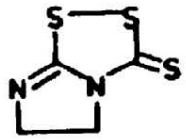
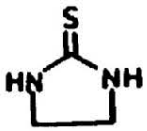
Figure A6.2(1)-1 ADME study in the rat

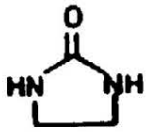
Mancozeb related compounds



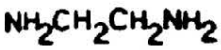
Ethylenebis(isothiocyanate)sulfide (EBIS)
red-brown color with F-N; UV



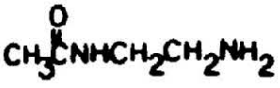
Ethylenethiourea (ETU)
blue color with F-N; pink color with DMCA; UV



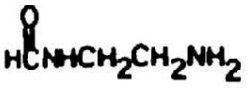
Ethyleneurea (EU)
yellow by Erhlich's reagent



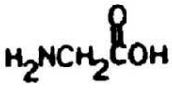
Ethylenediamine (EDA)
fluorescamine then UV



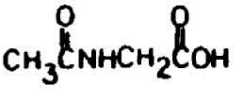
N-Acetylethylenediamine (N-AcEDA)
fluorescamine then UV



N-Formylethylenediamine (N-ForEDA)
fluorescamine then UV



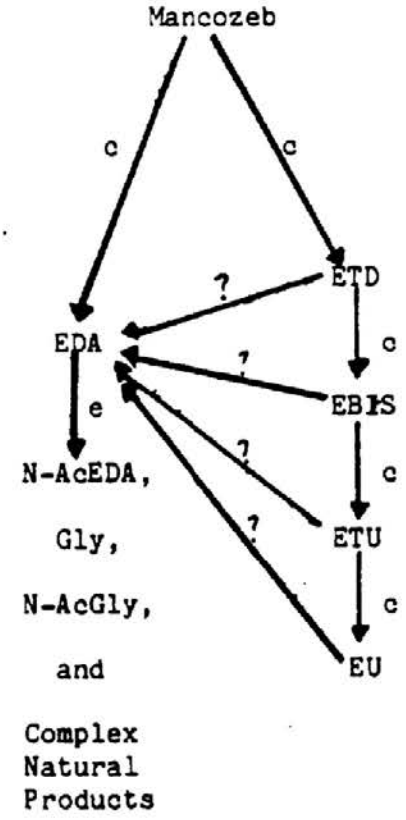
Glycine (Gly)
fluorescamine then UV



N-Acetylglycine (N-AcGly)
spray with 3N HCl, heat, spray with Ninhydrin, and heat again

Figure A6.2(1)-2 ADME study in the rat

Proposed degradation/metabolic pathway for mancozeb in the rat



c: known chemical conversions

e: enzymatic conversions

?: unknown whether these reactions could occur, either chemical enzymatically

Section A6.2(2) Metabolism studies in mammals

The disposition of 14C-Macozeb in the mouse

Annex Point IIA6.2

IUCLID 5.0/2

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1 REFERENCE

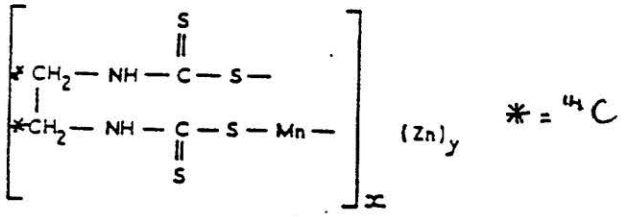
1.1	**Reference**	[redacted] (1990) The Disposition of [14C]-Mancozeb in the Mouse. Inveresk Research International. Report No. 4909. 1 October 1990 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Pennwalt Corporation
1.2.2	Companies with letter of access	Cerexagri SA
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Japanese guideline and EPA guideline n° 85-1. At the time the study was performed, no specific guideline was recommended for the EC registration. Nevertheless EPA guidelines are commonly accepted by several European countries for testing Pesticides. There is no major difference with the required EU guidelines.
2.2	**GLP**	Yes, the test was conducted in compliance with the EPA GLP Guidelines.
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Mancozeb
3.1.1	Radiolabelled test material	A single radiolabelled form of [ethylene-U-14C]-mancozeb was supplied by Amersham International plc:



Section A6.2(2) **Metabolism studies in mammals**

Annex Point IIA6.2 **The disposition of 14C-Macozeb in the mouse**

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3.1.1.1	Description	Not provided.
3.1.1.2	Lot/Batch number	Batch No.: B-DD
3.1.1.3	Purity	Radiochemical purity: at least 98%. Specific activity: 43.2 µCi/mg.
3.1.1.4	Stability	Not provided.
3.1.2	Non-radiolabelled test material	Mancozeb
3.1.2.1	Description	Not provided.
3.1.2.2	Lot/Batch number	Not provided.
3.1.2.3	Purity	Not provided.
3.1.2.4	Stability	Not provided.
3.2	**Test Animals**	*Non-entry field*
3.2.1	Species	Mouse
3.2.2	Strain	CD-1
3.2.3	Source	Charles River (UK) Limited, [redacted].
3.2.4	Sex	Male and female.
3.2.5	Age/weight at study initiation	Animals used were in the weight range 24-36g. Age not provided.
3.2.6	Number of animals per group	49 males and 49 females (see Table A6.2(2)-1 for allocation of animals to groups).
3.2.7	Control animals	None
3.3	**Administration/ Exposure**	Oral
3.3.1	Type	Gavage
3.3.2	Concentration	2.5 or 150 mg/kg bw. See Table A6.2(2)-1 for group allocation.
3.3.3	Vehicle	1% carboxymethylcellulose.
3.3.4	Total volume applied	0.1 or 0.14 ml
3.4	**Examinations**	
3.4.1	Samples	See also Table A6.2(2)-1 for group allocation. **Pre-trial group - Excretion Kinetics: Single Low Dose** (5 males) **Urine and faeces:** collected frozen at the following times post dose: 0-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 h. At 24, 48, 72, 96, 120, 144 and 168 h post dose the cages were washed with water and the washings retained. **CO_2 and CS_2:** collected 0-8 and 8-24 h post dose from one animal.

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Metabolism studies in mammals

The disposition of 14C-Macozeb in the mouse

Body fluids/tissues collected after 168 h: bone mineral, bone-marrow, brain, fat, heart, testes/ovaries, spleen, lungs, kidneys, liver, skeletal muscle, adrenals, thyroid, thymus, whole blood, plasma, gastro-intestinal tract, residual carcass.

Total radioactivity was measured in all samples of urine, faeces, cage wash, expired air, plasma, red blood cells and tissues.

Dosing Group A - Excretion Kinetics: Single Low Dose

(5 females)

Sampling as for Pre-trial group.

Dosing Group B - Excretion Kinetics: Multiple Low Dose

(5 males and 5 females)

Sampling as for Pre-trial group (CO_2 and CS_2 collected from one male and one female).

Dosing Group C - Excretion Kinetics: Single High Dose

(5 males and 5 females)

Sampling as for Pre-trial group (CO_2 and CS_2 collected from one male and one female).

In addition CO_2 and CS_2 collected at the 24-48 h and 48-72 h time points.

Dosing Group D - Blood Kinetics: Single Low Dose

(14 males and 14 females)

Two male and 2 female mice were sacrificed and blood samples were taken at each of the following times post dose: 0.25, 0.5, 1, 2, 4, 8 and 24 h. Levels of radioactivity in whole blood were determined.

Dosing Group E - Tissue Distribution by Quantitative Analysis: Single Low Dose

(12 males and 12 females)

Three male and 3 female mice were sacrificed at each of the following times post dose:

1 h (time of peak blood concentration)*
8 h (time of half peak blood concentration)*
24 h and 48 h
(* as determined by dosing group D)

At sacrifice the concentrations of radioactivity were determined in the body fluids/tissues outlined in the Pretrial Group.

Dosing Group F - Excretion of Total Radioactivity in Bile: Single Low Dose

(4 males and 4 females)

One male and one female animal were sacrificed at 1, 8, 24 and 48 h post dose. Immediately following sacrifice the entire gall bladder

was removed for determination of radioactivity. Excreta were collected at sacrifice or every 24 h, whichever was applicable. Gastro-intestinal tract was analysed separately from the residual carcass.

Dosing Group G - Tissue Distribution by Whole Body Autoradiography: Single Low Dose

(2 males and 2 females)

One male and one female animal were sacrificed at 1 h and 48 h post dose for whole body autoradiography.

Dosing Group H - Tissue Distribution by Whole Body Autoradiography: Single High Dose

(2 males and 2 females)

One male and one female animal were sacrificed at 1 h and 48 h post dose for whole body autoradiography.

Metabolic profiling

Metabolite profiling of representative sex specific urine pools (20% by volume, 0-8 and 8-24 h) was undertaken to determine the extent of [14C]-mancozeb metabolism and to quantify the byconversion of [14C]-mancozeb to [14C]-ethylenethiourea (ETU).

3.4.2 Analytics

Quantification of radioactivity

All samples were analysed by liquid scintillation counting (LSC).

Metabolic profiling

TLC method.

4 RESULTS AND DISCUSSION

4.1 Excretion kinetics

Following single and multiple oral administration radioactivity was rapidly eliminated in urine and faeces. Elimination of radioactivity in expired air was < 5 % in all groups. A mean of less than 1.4 % of the dose remained in carcass and tissues after 7 days.

4.2 Blood kinetics

Peak levels of total radioactivity were obtained at 1 and 2 hours post dosing, levels thereafter decreased rapidly.

4.3 Tissue distribution

Mean levels of total radioactivity were highest at 1 hour post dosing and thereafter decreased to level close to the detection limit by 24-48 hours.

4.4 Biliary excretion

Elimination of absorbed radioactivity via bile was insignificant.

4.5 **Bioconversion to ETU in urine**

Bioconversion to ETU in urine was low (less than 5%).

4.6 **Carbon disulfide evolution**

Low dose: conversion to carbon disulfide was at the limit of detection. High dose group showed ca 4 % conversion to carbon disulfide

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6.2(2) Metabolism studies in mammals

Annex Point IIA6.2

The disposition of 14C-Macozeb in the mouse

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5.1	**Materials and methods**	This study was performed to GLP and EPA test guidelines 85-1. The study investigates the absorption, distribution and elimination of [14C]-mancozeb in the CD-1 mouse following oral administration at low (2.5 mg/kg bw) and high (150 mg/kg bw) dose levels. The bioconversion of [14C]-ethylene thiourea was also investigated.
5.2	**Results and discussion**	Summarize relevant results; discuss dose-response relationship. Following single oral administration of [14C]-mancozeb to male and female mice (low dose 2.5 mg/kg bw) total radioactivity was absorbed and eliminated rapidly. Elimination of absorbed radioactivity via bile was negligible. Urinary excretion accounted for ca 30% of the administered dose representing the absorbed portion of the dose. Faecal elimination accounted for the majority of the dose (ca 60%) representing the non absorbed portion of the dose. The concentrations of total radioactivity in tissues peaked at the same time as that observed in whole blood (ca 1 h). Thereafter, levels of total radioactivity declined in all tissues. Insignificant amounts of the administered dose (ca 0.5%) remained in the carcass after 7 days and recovery of the administered dose was quantitative. The rates and routes of elimination were similar in males and females. Following repeated dosing at the low dose level and single administration of the high dose level (150 mg/kg) the rates and routes of elimination were similar to those observed following single oral administration at the low dose level. No sex related differences in any of the dosing groups were observed. The thyroid was occasionally associated with higher levels of total radioactivity in most dosing groups. These higher levels are probably caused by multiplication factors due to the small amount of tissue available for analysis. Levels of radioactivity were always less than twice background. Metabolism of absorbed [14C]-mancozeb was rapid and extensive with at least 4 major components observed in chromatograms after running in the first dimension. The bioconversion of [14C]-mancozeb to [14C]-Ethylenethiourea (ETU) was low. Less than 5% of the dose was accounted for as ETU following single and multiple oral administration at the low dose level and single oral administration at the high dose level. Similarly conversion of mancozeb to CS_2 was insignificant in these dosing groups.

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Metabolism studies in mammals

The disposition of 14C-Macozeb in the mouse

5.3	**Conclusion**	**Absorption:** rapid (max plasma and tissue concentrations after ca. one hour); ca. 30% bioavailability (based on urinary and biliary excretion). **Distribution:** radioactivity widely distributed, highest concentrations found in GI tract, major excretory organs, thyroid. **Excretion:** rapid, the majority of the radioactivity excreted with 24 hours; insignificant amounts of the administered dose remained in the carcass after 7 days; absorbed portion excreted via the urine; enterohepatic circulation negligible; rates and routes of elimination similar in males and females and after single and repeat dosing. **Metabolism:** bioconversion to ETU <5%; at least 3 other non-identified metabolites, conversion of mancozeb to CS_2 insignificant.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	Other conclusions: *(Adopt applicant's version or include revised version)*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	
	COMMENTS FROM
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*

Section A6.2(2) Metabolism studies in mammals

Annex Point IIA6.2

The disposition of 14C-Macozeb in the mouse

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Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.2(2)-1 The disposition of 14C-Macozeb in the mouse

Group allocation

Dosing Group	Investigation	Single or multiple dosing	Dose Level (mg/kg bw)	No of Males	No of Females
Pretrial Group	Excretion kinetics	Single	2.5	5	-
A	Excretion Kinetics	Single	2.5	-	5
B	Excretion Kinetics	Multiple*	2.5	5	5
C	Excretion Kinetics	Single	150	5	5
D	Blood Kinetics	Single	2.5	14	14
E	Tissue Distribution by Quantitative Analysis	Single	2.5	12	12
F	Excretion of Total Radioactivity in Bile	Single	2.5	4	4
G	Tissue Distribution by Whole Body Autoradiography	Single	2.5	2	2
H	Tissue Distribution by Whole Body Autoradiography	Single	150	2	2

* Multiple oral administrations of non-radiolabelled Mancozeb on Days 1-14 and [14C)]-Mancozeb on Day 15.

