5)	3 (2.6)	4 (7)	43 (7.3)
Pituitary: anterior adenoma	12.4 (7.5-18.8)	-	6 (5.3)	7 (12.3)	106 (17.9)
Thyroid : C-cell adenoma	**12.5 (3.8-21.3)***	**-**	**6 (5.3)**	**5 (8.8)**	**109 (18.4)**
Adrenal: pheochromocytoma	11.9 (1.3-22.5)*	3(3.8)	6 (5.3)	9 (15.8)	96 (16.3)
Subcutis : fibroma	7.2 (5.0-11.3)	-	2 (1.8)	6 (10.5)	61(10.3)

* Significant heterogeneity p<0.05 Fisher=s exact test.

2. Age-related occurrence of tumours in **female F344/DuCrj rats** at more than 5% incidence and variability in incidence among 12 control groups:

Histological types of tumours	**Overall incidence of tumours and variability in incidence**	**Age-related occurrence of tumours nbre affected (%)**			
		0-58 wk.	**59-84**	**85-97**	**98-110**
NE. of animals examined during each interval of age	959	204	141	45	568
Leukaemia (mononuclear cell)	9.5 (3.8-15)	- (-)	4 (2.8)	9 (20)	78 (13.7)
Uterus : endometrial polyp	12.6 (6.3-23.8)	3 (1.5)	9 (6.3)	3(6.7)	106(18.7)
Preputial gland : adenoma	5.3 (1.3-12.5)	1 (0.5)	3 (2.6)	4 (7)	43 (7.3)
Pituitary: anterior adenoma	28.2 (15-41.3)*	-	25 (17.6)	9 (20)	236 (41.5)
Thyroid : C-cell adenoma	**8.2 (2.5-16.3)**	**1 (1.3)**	**4 (2.8)**	**2 (4.4)**	**72 (12.7)**
Mammary gland : adenoma/fibroadenoama	13.2 (5-22.5)	-	3 (2.1)	2 (4.4)	122 (21.5)

* Significant heterogeneity p<0.05