Section A6.2(3) Metabolism studies in mammals

Annex Point IIA6.2

Metabolism of 14C-Macozeb in the mouse

IUCLID 5.0/3

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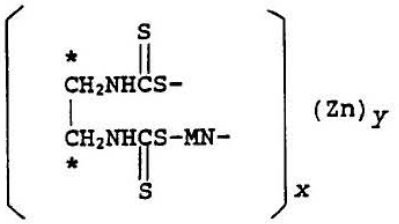
		1 REFERENCE
1.1	**Reference**	[redacted] (1992) Metabolism of [Ethylene-U-14C]-Mancozeb in the Mouse. Inveresk Research International Ltd/ XenoBiotic Laboratories Inc. NPC Project No. T91-3413. 9 July 1992. (unpublished) In-life part (Appendix 1): [redacted] (1992) The Metabolism of [Ethylene-U-14C]-Mancozeb in the Mouse. Inveresk Research International. Report No. 8519. 9 July 1992. (unpublished) Analytical part (Appendix II): [redacted] (1992) Metabolism of [Ethylene-U-14C]-Mancozeb in the Mouse Phase II – Metabolite Analysis and Characterisation. XenoBiotic Laboratories Inc. XBL Report No. 8519. 9 July 1992. (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Elf Atochem North America.
1.2.2	Companies with letter of access	Cerexagri SA
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	This study has been performed according EPA guideline n° 85-1. These guidelines are scientifically recognised in several countries. There are no major differences or deviations with the required EU guidelines. At the time the study was performed no particular guideline was required for EC registration.
2.2	**GLP**	Yes, the test was conducted in compliance with the EPA GLP Guidelines.
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Mancozeb
3.1.1	Radiolabelled test material	A single radiolabelled form of [ethylene-U-14C]-mancozeb was supplied by Amersham International plc:

Section A6.2(3) Metabolism studies in mammals

Annex Point IIA6.2

Metabolism of 14C-Macozeb in the mouse

IUCLID 5.0/3



* Denotes position of ^{14}C label

3.1.1.1	Description	Not provided.
3.1.1.2	Lot/Batch number	Batch No.: B-DD.
3.1.1.3	Purity	Radiochemical purity: at least 98%. No breakdown ETU was observed. Specific activity: 43.2 µCi/mg.
3.1.1.4	Stability	See point 3.2.2 for radiochemical purity of dosing suspensions.
3.1.2	Non-radiolabelled test material	Mancozeb
3.1.2.1	Description	Not provided.
3.1.2.2	Lot/Batch number	Not provided.
3.1.2.3	Purity	Not provided.
3.1.2.4	Stability	Not provided.
3.1.3	Reference Standards	Reference standards used in this study are identified in Figure A6.2(3)-2.
3.2	**Test Animals**	*Non-entry field*
3.2.1	Species	Mouse
3.2.2	Strain	CD-1
3.2.3	Source	Charles River (UK) Limited, [redacted].
3.2.4	Sex	Male and female.
3.2.5	Age/weight at study initiation	Male and female mice were in the weight range 26-33 g (corresponding to 32-72 days old) and 20-27 g (corresponding to 31-68 days old), respectively.
3.2.6	Number of animals per group	15 males and 15 females (see Table A6.2(3)-1 for allocation of animals to groups).
3.2.7	Control animals	Yes, 10 males.
3.3	**Administration/ Exposure**	Oral.
3.3.1	Type	Gavage
3.3.2	Concentration	

Section A6.2(3) **Metabolism studies in mammals**

Annex Point IIA6.2 Metabolism of 14C-Macozeb in the mouse

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Group	Target Dose (mg/kg bw)	Achieved Dose (mg/kg bw)		Radio-chemical purity (%)	Significant amounts of ETU
		Males	Females		
A	2.5	2.37	2.44	96.7	No
B	2.5	2.54	2.47	94.7	No
C	150	155.5	151.6	93.4	No

See Table A6.2(3)-1 for study design.

3.3.3 Vehicle: 1% sodium carboxymethylcellulose.

3.3.4 Total volume applied: 0.065 to 0.095 ml.

3.4 Examinations

3.4.1 Sampling: See Table A6.2(3)-1 for study design.

An additional group of 10 untreated, control male mice were placed in pairs into metabolism cages. Excreta were collected at ambient temperature over a 24 h period.

3.4.2 Analytics

Total Radioactive Residue Determination

Radioactivity in urine was determined directly by liquid scintillation counting (LSC). Faeces were homogenized in 4% ethylene diaminetetraacetic acid (EDTA), and triplicate aliquots were combusted in a Harvey Biological Sample Oxidizer.

Metabolite profiling

Urine was analyzed by two dimensional thin layer chromatography (2-D TLC) directly. Homogenized faeces were blended in methanol (MeOH). The extracted ($MeOH/H_20$) radioactivity ranged from 53.32% to 81.25% over the different groups. The faeces $MeOH/H_20$ extract was analyzed by 2-D TLC.

4 RESULTS AND DISCUSSION

4.1 Material Balance: Table A6.2(3)-2 summarizes the percent recoveries of the dosed radioactivity excreted in the urine and the faeces from the male and female mice from each group.

4.2 Metabolites in urine: The metabolites identified in the urine were Ethylenethiourea (ETU), ethylenethiuram monosulfide (EBIS)/ethylene thiourea-N-thiocarbamide (ETT), N-acetyl-ethylenediamine (N-acetyl-EDA), ethylenediamine (EDA), Ethyleneurea (EU), creatine, and allantoin.

These metabolites, expressed as percent of administered dose are presented in Table A6.2(3)-3.

In addition, six unknowns were detected in urine. A major unknown (Unknown A) was detected in amounts from 1.34 to 9.00% of the administered radiolabelled dose. After isolation from urine and characterization by mass spectrometry, the structure of Unknown A was tentatively proposed as the sulfoxide of Jaffe's base.

Section A6.2(3) **Metabolism studies in mammals**

Annex Point IIA6.2 Metabolism of 14C-Macozeb in the mouse

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4.3	**Metabolites in faeces**	The metabolites identified in the faeces were Ethylene thiourea (ETU), ethylenethiuram monosulfide (EBIS)/ethylene thiourea-N-thiocarbamide (ETT), ethylenediamine (EDA), and Ethyleneurea (EU) and N-acetyl-ethylenediamine (N-acetyl-EDA). These metabolites, expressed as percent of administered dose are presented in Table A6.2(3)-4. Additional unknown metabolites were found in the faeces. As seen in the urine, major unknown was characterized as Jaffe's base sulfoxide and was found in the faeces of Group C females. An Unknown G was not characterized in the group C males (0.07% of dose) and females (0.37% of dose). Several other unknowns noted in the faeces accounted for less than 6.28% of the total radiolabelled dose.
4.4	**Metabolic pathway**	A proposed metabolic pathway of 14C-mancozeb in the mouse is presented in Figure A6.2(3)-1.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	This study was performed to GLP and EPA test guidelines 85-1. Male and female mice were dosed with [Ethylene-U-14C]-Mancozeb in 3 dosing groups. Dosing Groups A and C received a single oral administration of [Ethylene-U-14C]-Mancozeb at the low (2.5 mg/kg bw) and high (150 mg/kg bw) dose levels, respectively. Animals in Dosing Group B received a single oral administration of [Ethylene-U-14C]-Mancozeb at the low dose level on Day 15 following 14 single daily administrations of non-radiolabelled Mancozeb at the low dose level. On each dosing occasion the dose suspension was kept cool in ice and the time between dose preparation and dose administration was kept to an absolute minimum. The radiochemical purity of [Ethylene-U-14C]-Mancozeb in the dose suspension after dosing was greater than 93% and no significant amounts of ETU were present. Urine and faeces samples generated from Dosing Groups A-C were collected frozen over a 24 h period for total radioactivity residue determination and metabolite profiling.
5.2	**Results and discussion**	The animals were observed to be healthy prior to, and during the experimental period. After oral administration of a low dose of mancozeb, more than 70% of the administered radioactivity was eliminated within 24 hours in the urine and faeces of the mouse. In the high dose group, ca. 50-64% of the administered radioactivity was eliminated in the urine and faeces. The major metabolites in urine were ethylenethiourea (ETU), ethylenethiuram monosulfide (EBIS)/ethylene thiourea-N-thiocarboxamide (ETT), *N*-acetyl-ethylenediamine (*N*-acetyl-EDA), ethylenediamine (EDA), and ethyleneurea (EU). In faeces, the

Section A6.2(3) **Metabolism studies in mammals**

Annex Point IIA6.2 Metabolism of 14C-Macozeb in the mouse

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		major metabolites were ETU, EBIS/ETT, and EU. The minor metabolites in urine were creatine and allantoin; and, in faeces, they were EDA and *N*-acetyl-EDA. The major unknown (Unknown A) found in urine was proposed to be a Jaffe's base sulfoxide. A proposed metabolic pathway of 14C-mancozeb in the mouse is presented in Figure A6.2(3)-1.
5.3	**Conclusion**	**Absorption:** based on urinary excretion only the bioavailability was ca. 30% (low dose) and 45% (high dose). **Distribution:** not investigated. **Excretion:** more than 70% and ca. 50-64% of administered radioactivity eliminated within 24 hours in low and high dose, respectively. **Metabolism:** The major metabolites in urine (the absorbed portion) were ETU, EBIS/ETT, *N*-acetyl-EDA, EDA, and EU; 3.9-9.2% (on a mole per mole basis) of the administered dose was recovered in the urine as ETU.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	Other conclusions: *(Adopt applicant's version or include revised version)*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	

Section A6.2(3) Metabolism studies in mammals

Annex Point IIA6.2

Metabolism of 14C-Macozeb in the mouse

IUCLID 5.0/3

	COMMENTS FROM
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.2(3)-1 Metabolism of 14C-Macozeb in the mouse

Study design

Dosing Group	Investigation	Samples taken	Single or multiple dosing	Dose Level (mg/kg bw)	No of Males	No of Females
A	Metabolism	Urine and faeces: collected frozen at 0-8 and 8-24 h post dose.	Single	2.5	5	5
B	Metabolism	Urine and faeces: collected frozen at 0-8 and 8-24 h post dose.	Multiple*	2.5	5	5
C	Metabolism	Urine and faeces: collected frozen at 0-8 and 8-24 h post dose.	Single	150	5	5

* Multiple oral administrations of non-radiolabelled Mancozeb on Days 1-14 and [Ethylene-U-14C)]-Mancozeb on Day 15.

Table A6.2(3)-2 Metabolism of 14C-Macozeb in the mouse

Total dose recovered in % of the dose administered

Group	Males		Females	
	Urine	Faeces	Urine	Faeces
A	29.58	68.31	32.31	41.30
B	23.06	61.48	33.47	44.01
C	50.69	13.30	40.64	9.06

Table A6.2(3)-3 Metabolism 14C-Macozeb in the mouse

Major urine metabolites in % of dose administered

Metabolite*	Males			Females		
	A	B	C	A	B	C
ETU	5.46	3.94	9.20	6.08	6.57	8.88
EBIS/ETT	3.61	3.25	2.59	3.48	5.74	3.25
N-Acetyl-EDA	2.46	0.85	10.58	3.89	1.71	0.85
EDA	1.47	1.00	4.57	2.22	1.85	4.23
EU	2.29	2.92	4.08	1.47	1.51	2.22
Creatine	0.58	0.27	2.81	0.85	0.37	1.73
Allantoin	0.47	0.9	1.19	0.19	N.D.	0.83

N.D. = Not detected.

Table A6.2(3)-3 Metabolism of 14C-Macozeb in the mouse

Major faecal metabolites in % of dose administered

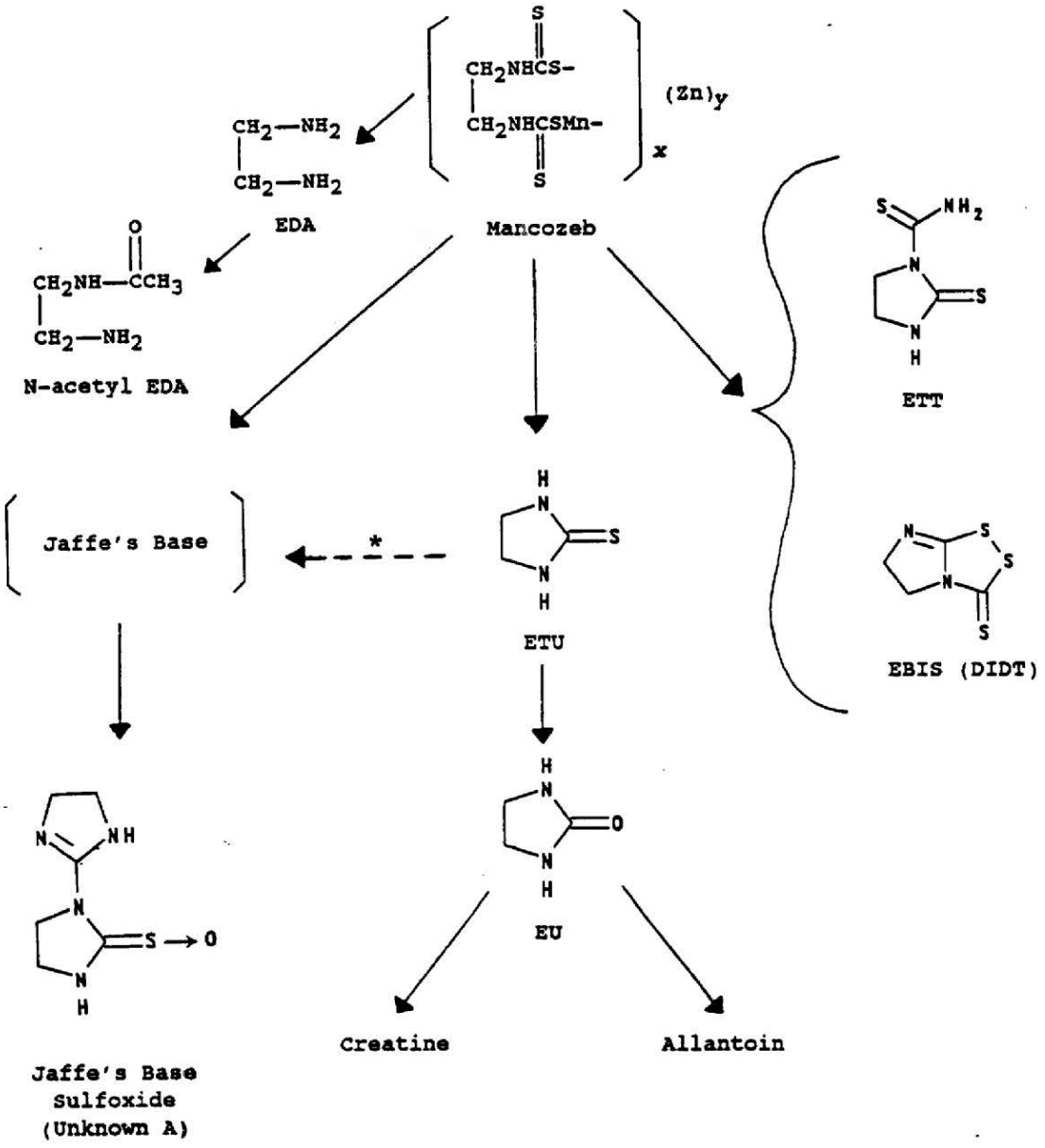
Metabolite*	Males			Females		
	A	B	C	A	B	C
ETU	6.35	17.38	2.59	8.07	8.85	1.64
EBIS/ETT	9.85	7.32	0.44	2.73	2.36	0.48
EDA	0.92	2.26	0.14	N.D.	1.89	0.70
EU	7.40	3.74	1.52	5.17	3.95	0.47
N-Acetyl-EDA	N.D.	N.D.	N.D.	N.D.	N.D.	0.45

N.D. = Not detected.

* See Figure A6.2(3)-2 for structures and chemical names of metabolites.

Figure A6.2(3)-1 Metabolism of 14C-Macozeb in the mouse

Proposed metabolic pathway for 14C Mancozeb in the mouse



* Dotted arrow denotes a probable inadvertent dimerization of ETU to Jaffe's Base in the course of metabolite isolation.

Figure A6.2(3)-2 Metabolism of 14C-Macozeb in the mouse

Structure and chemical names of metabolite standards

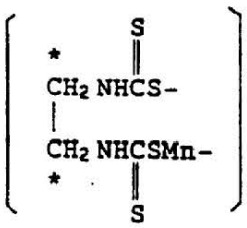
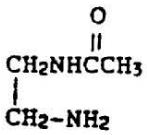
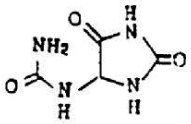
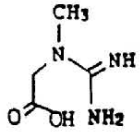
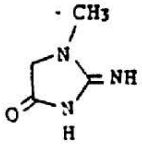
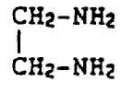
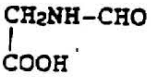
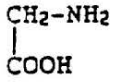
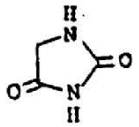
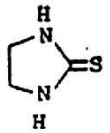
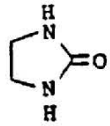
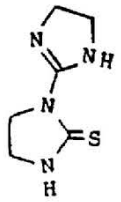
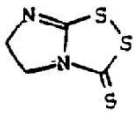
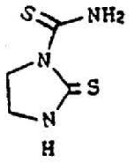
Structure	Code	Chemical Name	Abbreviation
 * Denotes position of ^{14}C label		Manganese ethylene bisdithiocarbamate complex with zinc salt	[Ethylene-U-^{14}C]-Mancozeb
	1	N-Acetyl-ethylenediamine	N-Acetyl-EDA
	2	Allantoin	
	3	Creatine	
	4	Creatinine	
	5	Ethylenediamine	EDA
	6	N-Formylglycine	N-Formyl-Gly
	7	Glycine	Gly

Figure A6.2(3)-2 Metabolism of 14C-Macozeb in the mouse

Structure and chemical names of metabolite standards (continued)

Structure	Code	Chemical Name	Abbreviation
	8	Hydantoin	HT
	9	Ethylenethiourea	ETU
	10	Ethyleneurea	EU
	11	3-(2-imidazolin-2-yl)-2-imidazolidinethione	Jaffe's Base
	12	Ethylenethiuram monosulfide	DIDT, EBIS
	13	Ethylene thiourea-N-thiocarboxamide	ETT, Carb-imide

Section A6.2(4) Metabolism studies in mammals

Rat *in vivo* dermal penetration with Mancozeb

Annex Point IIA6.2

IUCLID 5.0/4

Official use only

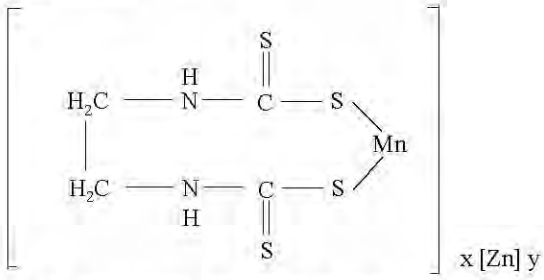
1 REFERENCE

1.1	**Reference**	[redacted] (2002) [14C]-Mancozeb - In vivo dermal absorption study in the male rat. Huntingdon Life Sciences Ltd. Laboratory Report No. EFA 041/022683. 30 April 2002. (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Rohm & Haas.
1.2.2	Companies with letter of access	Cerexagri SA
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	OECD Guideline of Testing of Chemicals, Toxicokinetics, 417, Adopted April 1984., OECD Test Guideline for the Testing of Chemicals, Draft New Guideline 427: Skin Absorption : In Vivo Method for the conduct of skin absorption studies (December 2000).
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Mancozeb
3.1.1	Radiolabelled test material	 x [Zn] y 14C - Mancozeb labelled in the ethylene positions
3.1.1.1	Description	Not provided.
3.1.1.2	Lot/Batch number	Batch No.: 88-32
3.1.1.3	Purity	Radiochemical purity: 97.7% Specific activity: 15.5 mCi/mmol (2112.7 kBq/mg)
3.1.1.4	Stability	The radiochemical purity of dose formulations was measured (see point 4.1).
3.1.2	Non-radiolabelled test material	Mancozeb
3.1.2.1	Description	Greyish yellow powder.
3.1.2.2	Lot/Batch number	Batch No.: 9903-261/31

Section A6.2(4) **Metabolism studies in mammals**

Annex Point IIA6.2 **Rat *in vivo* dermal penetration with Mancozeb**

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3.1.2.3	Purity	Report indicates 86.4%. However, the Certificate of Analysis indicates 86.0%.
3.1.2.4	Stability	Expiry date: May 2002 (six months after opening the container).
3.2	**Test Animals**	*Non-entry field*
3.2.1	Species	Rat
3.2.2	Strain	Sprague-Dawley (Crl:CD®BR).
3.2.3	Source	Charles River (UK) Ltd, [redacted]
3.2.4	Sex	Males
3.2.5	Age/weight at study initiation	6 - 8 weeks. 173 - 214 g.
3.2.6	Number of animals per group	4, see point 3.3.2 for group assignment.
3.2.7	Control animals	None
3.3	**Administration/ Exposure**	Dermal
3.3.1	Skin preparation	An area of dorsal skin (at least 3 x 4 cm) was clipped approximately 16 - 24 hours prior to dosing.
3.3.2	Group assignment	

Study section	Group No.	No. of animals (male)	Sacrifice time
Preliminary study	1 2	3 3	One each at 8h, 144h and 240h
Main study (high level dose)	3 4 5	4 4 4	8 h 72 h 144 h
Main study (low level study)	6 7 8	4 4 4	8 h 72 h 144 h

3.3.3	Duration	Rats were exposed for 8 hours (analogous to the length of a normal working day) and were observed over a period of 144 hours after the application.
3.3.4	Dose preparation	Mancozeb was formulated as a 80 Wettable Powder by mixing Mancozeb technical, [14C]-Mancozeb and an ingredient premix, with an amount of distilled water.
3.3.5	Vehicle	Distilled water.
3.3.6	Concentration	High dose: 1500 mg Mancozeb 80WP/ml (= 1200 mg Mancozeb/ml) Low dose: 1.75 mg Mancozeb 80WP/ml (= 1.4 mg Mancozeb/ml)
3.3.7	Dose volume	Each rat received 120 µl of the dose solution (equivalent to 10 µl/cm^2).
3.4	**Examinations**	

Section A6.2(4) **Metabolism studies in mammals**

Annex Point IIA6.2 **Rat *in vivo* dermal penetration with Mancozeb**

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3.4.1	Sampling	
3.4.1.1	Dressing and residual test material	At 8 hours the gauze, tape and filters were removed and retained for analysis. The treated area was washed with cotton wool swabs soaked in 1% v/v Tween 80 in order to remove and retain non-absorbed dose. The swabs were taken for analysis. Animals that were required to provide samples beyond 8 hours were then fitted with clean filters, gauze cover and tape/bandage and replaced in the metabolism cage.
3.4.1.2	Urine and faeces	Urine and faeces were collected separately from each animal into containers cooled in solid carbon dioxide at 0 to 8, 8 to 24, and at 24 hour intervals up to sacrifice. The interior of each metabolism cage was washed with water at each sample point and with 4% w/v EDTA solution after sacrifice, and the washings retained for analysis.
3.4.1.3	Air	Expired air was collected for the first 72 hours of the preliminary study only.
3.4.1.4	Blood	A terminal blood sample was taken from each animal.
3.4.1.5	Treated skin	At sacrifice, the treated area of skin was removed and tape-stripped. Tape-strips and residual treated skin were retained for analysis. The area of skin, approximately 1 cm in width, surrounding the site of dose application was removed to investigate leaching of the dose through the skin.
3.4.1.6	Other samples	The untreated skin, thyroid gland and the residual carcass were also taken for analysis. Dressings, saddles, filters and gauze covers removed from the animals were retained for analysis.
3.4.2	Analytics	Radioactivity was measured by liquid scintillation counting (LSC). Radiochemical purity analysis was determined by thin layer chromatography (TLC).

4 RESULTS AND DISCUSSION

4.1 **Dose Purity**

The radiochemical purity of [14C]-Mancozeb was measured in duplicate in the dose formulations by TLC at dose administration:

Study phase	Mean % Purity
Preliminary study high dose	99.1
Preliminary study low dose	95.8
Main study high dose	99.4
Main study low dose	96.8

The mean radiochemical purity of [14C]-Mancozeb in the high dose formulations was >99% throughout the study.

The mean radiochemical purity of [14C]-Mancozeb in the low dose formulations was less than 97%. However since the purity of the preliminary high dose formulation, prepared at the same time as the preliminary low dose formulation, was >99%, this indicates that the lower purity was caused by the hydrolytic degradation of the compound when mixed with water.

Section A6.2(4) **Metabolism studies in mammals**

Annex Point IIA6.2 **Rat *in vivo* dermal penetration with Mancozeb**

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4.2 Achieved Dose of [14C]-Mancozeb

The radioactivity in aliquots taken from the top, middle and bottom of the dose formulations, prior to dosing, were within 5% of the mean activity for the three regions. No appreciable concentration gradient was observed, therefore the dose formulations were considered to be homogenous.

The mean achieved doses of each group were as follows:

Group No.	Nominal Dose Level (mg/cm^2)	Animal Nos.	Achieved Dose		
			kBq	mg	mg/cm2
Preliminary study					
1	12	1-3	229.88 ± 6.18	95.47 ± 2.56	7.96 ± 0.21
2	0.014	4-6	459.64 ± 7.96	0.22 ± 0.00	0.018 ± 0.000
Main study					
3-5	12	7-18	268.25 ± 2.05	96.24 ± 0.74	8.02 ± 0.06
6-8	0.014	19-30	277.36 ± 12.12	0.16 ± 0.01	0.013 ± 0.001

The achieved doses for the high dose level were lower than expected because of the displacement factor which occurred due to the very high concentration of the formulation. The formulations were prepared using the ratio of 1200 mg Mancozeb to 1 ml water. It was not possible to prepare a more concentrated formulation which would be suitable for dermal dosing.

4.3 Distribution of radioactivity

4.3.1 Preliminary study

High dose level

The distribution of radioactivity 8, 144 and 240 hours after a single topical application of [14C]-Mancozeb at a nominal dose level of 12 mg/cm^2 to male rats is presented in table A6.2(4)-1.

Low dose level

The distribution of radioactivity 8, 144 and 240 hours after a single topical application of [14C]-Mancozeb at a nominal dose level of 0.014 mg/cm^2 to male rats is presented in table A6.2(4)-2.

The total mean radioactivity in the expired air traps up to 72 hours after dosing was 0.01% at both dose levels. Two of the six rats also showed clinical signs, including brown staining around the eyes and nose, due to the high humidity in the metabowls. Gas traps were removed at 72 hours and the affected rats had recovered by 168 hours. It was decided from these results that no expired air collections would be necessary in the main study

4.3.2 Main study

High dose level

The distribution of radioactivity 8, 72 and 144 hours after a single topical application of [14C]-Mancozeb at a nominal dose level of 12 mg/cm^2 to male rats is presented in table A6.2(4)-3.

Low dose level

The distribution of radioactivity 8, 72 and 144 hours after a single topical application of [^{14}C]-Mancozeb at a nominal dose level of 0.014 mg/cm^2 to male rats is presented in table A6.2(4)-4.

A summary of the mean concentration of radioactivity in blood at 8, 72, 144 and 240 hours after a single topical application of [^{14}C]-Mancozeb to male rats are presented in table A6.2(4)-5.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

This study was performed to GLP and complies with OECD Test Guidelines 417 and draft OECD Guidelines 427.

The rate and extent of absorption of radioactivity following dermal application of the fungicide Mancozeb has been studied using two concentrations of [14C]-Mancozeb 80 WP formulations. [14C]-Mancozeb was administered in a high level formulation (nominally 1200 mg Mancozeb/ml, equivalent to the commercial powder dissolved in a minimum quantity of water) to mimic the concentration to which the operator is exposed during the mixing/loading procedure and a diluted formulation (low level, 1.4 mg Mancozeb/ml) equivalent to the minimum strength in-use spray dilution.

A preliminary study was conducted on two groups of three male rats

- to determine the sacrifice times for the main study,
- to obtain an indication of the test substance absorbed through the skin and excreted, retained in the skin or remaining on the skin surface,
- to investigate the production of volatile metabolites in the expired air,
- to determine whether individual analysis of target organs, other than thyroid, was required in the main study.

The results from the preliminary study were used to determine the need to investigate the remaining material in the skin, and its localisation, and any requirement to examine the tissue distribution of the radioactivity.

Rats were exposed for 8 hours (analogous to the length of a normal working day) and were observed over a period of 240 hours after the application.

The main study involved three groups of four male rats at each dose level. The length of exposure was 8 hours and a group of animals was sacrificed 8, 72 and 144 hours after dose administration. At the end of the exposure period the remaining dose was washed off the skin with detergent solution. Urine, faeces and cage wash were collected at 8 hours, 24 hours and daily until termination. At termination the dose site was tape-stripped, to remove the stratum corneum, and the remaining treated skin, a small area of skin surrounding the dose site, untreated skin, thyroid and residual

carcass were retained for analysis.

5.2 Results and discussion

Preliminary study

The preliminary study showed that the total mean radioactivity in the expired air traps was very low after 72 hours at both dose levels. The animals sacrificed at 144 hours and 240 hours at each dose level showed similar absorption patterns with little, if any, evidence of additional absorption occurring between 144 and 240 hours at the high dose level. Most of the applied dose (>63%) was not absorbed. Less than 1.2% of the applied radioactive material was absorbed up to 240 hours. The amount remaining at the treated site was stable for the high dose group (0.3%) and decreased substantially for the low dose group from 23.29% at 8 hours to 3.48% at 240 hours, the majority being detected in the stratum corneum.

Main study

The patterns of absorption, distribution and excretion of [^{14}C]-Mancozeb after dermal application to rats at two concentrations, nominally 12 mg/cm^2 and 0.014 mg/cm^2 were broadly similar. The distribution of radioactivity with time is summarised below:

Group number	3	4	5	6	7	8
Sacrifice time (hours)	8	72	144	8	72	144
Nominal dose (mg/cm^2)	12	12	12	0.014	0.014	0.014
% Direct Absorption (A)	0.05	0.35	0.44	0.12	0.38	0.36
% In treated skin (after tape-stripping) (B)	0.05	0.08	0.02	0.13	0.16	0.24
Total % absorbed (A+B)	0.11	0.43	0.47*	0.24	0.54	0.60
% In stratum corneum (C)	0.90	0.75	0.35	4.52	4.03	2.97
Total % at dose site (B+C)	0.96	0.84	0.38	4.64	4.19	3.20
Total % non-absorbed	91.50	95.28	93.65	89.48	89.60	95.14
Overall % Recovered	92.52	96.46	94.47	94.25	94.17	98.71

* From the figures provided, total absorbed (direct absorption + radioactivity in treated skin) should normally be 0.44%+0.02%= 0.46%. The value here probably results from rounding.

Less than 0.6% of the applied radioactive material was absorbed at either dose concentration up to 144 hours after dosing.

Greater than 89% of the applied radioactivity was not absorbed at both the high and low dose levels. Following eight hours exposure, the majority of the dose was removed from the treated area by swabbing with a detergent solution. The mean amount of radioactivity removed from the animals treated with the high level formulation was somewhat higher (89.78% to 92.60% dose) than that removed from the low dose level animals (43.41% to 79.78% dose). However, a substantial proportion of the applied low dose was detected in the glass fibre filters (11.77% to 38.67%), and was attributable to flaking of the dried test material from the skin as the rat moved around.

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Metabolism studies in mammals

Rat *in vivo* dermal penetration with Mancozeb

At the high dose level, the applied material remaining at the dose site decreased from 0.96% at 8 hours to 0.38% after 144 hours. A high proportion of the dose which remained at the application site at sacrifice was detected in the stratum corneum. The dose remaining in the treated skin, following removal of the stratum corneum by tape-stripping, decreased from a mean of 0.08% to 0.02% between 72 and 144 hours post-dose application. At the low dose level the proportion remaining at the dose site at sacrifice was greater than following the high dose, decreasing from 4.64% dose at 8 hours to 3.20% at 144 hours. The dose remaining in the treated skin, following removal of the stratum corneum by tape-stripping, increased from 0.13% applied dose at 8 hours to 0.24% at 144 hours.

Direct absorption (radioactivity in the excreta, cage wash, untreated skin, thyroid and carcass) increased with time for the high dose level from 0.05% at 8 hours to 0.44% at 144 hours, whilst that for the low dose level reached a maximum of approximately 0.4% by 72 hours. The total absorbed dose after dermal application of [^{14}C]-Mancozeb can be considered to correspond to the direct fraction and the remaining material in the treated skin, which amounted to 0.11, 0.43 and 0.47% dose applied at 8, 72 and 144 hours respectively at the high dose level and 0.24, 0.54 and 0.60% at the corresponding times at the low dose level. Therefore if the total absorption was shown to increase with time following dermal application of [^{14}C]-Mancozeb at both dose concentrations, clearly the rate of absorption decreased substantially over the same period.

The level of direct absorption of [^{14}C]-Mancozeb into the rat following dermal application using the high level and the low level dose formulations has been shown in this study to be 0.44% and 0.36% respectively after 144 hours. At least 75% of the dose that had been absorbed was excreted, with <0.12% dose remaining in the carcass and tissues ([^{14}C]-Mancozeb in the thyroid did not exceed the background level in any group). Since the majority of the radioactivity recovered in the cage wash can be attributed to that excreted in urine, the proportion of recovered radioactivity attributable to renal excretion was 0.32% and 0.24% dose for the high and low dose levels respectively.

A proportion of the dose which remained in the treated skin following swabbing at 8 hours post-dose appeared to have been lost by desquamation and upward renewal of the stratum corneum layers and was recovered from the first tape-strip, dose site shavings and razor head wash.

Comments

Where possible results, conclusions, and evaluation (reliability, deficiency and guideline compliance) of this study are presented as in Annex B (and its addenda) of the PPP Monograph for Mancozeb. Tables have been added to facilitate the presentation of the results.

Section A6.2(4) **Metabolism studies in mammals**

Annex Point IIA6.2 **Rat *in vivo* dermal penetration with Mancozeb**

IUCLID 5.0/4

5.3	**Conclusion**	Following a single dermal application of [14C]-Mancozeb in a high level dose formulation (nominally 12 mg Mancozeb/cm^2) to male rats, 0.47% of the dose had been absorbed by 144 hours. A slightly higher proportion (0.6%) of the low level dose (nominally 0.014 mg Mancozeb/cm^2) was absorbed over the same period. Greater than 89% of both the high and low dose levels were associated with non-absorbed material from the skin surface (skin swabs, gauze wash, carbon filters, glass fibre filters, dose site shavings, razor heads and the first tape-strip). By 144 hours after dosing, the amount of radioactivity remaining in the treated skin was 0.02% and 0.24% of the high and low doses respectively and was considered to be absorbed. The majority of the dose that had been absorbed was excreted, predominantly in the urine, with <0.12% dose remaining in the carcass and tissues. Dermal absorption (radioactivity in faecal material or in tissues plus radioactivity remaining in treated skin after stripping) after 8 hours, analogous to the length of a normal working day, was 0.11% and 0.24% for the high and low level dose formulations, respectively.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	Other conclusions: *(Adopt applicant's version or include revised version)*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	

Section A6.2(4) Metabolism studies in mammals

Annex Point IIA6.2

Rat *in vivo* dermal penetration with Mancozeb

IUCLID 5.0/4

	COMMENTS FROM
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.2(4)-1 Rat in vivo dermal penetration with Mancozeb

Preliminary study

Summary of the distribution of radioactivity 8, 144 and 240 hours after a single topical application of [14C]-Mancozeb at a nominal dose level of 12 mg/cm^2 to male rats

Results are expressed as percent applied radiochemical dose

Animal Number Sacrifice time (h)	1 8	2 144	3 240
Urine	0.00	0.03	0.11
Faeces	ND	0.02	0.09
Cage Wash	ND	0.09	0.13
Expired Air	0.02	ND	ND
Tissues	0.05	0.04	0.04
TOTAL DIRECT ABSORPTION	**0.07**	**0.17**	**0.36**
Treated skin	0.01	ND	0.02
Scissor Wash	ND	ND	ND
Stratum Corneum	0.26	0.12	0.28
TOTAL AT DOSE SITE	**0.27**	**0.12**	**0.30**
TOTAL ABSORBED (Direct absorption + treated skin)	**0.09**	**0.17**	**0.38**
Razor Head Wash	NS	0.02	0.02
Dose Site Shavings	NS	0.07	0.24
Surface Tape Strip	0.09	0.01	0.05
Carbon Filters	0.05	0.07	0.49
Skin Swabs	90.52	104.98	103.64
Gauze Wash	0.17	0.59	0.72
TOTAL NON-ABSORBED	**90.83**	**105.73**	**105.16**
OVERALL RECOVERY	**91.17**	**106.02**	**105.82**

ND Results within background range
NS No Sample

Table A6.2(4)-2 Rat in vivo dermal penetration with Mancozeb

Preliminary study

Summary of the distribution of radioactivity 8, 144 and 240 hours after a single topical application of [14C]-Mancozeb at a nominal dose level of 0.014 mg/cm^2 to male rats

Results are expressed as percent applied radiochemical dose

Animal Number Sacrifice time (h)	4 8	5 144	6 240
Urine	0.01	0.28	0.41
Faeces	ND	0.07	0.06
Cage Wash	ND	0.10	0.15
Expired Air	ND	0.02	0.01
Tissues	0.03	0.54	0.04
TOTAL DIRECT ABSORPTION	**0.04**	**1.00**	**0.69**
Treated skin	0.33	0.13	0.10
Scissor Wash	0.01	0.00	ND
Stratum Corneum	22.96	4.94	3.37
TOTAL AT DOSE SITE	**23.29**	**5.07**	**3.48**
TOTAL ABSORBED (Direct absorption + treated skin)	**0.37**	**1.13**	**0.79**
Razor Head Wash	NS	0.01	0.08
Dose Site Shavings	NS	1.02	2.35
Surface Tape Strip	1.03	1.04	0.72
Carbon Filters	5.11	1.15	0.42
Skin Swabs	55.58	81.54	80.01
Gauze Wash	2.20	1.61	4.10
TOTAL NON-ABSORBED	**63.92**	**86.36**	**87.67**
OVERALL RECOVERY	**87.25**	**92.44**	**91.84**

ND Results within background range
NS No Sample

Table A6.2(4)-3 Rat in vivo dermal penetration with Mancozeb

Main study

Summary of the mean distribution of radioactivity 8, 72 and 144 hours after a single topical application of [14C]-Mancozeb at a nominal dose level of 12 mg/cm^2 to male rats

Results are expressed as percent applied radiochemical dose

Group Number Sacrifice time (h)	3 8			4 72			5 144		
Urine	0.00	±	0.00	0.03	±	0.01	0.05	±	0.04
Faeces	0.00	±	0.00	0.02	±	0.01	0.01	±	0.00
Cage Wash	ND	±	-	0.13	±	0.06	0.27	±	0.15
Tissues	0.05	±	0.03	0.17	±	0.14	0.11	±	0.08
TOTAL DIRECT ABSORPTION	**0.05**	**±**	**0.03**	**0.35**	**±**	**0.10**	**0.44**	**±**	**0.21**
Treated skin	0.05	±	0.03	0.08	±	0.10	0.02	±	0.02
Scissor Wash	0.01	±	0.01	ND	±	-	0.01	±	0.01
Stratum Corneum	0.90	±	0.97	0.75	±	0.36	0.35	±	0.13
TOTAL AT DOSE SITE	**0.96**	**±**	**1.00**	**0.84**	**±**	**0.42**	**0.38**	**±**	**0.16**
TOTAL ABSORBED (Direct absorption + treated skin)	**0.11**	**±**	**0.06**	**0.43**	**±**	**0.19**	**0.47**	**±**	**0.22**
Razor Head Wash		NS		0.07	±	0.10	0.01	±	0.01
Dose Site Shavings		NS		0.23	±	0.20	0.11	±	0.04
Surface Tape Strip	0.17	±	0.16	0.26	±	0.20	0.07	±	0.03
Carbon Filters	0.01	±	0.01	0.02	±	0.03	0.01	±	0.01
Glass Fibre Filter	0.31	±	0.36	0.24	±	0.10	0.16	±	0.11
Skin Swabs	89.78	±	1.39	92.60	±	3.31	92.04	±	3.41
Gauze Wash	1.23	±	0.69	1.94	±	0.98	1.26	±	0.94
TOTAL NON-ABSORBED	**91.50**	**±**	**0.67**	**95.28**	**±**	**4.44**	**93.65**	**±**	**3.35**
OVERALL RECOVERY	**92.52**	**±**	**0.45**	**96.46**	**±**	**4.47**	**94.47**	**±**	**3.22**

ND Results within background range
NS No Sample

Table A6.2(4)-4 Rat in vivo dermal penetration with Mancozeb

Main study

Summary of the mean distribution of radioactivity 8, 72 and 144 hours after a single topical application of [14C]-Mancozeb at a nominal dose level of 0.014 mg/cm^2 to male rats

Results are expressed as percent applied radiochemical dose

Group Number Sacrifice time (h)	6 8			7 72			8 144		
Urine	0.01	±	0.01	0.23	±	0.04	0.20	±	0.01
Faeces	0.00	±	0.00	0.06	±	0.01	0.06	±	0.02
Cage Wash	ND	±	-	0.02	±	0.01	0.04	±	0.07
Tissues	0.10	±	0.06	0.07	±	0.07	0.07	±	0.03
TOTAL DIRECT ABSORPTION	**0.12**	**±**	**0.06**	**0.38**	**±**	**0.11**	**0.36**	**±**	**0.06**
Treated skin	0.13	±	0.02	0.16	±	0.06	0.24	±	0.38
Scissor Wash	ND	±	-	ND	±	-	ND	±	-
Stratum Corneum	4.52	±	1.11	4.03	±	0.16	2.97	±	0.92
TOTAL AT DOSE SITE	**4.64**	**±**	**1.13**	**4.19**	**±**	**0.19**	**3.20**	**±**	**1.23**
TOTAL ABSORBED (Direct absorption + treated skin)	**0.24**	**±**	**0.06**	**0.54**	**±**	**0.16**	**0.60**	**±**	**0.43**
Razor Head Wash		NS		0.15	±	0.02	0.06	±	0.04
Dose Site Shavings		NS		2.04	±	0.72	1.06	±	0.32
Surface Tape Strip	0.51	±	0.14	0.70	±	0.35	0.64	±	0.26
Carbon Filters	0.52	±	0.14	0.72	±	0.18	0.16	±	0.07
Glass Fibre Filter	36.31	±	5.75	38.67	±	7.12	11.77	±	17.21
Skin Swabs	48.79	±	4.94	43.41	±	5.63	79.78	±	14.14
Gauze Wash	3.37	±	1.04	3.92	±	1.72	1.66	±	0.44
TOTAL NON-ABSORBED	**89.48**	**±**	**1.69**	**89.60**	**±**	**2.38**	**95.14**	**±**	**4.97**
OVERALL RECOVERY	**94.25**	**±**	**1.64**	**94.17**	**±**	**2.64**	**98.71**	**±**	**4.79**

ND Results within background range
NS No Sample

Table A6.2(4)-5 Rat in vivo dermal penetration with Mancozeb

Main study

Summary of the mean concentration of radioactivity in blood at 8, 72, 144 and 240 hours after a single topical application of [14C]-Mancozeb to male rats

Results are expressed as nanogram equivalents per gram of blood and as percent applied radiochemical dose

Group number	Sacrifice time (h)	Nominal dose level (mg/cm^2)	Animal number	Concentration of [^{14}C]-Mancozeb in blood (ng eq/g)	Group mean	% Applied radioactivity in blood *	Group mean
1	8	12	1	ND		ND	
	144		2	1035.85		0.01	
	240		3	1152.03	-	0.02	-
2	8	0.014	4	3.43		0.02	
	144		5	0.89		0.01	
	240		6	3.09	-	0.02	-
3	8	12	7	402.00		0.01	
			8	ND		ND	
			9	ND		ND	
			10	ND	100.50 ± 201.00	ND	0.00 ± 0.00
4	72	12	11	517.38		0.01	
			12	ND		ND	
			13	ND		ND	
			14	ND	129.35 ± 258.69	ND	0.00 ± 0.00
5	144	12	15	ND		ND	
			16	ND		ND	
			17	765.09		0.01	
			18	394.68	289.94 ± 367.37	0.00	0.00 ± 0.01
6	8	0.014	19	1.06		0.01	
			20	ND		ND	
			21	ND		ND	
			22	ND	0.26 ± 0.53	ND	0.00 ± 0.00
7	72	0.014	23	ND		ND	
			24	ND		ND	
			25	ND		ND	
			26	ND	ND ± -	ND	ND ± -
8	144	0.014	27	ND		ND	
			28	ND		ND	
			29	ND		ND	
			30	ND	ND ± -	ND	ND ± -

ND Results within background range
* Calculated assuming total blood weight is 7% of bodyweight.
0.00 < 0.005% dose

Section A6.3.1
Annex Point IIA6.3
IUCLID 5.4/1

Repeated dose toxicity (oral)
4 week rat dietary toxicity study with zineb

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (2002) Zineb Nautec: 4-Week Toxicity Study by Oral Route (Dietary Admixture) in Rats. CIT. Laboratory Study No. 22521 TSR. 11 September 2002. (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Cerexagri
1.2.2	Companies with letter of access	-
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	OECD 407 (1995).
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Zineb Nautec (equivalent to Zineb technical)
3.1.1	Lot/Batch number	Batch No.: 054072.
3.1.2	Specification	Deviating from specification given in section 2 as follows.
3.1.2.1	Description	White powder.
3.1.2.2	Purity	95.4%.
3.1.2.3	Stability	Expiry date: 12 April 2002 (In-life study phase complete on 26 February 2002).
3.2	**Test Animals**	*Non-entry field*
3.2.1	Species	Rat
3.2.2	Strain	Sprague-Dawley, Crl CD® (SD) IGS BR
3.2.3	Source	Charles River Laboratories, [redacted].
3.2.4	Sex	Male and female.
3.2.5	Age/weight at study initiation	Animals were approximately 6 weeks old and had a mean body weight of 224 g (range: 176 g to 254 g) for the males and 175 g (range: 159 g to 204 g) for the females.

Section A6.3.1 Repeated dose toxicity (oral)

Annex Point IIA6.3 **4 week rat dietary toxicity study with zineb**

IUCLID 5.4/1

3.2.6 Number of animals per group

Group allocation was as follows:

Group	Weeks on study	Dose Level (ppm)			
		0	50	400	3200
Principal	4	5M/5F	5M/5F	5M/5F	5M/5F
Satellite A*	1	5M/5F	5M/5F	5M/5F	5M/5F
Satellite B*	2	5M/5F	5M/5F	5M/5F	5M/5F
Satellite C*	3	5M/5F	5M/5F	5M/5F	5M/5F

*Satellite animals were used for thyroid and liver enzyme plasma levels and microscopic examination of thyroid and liver at interim necropsies.

3.2.7 Control animals: Yes, see point 3.2.6.

3.3 Administration/ Exposure: Oral

3.3.1 Duration of treatment: Up to 4 weeks (see point 3.2.6).

3.3.2 Frequency of exposure: Daily via the diet.

3.3.3 Postexposure period: None

3.3.4 Oral

3.3.4.1 Type: Via the diet.

3.3.4.2 Concentration

Group No.	1	2	3	4
Dose Level (ppm)	0	20	400	3200
Dose Level (mg/kg bw/day) - males	0	4.7	37.9	302.3
Dose Level (mg/kg bw/day) - females	0	5.1	40.3	317.9

Accuracy, homogeneity and stability of diet preparations were analysed and found to be acceptable.

3.3.4.3 Vehicle: None

3.3.4.4 Concentration in vehicle: No vehicle used. Test material mixed directly in the diet.

3.3.4.5 Total volume applied: Not applicable.

3.3.4.6 Controls: Yes, received control diet.

3.4 Examinations

3.4.1 Observations

Section A6.3.1 **Repeated dose toxicity (oral)**

Annex Point IIA6.3 **4 week rat dietary toxicity study with zineb**

IUCLID 5.4/1

3.4.1.1	Clinical signs	General clinical signs Each animal was observed at least once a day. Detailed clinical observation (principal animals) Once prior to the first exposure and once a week thereafter, detailed clinical observations were made on all animals outside the home cage, in a standard arena. Observations included changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g. lachrymation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypes (e.g. excessive grooming, repetitive circling) or bizarre behaviour (e.g. self-mutilation, walking backwards) were also recorded. Functional observation battery (FOB) (principal animals) Each animal of all groups was evaluated once in week 4. This included a detailed clinical examination, measurement of reactivity to manipulation or to different stimuli and motor activity. *Detailed clinical examination* Each animal of all groups was observed in the cage ("touch escape" or ease of removal from the cage), in the hand (fur appearance, salivation, lachrymation, piloerection, exophthalmos, reactivity to handling, pupil size [presence of myosis or mydriasis]) and in the standard arena (grooming, palpebral closure, defecation, and urination, tremors, twitches, convulsions, gait, arousal [hypo- and hyper-activity], posture, stereotypy, behaviour and breathing, ataxia, hypotonia). *Reactivity to manipulation or to different stimuli* The touch response, forelimb grip strength, papillary reflex, visual stimulus response, auditory startle reflex, tail pinch response, righting reflex, landing foot splay, and at the end of observation, rectal temperature were recorded. *Motor activity* Motor activity of each animal was measured once by automated infra-red sensor equipment over a 15-minute period once.
3.4.1.2	Mortality	Each animal was checked at least twice a day for mortality or signs of morbidity.
3.4.2	Body weight	The body weight of each animal was recorded once before allocation of the animals to groups, on the first day of treatment, and then once a week (where relevant) until the end of the study.
3.4.3	Food consumption	The quantity of food consumed by the animals of each cage was recorded twice a week, over a 3- or 4-day period, during the study. Food consumption was calculated per animal and per day.
3.4.4	Water consumption	Not performed.
3.4.5	Ophthalmoscopic examination	Not performed.
3.4.6	Haematology	The following parameters were determined for all surviving principal animals at termination: erythrocytes, haemoglobin, mean cell volume, packed cell volume, mean cell haemoglobin concentration, mean cell haemoglobin, thrombocytes, leucocytest, differential white cell count

		with cell morphology, prothrombin time.
3.4.7	Clinical Chemistry	The following parameters were determined for all surviving principal animals at termination: sodium, potassium, chloride, calcium, inorganic phosphorus, glucose, urea, creatinine, total bilirubin, total protein, albumin, albumin/globulin ratio, cholesterol, triglycerides, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALAT). The following parameters were also determined for animals of satellite A, B or C at the beginning of weeks 2, 3 or 4, respectively: alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase.
3.4.8	Urinalysis	Not described.
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	The following organ weights were recorded at scheduled necropsies: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testes, thymus, thyroid with parathyroids, uterus.
3.5.2	Gross and histopathology	A complete macroscopic post-mortem examination was performed on all study animals. This included examination of the external surfaces, all orifices, the cranial cavity, the external surfaces of the brain and spinal cord, the thoracic, abdominal and pelvic cavities with their associated organs and tissues and the neck with its associated organs and tissues. A microscopic examination was performed on all tissues listed below for principal animals of the control and high-dose groups killed at end of the 4-week treatment period, the liver and the thyroids of satellite animals of the control and high-dose groups killed in weeks 2, 3, and 4 of the treatment period, the thymus of all principal animals, the macroscopic lesions of all study animals. Adrenals, Aorta, Brain (including medulla/pons cerebellar and cerebral cortex), Cecum, Colon, Duodenum, Epididymides, Esophagus, Eyes with Harderian glands, Femoral bone with articulation, Heart, Ileum, Jejunum, Kidneys, Liver, Lungs with bronchi, Lymph nodes (mandibular and mesenteric), Mammary glands/area, Ovaries (including oviducts), Pancreas, Pituitary gland, Prostate (dorso-lateral and ventral), Rectum, Salivary glands (sublingual and submandibular), Sciatic nerve, Seminal vesicles (including coagulation gland), Skeletal muscle, Skin, Spinal cord (cervical, thoracic and lumbar), Spleen, Sternum with bone marrow, Stomach with forestomach, Testes, Thymus, Thyroids with parathyroids, Tongue, Trachea, Urinary, bladder, Uterus (horns and cervix), Vaginia.
3.5.3	Other examinations	<u>Thyroid hormone assays</u> The following parameters were determined at the beginning of weeks 2, 3 or 4 (satellite A, B or C, respectively), and at the beginning of week 5 (principal): Triiodothyonine (T3), Thyroxine (T4), Thyroid stimulating hormone (TSH).
3.5.4	Statistics	Parameters were analysed with recognised statistical techniques.
3.6	**Further remarks**	None.

4 RESULTS AND DISCUSSION

4.1 Observations

4.1.1 Clinical signs

Clinical signs

No clinical signs were observed in any group.

Functional observation battery (FOB)

There was no evidence of disturbance of either autonomal or physiological functions at any dose level.

Motor activity

There were no differences in the measured motor activity which could be attributed to treatment with the test item.

4.1.2 Mortality

No deaths occurred in this study.

4.2 Body weight gain

The body weight and body weight changes are summarized in the following table:

Concentration (ppm)	0	50	400	3200
Males (N=5)				
body weight (g)				
. on day 1	224	225	226	220
. on day 29	391	364	370	361
body weight change				
. days 29 vs. 1 (g)	+167	+139	+144	+141
. days 29 vs. 1 (% vs. controls)	-	-17	-14	-16
Females (N=5)				
body weight (g)				
. on day 1	184	180	177	177
. on day 29	258	252	245	241
body weight change				
. days 29 vs. 1 (g)	+74	+72	+68	+64
. days 29 vs. 1 (% vs. controls)	-	-3	-8	-14

Compared to controls, the body weight gain of males treated at 50, 400 or 3200 ppm and that of females treated at 3200 ppm was lower.

4.3 Food consumption and compound intake

The amount of food consumed (g/animal/day) during the whole treatment period (days 1 to 29) is summarized in the following table:

Concentration (ppm)	0	50	400	3200
Males (N=5)	28.3	27.7	27.9	26.9
Females (N=5)	22.2	22.0	21.2	20.7

Compared to controls, a tendency to lower mean food consumption was observed in treated groups at 3200 ppm.

4.4 Ophtalmoscopic examination

Not examined.

Section A6.3.1 **Repeated dose toxicity (oral)**

Annex Point IIA6.3 **4 week rat dietary toxicity study with zineb**

IUCLID 5.4/1

4.5 Blood and urine analysis

4.5.1 Haematology

There were no treatment-related differences in the haematological parameters at the end of the treatment period.

4.5.2 Clinical chemistry

There were no treatment-related differences in the blood biochemical parameters at the end of the treatment period.

The few differences noted in these parameters were considered to be of no toxicological importance, although they attained statistical significance, since they were slight and the individual values were within the range of our historical background data (e.g. decrease in plasma levels of inorganic phosphorus in females treated at 3200 ppm).

The mean values of ALP, ASAT and ALAT activities are summarized in the following table:

Sex	Males				Females			
Concentration (ppm)	0	50	400	3200	0	50	400	3200
ALP (IU/L)								
day 9	538	484	517	629	299	263	326	289
day 16	440	465	447	470	253	232	257	209
day 23	403	357	428	391	217	224	223	248
day 30	345	332	302	289	191	159	186	164
ASAT (IU/L)								
day 9	51	50	50	59	51	52	57	52
day 16	62	48*	44*	46	57	60	58	59
day 23	56	54	51	48	51	57	56	55
day 30	53	51	46	48	51	49	47	46
ALAT (IU/L)								
day 9	14	17	14	18	7	9	7	9
day 16	16	18	19	27	12	8	12	8
day 23	19	17	19	15	9	14	20	10
day 30	17	16	16	16	12	10	12	10

* p < 0.05

The liver enzyme activities of treated animals were similar to that of controls as measured on days 9, 16, 23 or 30.

4.5.3 Urinalysis

Neither qualitative nor quantitative treatment-related changes were observed.

4.6 Sacrifice and pathology

Section A6.3.1 **Repeated dose toxicity (oral)**

Annex Point IIA6.3 **4 week rat dietary toxicity study with zineb**

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4.6.1 Organ weights

The main differences (in %) in organ weights between the treated and control animals are recorded below:

Sex:	Males:			Females:		
Concentration (ppm)	50	400	3200	50	400	3200
Thymus						
. satellite A (week 1)						
- absolute	-20	-18	-33*	-1	-5	-25
- relative	-19	-15	-32*	+3	-1	-19
. satellite B (week 2)						
- absolute	-7	-3	-25*	+10	-4	-11
- relative	-4	+1	-20*	+12	-6	-9
. satellite C (week 3)						
- absolute	+6	-1	-16	+12	+3	-20
- relative	+12	+0	-18	+4	-2	-20
. principal (week 4)						
- absolute	-4	-1	-43*	-18	-34**	-38**
- relative	+3	+4	-39**	-17	-31*	-35**
Thyroids						
. satellite A (week 1)						
- absolute	-1	+16	+23			
- relative	+1	+21	+28*			

*: p = 0.05; **: p = 0.01

The decrease in both absolute and relative thymus weights in principal and satellite A and B animals of group 4, and principal females of groups 3 and 4 was considered to be treatment-related.

The increase in relative thyroid gland weight in males of group 4 of Satellite group A was considered to be related to the lower body weight gain in these males.

4.6.2 Gross and histopathology

Macroscopic post-mortem examination

Main necropsy findings consisted of small size of the thymus as indicated in the following table:

Sex:	Males:				Females:			
Concentration (ppm):	0	50	400	3200	0	50	400	3200
Satellite A (week 1)	1/5	1/5	1/5	5/5	-	-	-	-
Principal (week 4)	-	-	-	1/5	-	-	-	-

- 0/5

A depressed brownish/blackish focus measuring approximately 0.2 cm in diameter was found in the stomach mucosa of one female of group 4.

Microscopic examination

Stomach

Moderate erosion of the fundic mucosa was diagnosed in one female of group 4 (principal group). This correlated with the brownish/blackish focus.

Thymus

Minimal to slight thymic atrophy/involution was diagnosed in one male of group 1, one male of group 3, three males of group 4 (satellite group A), and all males and all females of group 4 (principal group). This

change was minimal in all rats, except for one male in group 3 (satellite group A) and one male and female of group 4 (principal group), in which this lesion was scored slight.

Minimal lymphocytolysis was associated with the thymic atrophy in one male each of groups 1, 3, and 4 (satellite group A) and one male and two females of group 4 (principal group). Minimal lymphocytolysis, unassociated with thymic atrophy/involution, was diagnosed in one female each of groups 2 and 3 (principal group).

4.7 Other

Thyroid hormone assays

The mean values of T3, T4 and TSH plasma levels are summarized in the following table:

Sex	Males				Females			
Concentration (ppm)	0	50	400	3200	0	50	400	3200
T3 (nmol/L)								
day 9	1.03	0.72	0.86	1.12	1.17	0.82	0.87	0.81
day 16	1.04	0.94	0.87	1.01	0.98	0.72	1.01	0.95
day 23	0.93	0.90	0.85	0.87	0.91	0.98	0.92	0.89
day 30	0.82	0.92	0.99	0.91	0.85	1.00	1.03	0.81
T4 (nmol/L)								
day 9	55.4	49.0	60.6	50.0	54.8	43.5	35.2*	29.1**
day 16	69.7	60.8	53.4	37.1**	46.7	30.5	42.8	24.9
day 23	56.2	55.5	51.9	34.5*	36.2	44.1	38.0	31.9
day 30	50.5	50.1	45.6	42.7	37.0	38.4	41.4	28.4
TSH (ng/mL)								
day 9	19.3	18.6	11.8	11.6	9.8	8.6	8.6	8.8
day 16	25.3	13.9	9.9	15.8	7.6	6.5	6.9	6.9
day 23	10.4	12.4	17.0	15.0	7.3	7.8	7.2	7.6
day 30	20.2	15.2	16.9	11.0	9.2	7.3	7.1	7.9

*: p < 0.05; **: p < 0.01

Compared to controls, some differences in T4 plasma levels attained statistical significance. Considering the great inter-individual variability of this parameter and the absence of time-related effect, these differences were not considered to be of toxicological importance.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

This study was conducted according to GLP and OECD Guideline 407 (1995).

The objective of this study was to derive time-dose-response information on the toxicity of the test item, Zineb nautec, following daily oral (dietary) administration to rats for up to 4 weeks.

Eighty male and 80 female Sprague-Dawley rats were assigned to one control and three treated groups. Each group [1 (control), 2 (50 ppm), 3 (400 ppm) and 4 (3200 ppm)] comprised principal, satellite A, satellite B and satellite C animals; each of five males and five females. Satellites A, B and C were sacrificed at the beginning of weeks 2, 3 and 4, respectively. The test item was mixed with the diet at constant concentrations of 50, 400 or 3200 ppm. The animals were checked twice daily for mortality and clinical signs were observed once a day. The neurotoxicity was assessed by a detailed clinical observation which was performed before the beginning of the study and at weekly intervals, and by a functional observation battery and the recording of

		the motor activity performed at the end of the treatment period. Body weight was recorded before the beginning of the study and then once a week. Food consumption was recorded once a week. Haematological and blood biochemical parameters were determined during week 5 on principal animals of each sex and group. Blood was also taken before the scheduled necropsy of satellite and principal animals for the determination of thyroid plasma levels (T3, T4 and TSH) and liver enzyme activities (alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase). At scheduled sacrifice, a macroscopic post-mortem examination was performed on all animals, designated organs were weighed and preserved. A microscopic examination was carried out on selected organs for the principal animals of the control and high-dose groups, the liver and the thyroids of satellite animals of the control and high-dose groups, the thymus of all principal animals and all macroscopic lesions.
5.2	**Results and discussion**	There was no mortality or clinical signs of treatment. There were no relevant differences in the neurotoxicological parameters as evaluated by the FOB. When compared to controls, the body weight gain of males treated at 50, 400 or 3200 ppm and that of females treated at 3200 ppm was lower. Food consumption of treated animals at 3200 ppm was lower than that of controls. There were no treatment-related variations in hematological or blood biochemical parameters in any group. There were no treatment-related differences in the thyroid plasma levels. There were no treatment-related differences in the liver enzyme activities. Decreased absolute and relative thymic weights were observed in principal and satellite A and B animals of group 4 (3200 ppm); and principal females of group 3 (400 ppm). This decrease in absolute and relative thymic weights was considered to correlate with the histopathologic change "thymic atrophy". Reduction of the thymus size was sometimes noted in a few treated males in weeks 1 and 4. After the 4-week treatment period, the following changes in the stomach and thymus were noted: moderate erosion of the gastric fundic mucosa in one female of group 4 (principal group); minimal to slight thymic atrophy in one male of group 1, one male of group 3, three males of group 4 (satellite group A), and all males and all females of group 4 (principal group), minimal lymphocytolysis associated with thymic atrophy in one male each of groups 1, 3, and 4 (satellite group A) and one male and two females of group 4 (principal group). This finding was diagnosed in one female each of groups 2 and 3 (principal groups), unassociated with the thymic atrophy. The fundic mucosal erosion and the thymic atrophy were considered likely to represent non-specific stress-related responses associated with the administration of the test item.
5.3	**Conclusion**	The test item when given by dietary admixture to Sprague-Dawley rats for 4 weeks at the concentrations of 50, 400 or 3200 ppm was clinically well tolerated at all dose-levels. At 50 ppm, the only adverse effect was a decrease in body weight gain in males. At 400 ppm, decrease in body weight gain was noted among treated

Section A6.3.1 **Repeated dose toxicity (oral)**

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		males and decrease of the thymus weights (absolute and relative) was observed in females. At 3200 ppm, decrease in body weight gain and food consumption were noted among treated males and females, decrease of the thymus weights (absolute and relative) was noted in both sexes, and the incidence of thymic atrophy was higher among animals of this group.
5.3.1	LO(A)EL	**Females = 3200 ppm** (decreased body weight). **Males = 50 ppm** (decreased body weight gain).
5.3.2	NO(A)EL	**Females = 400 ppm** (≈ 40.3 mg/kg bw /day) **Males <50 ppm** (≈ 4.7 mg/kg bw/day)
5.3.3	Other	None
5.3.4	Reliability	1
5.3.5	Deficiencies	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	LO(A)EL: NO(A)EL: Other conclusions: *(Adopt applicant's version or include revised version)*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*

Section A6.3.1 Repeated dose toxicity (oral)

Annex Point IIA6.3

4 week rat dietary toxicity study with zineb

IUCLID 5.4/1

Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.3.2(1)
Annex Point IIA6.3
IUCLID 5.4/11

Repeated dose toxicity (dermal)

Rat 4 week dermal toxicity study with Mancozeb

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		1 REFERENCE
1.1	**Reference**	[redacted] (1988) Mancozeb: 4-Week Repeat Dermal Toxicity Study in Rats. Hazleton Laboratories America. HLA Study No. 417-432, R&H Report No. 88RC-0007. 6 April 1988. (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Rohm and Haas Company.
1.2.2	Companies with letter of access	Cerexagri SA.
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	This study has been performed according to the E.P.A. Pesticide assessment Guidelines, n° 82-2. These guidelines are scientifically recognised in several countries. There is no major difference with the required EU guidelines. At the time the study was performed no particular guideline was required for EC registration.
2.2	**GLP**	Yes, the test was conducted in compliance with the EPA GLP Guidelines.
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Mancozeb (Dithane® M-45)
3.1.1	Lot/Batch number	Lot No.: 76777.
3.1.2	Specification	Deviating from specification given in section 2 as follows.
3.1.2.1	Description	A yellowish powder.
3.1.2.2	Purity	82.4%
3.1.2.3	Stability	Not provided.
3.2	**Test Animals**	*Non-entry field*
3.2.1	Species	Rat
3.2.2	Strain	Sprague-Dawley, Crl: CD®BR rats.
3.2.3	Source	Charles River Laboratories Inc., [redacted].
3.2.4	Sex	Male and female.
3.2.5	Age/weight at study initiation	8 weeks old. Males weighed 214.7 to 276.5 g; females weighed 158.9 to 197.0 g.
3.2.6	Number of animals per group	10 animals/sex/group.
3.2.7	Control animals	Yes, 10 males and 10 females.
3.3	**Administration/**	Dermal

Section A6.3.2(1) Repeated dose toxicity (dermal)

Annex Point IIA6.3

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Rat 4 week dermal toxicity study with Mancozeb

	Exposure	
3.3.1	Duration of treatment	4 weeks
3.3.2	Frequency of exposure	The animals were dosed only on week days
3.3.3	Postexposure period	None
3.3.4	**Dermal**	
3.3.4.1	Area covered	4 x 4 inch.
3.3.4.2	Occlusion	Occlusion
3.3.4.3	Concentration	0, 10, 100 and 1000 mg/kg bw/day. Analysis of dosing suspension revealed acceptable accuracy and homogeneity.
3.3.4.4	Vehicle	Distilled water.
3.3.4.5	Total volume applied	1.5 or 2.5 ml/kg bw.
3.3.4.6	Duration of exposure	6 hours
3.3.4.7	Removal of test substance	The application site was wiped with a soft paper tissue moistened with distilled water.
3.3.4.8	Controls	Control received vehicle only.
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Systemic During the 6-hour exposure period, animals were observed once every hour for signs of discomfort. Detailed physical examinations were recorded weekly. Local On each treatment day immediately before application of the test material, the application site was observed for signs of dermal response. Skin irritation was also evaluated ca. 24h after the last treatment. Erythema and oedema were scored according to the Draize scale.
3.4.1.2	Mortality	Animals were examined twice daily for mortality and moribundity.
3.4.2	Body weight	Recorded weekly.
3.4.3	Food consumption	Recorded daily.
3.4.4	Water consumption	Quantitative measurements were not performed.
3.4.5	Ophthalmoscopic examination	Not performed.
3.4.6	Haematology	Animals examined and frequency: all animals, prior to termination Parameters: hematocrit, RBC, hemoglobin, MCV, MCH, MCHC, WBC, platelet count, leucocyte differential count and cell morphology.

Section A6.3.2(1) **Repeated dose toxicity (dermal)**

Annex Point IIA6.3 **Rat 4 week dermal toxicity study with Mancozeb**

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3.4.7	Clinical Chemisty	Animals examined and frequency: all animals, prior to termination. Parameters: sodium, chloride, total protein, albumin, globulin, calcium, alkaline phosphatase, BUN, inorganic phosphorus, total bilirubin, creatinine, glucose, total cholesterol, aspartate aminotransferase, alanine aminotransferase, triglycerides.
3.4.8	Urinalysis	Not performed.
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	The following organ weights were recorded at scheduled necropsies: brain, heart, kidneys, liver, adrenals, ovaries, thyroid with parathyroid and testes with epididymides.
3.5.2	Gross and histopathology	Gross pathology Necropsy were conducted on each animal, and included examination of: the external surfaces, all orifices, cranial cavity, carcass, external surface of the brain and spinal cord, the nasal cavity and paranasal sinuses, the thoracic, abdominal and pelvic cavities and their viscera, the cervical tissues and organs. Histopathology Histopathological examination was performed on the adrenal gland, thyroid, lungs, liver, kidney, treated skin, and untreated skin (back of the outer thigh, left hindleg) from the highest dose group and the control group.
3.5.3	Other examinations	Thyroid function T3, T4 and TSH levels were assessed at the end of the study for all animals.
3.5.4	Statistics	Parameters were analysed with recognised statistical techniques.
3.6	**Further remarks**	
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Systemic Clinical observation showed no evidence of any compound related effects. Local Only transient findings of erythema were noted. No other signs of dermal irritation were observed throughout the study.
4.1.2	Mortality	No compound related death occurred during the study.
4.2	**Body weight gain**	Mean body weights and body weight gains were comparable to the control.
4.3	**Food consumption and compound intake**	No apparent compound related effects were observed.
4.4	**Ophtalmoscopic examination**	Not examined.

Section A6.3.2(1) **Repeated dose toxicity (dermal)**

Annex Point IIA6.3 **Rat 4 week dermal toxicity study with Mancozeb**

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4.5	**Blood and urine analysis**	
4.5.1	Haematology	No compound related effects were observed.
4.5.2	Clinical chemistry	No compound related effects were observed.
4.5.3	Urinalysis	Not investigated.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	Incidental significant positive trends were noted in the relative kidney and ovary weight data for the females.
4.6.2	Gross and histopathology	Histopathology did not show Mancozeb related skin alteration. Minimal to slight keratosis, and acantosis was observed in all groups and was considered as related to application procedures and unrelated to compound.
4.7	**Other**	Thyroid function No compound related effects were observed.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	This study was conducted according to GLP and EPA 82-2. Mancozeb (Dithane® M-45) was evaluated for dermal irritation and systemic toxicity when applied to the dorsal intact skin of male and female Sprague-Dawley rats (10/sex/group) in aqueous suspensions providing dose levels of 10, 100, and 1000 mg/kg bw/day. Dermal applications were repeated for a total of 20 or 21 six-hour exposure periods. The vehicle, distilled water, was similarly applied to another group of 10 male and 10 female rats that served as a control (0 mg/kg bw/day). Dose volumes were 2.5 ml/kg bw/day for the highest dose group and 1.5 ml/kg bw/day for the other groups. Survival, clinical signs, body weights, food consumption, skin reactions, results, of terminal clinical pathology studies (haematology, serum chemistry, thyroid function tests), absolute and relative (organ to body weight ratio) organ weight data, and macroscopic and microscopic tissue findings were evaluated.
5.2	**Results and discussion**	There was no evidence of any compound-related effects in survival data, clinical findings, body weight values, or food consumption measurements. Skin irritation (Draize scale) was limited to observations of slight erythema in two of ten 100 mg/kg bw/day males on a single day (Day 5), two of ten 1000 mg/kg bw/day males on Day 4 or 5, and one of ten 1000 mg/kg bw/day females on Days 3-6 of application. These transient gross findings did not provide definitive evidence of dermal irritation attributable to the application of mancozeb. Yellow staining of the application sites was consistently evident at 1000 mg/kg bw/day (males and females) and sporadically noted at 100 mg/kg bw/day. This is attributed to staining by mancozeb, a yellowish compound. Haematology, serum chemistry, and thyroid function (triiodothyronine [T3], thyroxine [T4], and thyroid stimulating hormone [TSH] assays) showed no evidence of any dose- or compound-related effects.

Section A6.3.2(1) **Repeated dose toxicity (dermal)**

Annex Point IIA6.3 **Rat 4 week dermal toxicity study with Mancozeb**

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		Incidental statistically significant increases were found in the mean absolute leukocyte and lymphocyte counts of the 100 mg/kg bw/day males and the mean T3 value of the 1000 mg/kg bw/day males. These findings are not considered to be indicative of toxicity. There were no meaningful or statistically significant differences between mean absolute and relative organ weight values of control and test groups of rats. At necropsy, treated skins of the majority (7 of 10 per each sex) of the 100 mg/kg bw/day rats and all (20 of 20) 1000 mg/kg bw/day rats were noted to appear dark or had darkened areas (generally described to be yellow). Yellow to brown darkening of the exposure site was also noted in 4 of 10 control males and 4 of 10 male and 1 of 10 female 10 mg/kg rats. Histopathology showed minimal, to slight increased keratin production (hyperkeratosis) and thickening of the epidermis (acanthosis) that was not meaningfully different in incidence or severity between control and test groups, and these findings are attributed to application procedures. There were no microscopic findings (treated and untreated skin, thyroid, lung, liver, kidneys, and adrenals were examined) attributable to the dermal application of mancozeb. Comments Wherever possible results, conclusions, and evaluation (reliability, deficiency and guideline compliance) of this study are presented as in Annex B (and its addenda) of the PPP Monograph for Mancozeb.
5.3	**Conclusion**	Up to 21 six-hour dermal exposures to mancozeb over 4 weeks at dose levels of up to 1000 mg/kg bw/day produced no systemic or local adverse effects.
5.3.1	LO(A)EL	> 1000 mg/kg bw/day (no adverse findings at any dose level).
5.3.2	NO(A)EL	= 1000 mg/kg bw/day
5.3.3	Other	None
5.3.4	Reliability	1
5.3.5	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	LO(A)EL: NO(A)EL:

Section A6.3.2(1) Repeated dose toxicity (dermal)

Annex Point IIA6.3

Rat 4 week dermal toxicity study with Mancozeb

IUCLID 5.4/11

	Other conclusions: *(Adopt applicant's version or include revised version)*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.3.2(2)
Annex Point IIA6.3
IUCLID 5.4/12

Repeated dose toxicity (dermal)

Rabbit 21 day dermal toxicity study with Mancozeb

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1 REFERENCE

1.1	**Reference**	[redacted] (1988) Mancozeb Technical: Twenty-One Day Dermal Toxicity Study in Rabbits. Huntingdon Research Centre Ltd. Report No. 62/87964. 3 October 1988. (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Pennwalt Corporation.
1.2.2	Companies with letter of access	Cerexagri SA.
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	This study has been performed according to the E.P.A. Pesticide assessment Guidelines, n° 82-2. These guidelines are scientifically recognised in several countries. There is no major difference with the required EU guidelines. At the time the study was performed no particular guideline was required for EC registration.
2.2	**GLP**	Yes, the test was conducted in compliance with the EPA GLP Guidelines.
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Mancozeb technical
3.1.1	Lot/Batch number	Not provided.
3.1.2	Specification	Deviating from specification given in section 2 as follows.
3.1.2.1	Description	A greenish-yellow powder.
3.1.2.2	Purity	Not provided.
3.1.2.3	Stability	Not provided.
3.2	**Test Animals**	*Non-entry field*
3.2.1	Species	Rabbit.
3.2.2	Strain	New Zealand White.
3.2.3	Source	Interfauna, [redacted]
3.2.4	Sex	Male and female.
3.2.5	Age/weight at study initiation	On arrival (7 days prior to commencement of dosing), the rabbits were approximately 10 to 12 weeks old and in a weight range of 2.2 to 2.8 kg.
3.2.6	Number of animals per group	5 animals/sex/group.
3.2.7	Control animals	Yes, 5 males and 5 females.

Section A6.3.2(2) **Repeated dose toxicity (dermal)**

Annex Point IIA6.3 **Rabbit 21 day dermal toxicity study with Mancozeb**

IUCLID 5.4/12

3.3	**Administration/ Exposure**	Dermal
3.3.1	Duration of treatment	21 days.
3.3.2	Frequency of exposure	Daily.
3.3.3	Postexposure period	None
3.3.4	**Dermal**	
3.3.4.1	Area covered	Ca. 10% of total body surface.
3.3.4.2	Occlusion	Occlusion
3.3.4.3	Concentration	0, 62.5, 250, 1000 mg/kg bw/day.
3.3.4.4	Vehicle	Distilled water.
3.3.4.5	Total volume applied	The test substance was moistened with distilled water: 62.5 and 250 mg/kg bw/day: 1 ml 1000 mg/kg bw/day: 2 ml
3.3.4.6	Duration of exposure	6 hours
3.3.4.7	Removal of test substance	Treated skin was washed with warm water (30-40°C) and gently blotted dry.
3.3.4.8	Controls	Control animals were similarly dosed with distilled water (1 ml/kg bw/day).
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Systemic All animals were observed daily for signs of ill health, behavioural changes or toxicosis. Local Local irritation was recorded immediately prior to the first daily application of the test substance and subsequently daily. Local dermal reactions (erythema and oedema) resulting from treatment were assessed according to a modified Draize scoring system.
3.4.1.2	Mortality	Animals were examined twice daily for mortality and moribundity.
3.4.2	Body weight	Recorded weekly.
3.4.3	Food consumption	Recorded weekly.
3.4.4	Water consumption	Quantitative measurements were not performed.
3.4.5	Ophthalmoscopic examination	Not performed.
3.4.6	Haematology	Animals examined and frequency: all animals, prior to termination. Parameters: packed cell volume, haemoglobin, red blood cell count, platelet count, mean corpuscular haemoglobin concentration, mean

		corpuscular volume, total white cell count, differential count, cell morphology, thrombotest.
3.4.7	Clinical Chemistry	Animals examined and frequency: all animals, prior to termination Parameters: glucose, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, total bilirubin, cholesterol, urea nitrogen, total protein, albumin, globulin, albumin/globulin ratio, sodium, potassium, calcium, chloride, inorganic phosphorus, creatinine.
3.4.8	Urinalysis	Not performed.
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	The following organ weights were recorded at scheduled necropsies: adrenals, kidneys, liver, ovaries, testes (with epididymides).
3.5.2	Gross and histopathology	Gross pathology All animals were subjected to a gross necropsy. Histopathology Kidneys and liver was examined histopathologically from all control and high dose animals. Skin (treated and untreated) was examined from all animals.
3.5.3	Other examinations	Thyroid function Tri-iodothyronine (T3) and thyroxine (T4) levels were assessed at the end of the study for all animals.
3.5.4	Statistics	Parameters were analysed with recognised statistical techniques.
3.6	**Further remarks**	
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Systemic Clinical observation showed no evidence of any compound related effects. Local dermal reactions No dermal irritation was observed for control rabbits receiving distilled water during the three-week treatment period. Slight dermal irritation developed in all rabbits in the treatment groups by Day 16. For most rabbits, slight oedema developed a few days following the initial observation of slight erythema. The degree of dermal irritation did not appear to be dosage-related. In most instances, slight irritation persisted to the study termination. Irritation progressed, becoming well-defined, for one rabbit only (1000 mg/kg bw/day group) on Days 18 to 20 and Day 22. Other dermal reactions: Yellow staining of the treated skin site was observed for all rabbits receiving Mancozeb technical. Sloughing of the treated skin was also observed amongst rabbits receiving Mancozeb technical. Sloughing did not appear to be dosage-related and was noted, for periods of one to several days, amongst

		rabbits of all dosage groups.
4.1.2	Mortality	Mortalities One animal died at 62.5 mg/kg bw/day. However, this death was not attributed to treatment.
4.2	**Body weight gain**	During the treatment period slightly lower bodyweight gains were recorded amongst rabbits receiving Mancozeb technical in comparison with those receiving distilled water, with statistical significance ($P<0.05$) being achieved during Weeks 1 and 2 for male rabbits receiving 1000 mg/kg bw/day and also for female rabbits in this high dosage group during Week 3 ($P<0.05$). The trend to lower bodyweight gains for male rabbits can be related to the lower food consumption of male rabbits receiving Mancozeb technical in comparison with controls. However, in the absence of any definite toxicological findings, and in view of the dermal irritation recorded for rabbits receiving Mancozeb technical, the apparent effect on bodyweight gains and food consumption was considered to be related to the probable discomfort of the treated animals and unlikely to be directly related to treatment with Mancozeb technical.
4.3	**Food consumption and compound intake**	An apparent, but not statistically significant, trend to lower food consumption was generally recorded throughout the treatment period for male rabbits receiving Mancozeb technical in comparison with controls, particularly for those in the high dosage group. This apparent effect on food consumption can be related to the slightly lower bodyweight gains which were recorded for male rabbits receiving Mancozeb technical. However, as described in the previous section, the apparent effects on bodyweight gains and food consumption may have resulted from the probable discomfort of treated rabbits and was unlikely to be directly related to treatment with Mancozeb technical. Food consumption for female rabbits receiving Mancozeb technical was similar to that of the controls receiving distilled water throughout the three-week treatment period.
4.4	**Ophtalmoscopic examination**	Not performed.
4.5	**Blood and urine analysis**	
4.5.1	Haematology	No changes were observed in any haematological parameter that were considered to be of toxicological importance. Statistically significantly lower ($P<0.05$) thrombotest times were recorded for male rabbits receiving 1000 mg/kg bw/day in comparison with controls. However, this apparent shift in thrombotest times was small in magnitude, was not observed for female rabbits and was considered, therefore, to have probably arisen by chance.
4.5.2	Clinical chemistry	In comparison with control animals, higher urea nitrogen and creatinine levels were recorded for male rabbits in the intermediate and high dosage groups with statistical significance being achieved for creatinine levels of male rabbits receiving 250 mg/kg bw/day ($P<0.05$) or 1000 mg/kg bw/day ($P<0.01$). Individual urea nitrogen and creatinine levels for three male rabbits in the high dosage group were comparatively higher than the corresponding control values. However, this trend to

		higher urea nitrogen and creatinine levels was considered to result from the lower food intake of treated male rabbits in comparison with controls and unlikely to be directly related to treatment with Mancozeb technical. The apparent shifts in urea nitrogen and creatinine levels were not observed for female rabbits. Higher alanine aminotransferase levels were recorded for male rabbits receiving Mancozeb technical in comparison with controls, with statistical significance being achieved ($P<0.05$) for rabbits in the high dosage group. For treated female rabbits however, alanine aminotransferase levels were lower than those of the controls and, in the absence of any histopathological findings in the liver, the effect on male alanine aminotransferase levels was considered unlikely to be of toxicological importance. A slight, but not statistically significant trend to lower thyroxine (T4) and tri-iodothyronine (T3) levels was observed for male rabbits receiving Mancozeb technical, 250 or 1000 mg/kg bw/day in comparison with controls. However, the apparent shifts in these parameters were very low in magnitude, were not observed for female rabbits and were, therefore, considered to be of no toxicological importance. Slightly higher cholesterol levels were recorded for male and female rabbits receiving 250 or 1000 mg/kg bw/day in comparison with those receiving distilled water. Statistical significance was not, however, achieved and the apparent effect on this parameter was considered low in magnitude and unlikely to be of toxicological importance.
4.5.3	Urinalysis	Not investigated.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	Lower liver weights were recorded at termination for male (adjusted weights) and female (unadjusted weights) rabbits receiving Mancozeb technical in comparison with those receiving distilled water, with statistical significance being achieved for female rabbits in all dosage groups ($P<0.05$). However, the apparent shift to lower liver weights for both male and female rabbits was considered low in magnitude and, in the absence of any histopathological findings, was not considered to be treatment-related.
4.6.2	Gross and histopathology	<u>Macroscopic pathology</u> No macroscopic abnormalities were observed at termination that were considered to be related to treatment with Mancozeb technical. <u>Microscopic pathology</u> The following treatment-related changes were detected at treated skin sites: Minimal acanthosis in a proportion of male and female animals from all treatment groups compared to one control female. This was associated with hyperkeratosis in one female receiving 250 mg/kg bw/day and the majority of females receiving 1000 mg/kg bw/day. Focal erosion in one female receiving 250 mg/kg bw/day and the majority of females receiving 1000 mg/kg bw/day. level (1000 mg/kg bw/day).

Minimal inflammation of the superficial dermis, usually diffuse, in the majority of male and female animals in all treatment groups compared to one control female.

4.7 Other

Thyroid function

No compound related effects were observed.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

This study was conducted according to GLP and EPA guideline 82-2.

Mancozeb technical was administered to the intact skin of groups of 5 male and 5 female rabbits, daily, for twenty-one consecutive days at dosage levels of 0, 62.5, 250 or 1000 mg/kg bw/day. The test substance was applied moistened with distilled water.

The following parameters were investigated for all animals: survival, clinical signs, body weights, food consumption, skin reactions, clinical pathology (haematology, serum chemistry, thyroid function tests), organ weight data, macroscopic findings and microscopic evaluation of the treated and untreated skin. In addition microscopic evaluation of kidney and liver was performed for control and high dose animals.

5.2 Results and discussion

No dermal irritation was observed for control rabbits receiving distilled water. Treatment-related dermal irritation developed for all rabbits receiving Mancozeb technical by Day 16 and persisted, in most instances, to the study termination. This dermal irritation remained slight for the majority of rabbits, progressing to well-defined for one rabbit in the high dosage group only.

Sloughing and yellow staining of the treated skin were also noted for all rabbits receiving Mancozeb technical.

Treatment-related microscopic changes were confined to the treated skin of rabbits receiving Mancozeb technical and were as follows:

- Minimal acanthosis in a proportion of male and female rabbits from all treatment groups. This was associated with hyperkeratosis and/or focal erosion in one female receiving 250 mg/kg bw/day and in the majority of females receiving 1000 mg/kg bw/day.
- Minimal inflammation of the superficial dermis, usually diffuse, in the majority of rabbits receiving Mancozeb technical.

In all other respects including bodyweight changes, food consumption, haematology, biochemistry (including thyroid function), macroscopic and microscopic (excluding treated skin) pathology, no changes were observed for rabbits that were considered to be directly related to treatment with Mancozeb technical.

In the absence of any overt signs of toxicity and in view of the dermal irritation elicited by Mancozeb technical, apparent changes in bodyweight gains, food consumption and blood urea nitrogen and creatinine levels were considered to result from the probable discomfort of treated rabbits and to be of no toxicological importance.

5.3 Conclusion

Administration of mancozeb to the intact skin of New Zealand White rabbits, daily (6hrs/day), for twenty-one consecutive days at dose levels of up to 1000 mg/kg bw/day produced some dermal irritation but no systemic adverse effects.

Section A6.3.2(2) Repeated dose toxicity (dermal)

Annex Point IIA6.3

Rabbit 21 day dermal toxicity study with Mancozeb

IUCLID 5.4/12

5.3.1	LO(A)EL	> 1000 mg/kg bw/day (no systemic adverse findings at any dose level).
5.3.2	NO(A)EL	= 1000 mg/kg bw/day
5.3.3	Other	None
5.3.4	Reliability	1
5.3.5	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	LO(A)EL: NO(A)EL: Other conclusions: *(Adopt applicant's version or include revised version)*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section IIIA 6.3.3 **Annex Point IIA, VI.6.3**	**(Sub)heading** *(specify where appropriate)* **Repeated dose toxicity (inhalation)**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	A subchronic inhalation toxicity study is available with Mancozeb.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*Give date of action*	
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*	
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.4.1(1)
Annex Point IIA6.4
IUCLID 5.4/5

Subchronic oral toxicity

13 week rat dietary toxicity study with Mancozeb

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1989) Mancozeb Technical: Toxicity to Rats by Dietary Administration for 13 Weeks with 4 Week Recovery Period. Huntingdon Research Centre Ltd. Report No.: PWT 46/87924. 12 July 1989. (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Pennwalt Corporation.
1.2.2	Companies with letter of access	Cerexagri SA
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	This study has been performed according to the E.P.A. and OECD guidelines n° 408. These guidelines are scientifically recognised in several countries. There is no major difference with the required EU guidelines. At the time the study was performed no particular guideline was required for EC registration.
2.2	**GLP**	Yes, the test was conducted in compliance with the EPA GLP Guidelines.
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Mancozeb technical
3.1.1	Lot/Batch number	Batch n° BLI 850 930.
3.1.2	Specification	Deviating from specification given in section 2 as follows.
3.1.2.1	Description	A greenish-yellow powder.
3.1.2.2	Purity	88.2%
3.1.2.3	Stability	The batch of test material used was known to be stable for the duration of the study. However, stability of the test material was re-confirmed prior to the start of the study and at three monthly intervals thereafter.
3.2	**Test Animals**	*Non-entry field*
3.2.1	Species	Rat
3.2.2	Strain	Crl:CD(SD)BR
3.2.3	Source	Charles Rivers Laboratories [redacted].
3.2.4	Sex	Male and female.
3.2.5	Age/weight at study initiation	Animals were 5 to 6 weeks old. Group mean body weights on day -1 were 169 g for males and 133 to 134 g for females.

Section A6.4.1(1) **Subchronic oral toxicity**

Annex Point IIA6.4 **13 week rat dietary toxicity study with Mancozeb**

IUCLID 5.4/5

3.2.6 Number of animals per group

Allocation to dose groups was as follows:

Dose Level (ppm)	0	28	113	454
Main study animals	10 M/10 F	10 M/10 F	10 M/10 F	10 M/10 F
Recovery animals	10 M/10 F	-	-	10 M/10 F

3.2.7 Control animals

Yes, see point 3.2.7.

3.3 Administration/ Exposure

Oral

3.3.1 Duration of treatment

13 weeks.

3.3.2 Frequency of exposure

Daily via the diet.

3.3.3 Postexposure period

4 weeks (control and high dose groups only).

3.3.4 Oral

3.3.4.1 Type

Via the diet.

3.3.4.2 Concentration

Group No.	1	2	3	4
Dose Level (ppm)	0	28	113	454
Dose Level (mg/kg bw/day) - males	0	1.7	7.0	29.2
Dose Level (mg/kg bw/day) - females	0	2.1	8.4	33.4

Treatment levels are expressed in terms of the material as supplied, Mancozeb technical (88.2%).

Accuracy, homogeneity and stability of diet preparations were analysed and found to be acceptable.

3.3.4.3 Vehicle

None

3.3.4.4 Concentration in vehicle

No vehicle used. Test material mixed directly in the diet.

3.3.4.5 Total volume applied

Not applicable.

3.3.4.6 Controls

Yes, received control diet.

3.4 Examinations

3.4.1 Observations

3.4.1.1 Clinical signs

All signs of ill health, together with any behavioural changes or reaction to treatment were recorded for individual animals. These detailed examinations were carried out daily.

3.4.1.2 Mortality

Each animal was checked at least twice a day for mortality or signs of morbidity.

3.4.2 Body weight

The weight of each rat was recorded at the time of allocation of animals to groups, on the day of commencement of treatment, and once a week thereafter.

3.4.3 Food consumption

The quantity of food consumed by each cage of rats was recorded on a

		weekly basis.
3.4.4	Water consumption	Daily monitoring by visual appraisal of the water bottles was maintained throughout the study. Water consumption was measured accurately, by weight, over daily periods during Week 12 for all cages in all groups.
3.4.5	Ophthalmoscopic examination	Before treatment commenced and during Week 13 the eyes of all animals in the control and high dosage level groups were examined by means of a Keeler indirect ophthalmoscope.
3.4.6	Haematology	Animals examined and frequency: samples were taken from 10 male and 10 female rats from each group during Week 13; (for Groups 1 and 4: samples were taken from 5 male and 5 female rats scheduled to be killed after 13 weeks of treatment, and from 5 male and 5 female rats allocated to the recovery group). In addition were samples taken from all recovery group rats in Week 17. Parameters: packed cell volume, haemoglobin, red cell count, mean corpuscular haemoglobin concentration, mean corpuscular volume, total white cell count, platelet count, differential white cell counts, cell morphology, thrombotest.
3.4.7	Clinical Chemistry	Animals examined and frequency: as for haematology. Parameters: total protein, albumin, globulin, urea nitrogen, creatinine, sodium, potassium, calcium, inorganic phosphorus, chloride, cholesterol, glucose, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, total bilirubin.
3.4.8	Urinalysis	Animals examined and frequency: as for haematology. Parameters: Appearance, colour, volume, pH, specific gravimetry, protein, glucose, ketones, bile pigment, urobilinogen, haem pigments, epithelial cells, leukocytes, erythrocyte, organisms, renal tubule casts, sperm.
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	The following organ weights were recorded at scheduled necropsies: adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, thyroid.
3.5.2	Gross and histopathology	Gross pathology Each necropsy included examination of all superficial tissues visually and by palpation. The cranial roof was removed to allow observation of the brain, the pituitary gland and the cranial nerves. Thoracic and abdominal viscera were examined. Histopathology Adrenals**, alimentary tract*(oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, and rectum), aorta*, brain**(medullary, cerebellar and cortical sections), eyes**, femur (with joint), Harderian gland, head (to preserve nasal cavity, paranasal sinuses, oral cavity, nasopharynx, middle ear, teeth, lachrymal gland and Zymbal's gland), heart*, kidneys*, larynx and pharynx, liver*, lungs*(all lobes and mainstem bronchi), lymph nodes**(cervical and mesenteric), mammary gland**, ovaries*, pancreas**, pituitary**, prostate* salivary gland**, sciatic nerve**, seminal vesicles, skeletal muscle**, skin, spinal column**(to preserve samples of spinal cord from cervical, thoracic and lumbar levels), spleen*, sternum*(for bone and marrow), testes*(with epididymides), thymus**(where present), thyroid*(with parathyroid), tongue, trachea**,

		urinary bladder**, uterus*(corpus and cervix), vagina, any other macroscopically abnormal tissues*. Histopathological examination was performed on all tissues marked '*' from all animals and on tissues marked '**' from animals in the control and high dosage groups, killed at Week 13.
3.5.3	Other examinations	Thyroid hormone assays Animals examined and frequency: as for haematology. Parameters: Thyroxine (T4), Triiodothyonine (T3).
3.5.4	Statistics	All parameters were analysed with recognised statistical techniques.
3.6	**Further remarks**	None.

4 RESULTS AND DISCUSSION

4.1	**Observations**	
4.1.1	Clinical signs	There were no signs indicative of a reaction to treatment.
4.1.2	Mortality	There were no deaths in this study.
4.2	**Body weight gain**	A significant reduction in bodyweight gain was noted for females receiving 454 ppm. A slight, non-significant reduction was also noted for males at the same treatment level. A contrasting effect was seen in the recovery period, when females previously receiving 454 ppm showed an increase in bodyweight gain. See Table A6.4.1(1)-1.
4.3	**Food consumption and compound intake**	Food consumption/ food efficiency There was no effect of treatment on food consumption, although a marginally inferior efficiency of food utilisation was noted for animals receiving 454 ppm. An increased efficiency was noted during the recovery period, for animals that had previously received 454 ppm. Compound intake See point 3.3.4.2 for achieved dosages. A 14-24% higher dietary intake of Mancozeb technical was recorded for females compared to males over the treatment period.
4.4	**Water consumption**	A slightly increased water intake was seen for males receiving 454 ppm during Week 12. See Table A6.4.1(1)-2.
4.5	**Ophthalmoscopic examination**	No treatment-related ocular lesions were observed.
4.6	**Blood and urine analysis**	

Section A6.4.1(1) **Subchronic oral toxicity**

Annex Point IIA6.4 **13 week rat dietary toxicity study with Mancozeb**

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4.6.1 Haematology

A marginal reduction in neutrophil numbers was noted for females receiving 454 ppm, or 113 ppm at the end of the treatment period. This change was no longer apparent at the end of the recovery period. (see table below).

	Week	Sex	Dose Level (ppm)			
			0	28	113	454
Neutophil levels $x10^3/mm^3$	12	M	1.85	1.73	2.12	1.51
		F	1.02	0.88	0.64	0.61*
	17	M	1.95	-	-	2.69
		F	0.91	-	-	1.10

* $P<0.05$ in comparison with control.

4.6.2 Clinical chemistry

Standard parameters showed no treatment related changes.

4.6.3 Urinalysis

Investigations performed in Weeks 13 and 17 of the study revealed no treatment-related effects.

4.7 Sacrifice and pathology

4.7.1 Organ weights

There was no evidence of any effect of treatment on organ weights for rats killed either at the end of the treatment or recovery periods.

4.7.2 Gross and histopathology

Macroscopic findings

Examination of rats killed either at the end of the treatment or recovery periods revealed no evidence of any treatment-related findings.

Microscopic findings

Examination of tissues from all rats killed at termination revealed no findings considered indicative of a reaction to treatment with Mancozeb technical

Section A6.4.1(1) **Subchronic oral toxicity**

Annex Point IIA6.4 **13 week rat dietary toxicity study with Mancozeb**

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4.8 Other

Thyroid hormone assays

Plasma T4 levels were reduced for males receiving 454 ppm and females receiving 113 or 454 ppm. Plasma T3 levels were marginally increased for females receiving 454 ppm. These findings are considered to be of equivocal toxicological significance. Investigations during Week 17 revealed values similar to those of control animals for all parameters in rats previously receiving 454 ppm. (see table below).

	Week	Sex	Dose Level (ppm)			
			0	28	113	454
T3 levels ng/dl	12	M	36	35	36	38
		F	54	50	52	66*
	17	M	40	-	-	36
		F	46	-	-	45
T4 µg/dl	12	M	4.3	4.2	4.4	3.5**
		F	3.3	3.1	2.8	2.8
	17	M	4.0	-	-	3.8
		F	2.6	-	-	2.8

* $P<0.05$ in comparison with control.

** $P<0.01$ in comparison with control.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

This study was conducted according to GLP and OECD Guideline 408 (1981).

Ten male and 10 female Crl: CD(SD)BR rats received Mancozeb technical at 0, 28, 113 or 454 ppm in the diet for a scheduled 13 week treatment period. An additional 10 males and 10 females were allocated to the control and high dose groups and kept on study for a further 4 weeks without treatment to investigate recovery. Clinical examinations were performed daily. Food consumption was measured daily for each animal. Water consumption was monitored visually throughout the study and quantitatively during week 12. Body weight was recorded weekly for each animal. Ophthalmological examinations were performed pretest and at week 13. Clinical pathology investigations (including measurements of thyroid hormones T3 and T4) were performed after 13 or 17 weeks. All animals were subjected to a gross necropsy examination, selected organs were weighed and selected tissue examined histopathologically.

5.2 Results and discussion

The results of this study indicate a minimal effect of Mancozeb technical among males and females receiving 454 ppm, characterised by a reduced weight gain chiefly during the treatment period. Other changes from controls were considered to be equivocal and included increases in T3, and suppression of T4 and neutrophils. The latter two changes also affecting females receiving 113 ppm.

5.3 Conclusion

The dietary inclusion level of 28 ppm (equivalent to 1.7 mg/kg bw/day for males and 2.1 mg/kg bw/day for females) was established as a clear

Section A6.4.1(1) **Subchronic oral toxicity**

Annex Point IIA6.4 **13 week rat dietary toxicity study with Mancozeb**

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		"no-effect" level in this study.
5.3.1	LO(A)EL	113 ppm (non significant reduction of neutrophils and T4 in females considered as equivocal).
5.3.2	NO(A)EL	28 ppm (equivalent to 1.7 mg/kg bw/day for males and 2.1 mg/kg bw/day for females)
5.3.3	Other	None
5.3.4	Reliability	1
5.3.5	Deficiencies	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	LO(A)EL: NO(A)EL: Other conclusions: *(Adopt applicant's version or include revised version)*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*

Section A6.4.1(1) **Subchronic oral toxicity**

Annex Point IIA6.4 13 week rat dietary toxicity study with Mancozeb

IUCLID 5.4/5

Remarks

Table A6.4.1(1)-1 13 week rat dietary toxicity study with Mancozeb
Body weight – group mean values (g)

Week	Group and Dosage (ppm)							
	1♂ Control	2♂ 28	3♂ 113	4♂ 454	1♀ Control	2♀ 28	3♀ 113	4♀ 454
Pre-dose								
-1	169	169	169	169	134	133	133	133
Dosing								
0	238	226	243	239	172	170	170	173
1	297	282	302	298	195	191	192	191
2	349	336	354	344	218	212	215	211
3	386	378	392	375	232	227	228	223
4	418	412	425	401	248	238	241	236
5	450	444	459	427	261	250	257	248
6	473	475	484	453	270	263	265	258
7	498	503	516	477	277	273	274	262
8	526	532	544	502	287	282	283	272
9	544	556	566	515	293	288	292	277
10	567	577	577	535	298	300	302	285
11	585	595	603	550	302	301	302	286
12	595	609	617	561	310	305	306	290
13	598	602	613	567	306	299	296	287
Withdrawal								
13	603			571	319			289
14	620			590	327			301
15	628			598	327			301
16	650			614	334			310
17	641			615	326			307
#Mean gain								**
Week 0-13:	360	376	370	328	134	129	126	114
SD:	64	50	59	47	20	26	23	17
#Mean gain								+
Week 13-17:	39			43	7			18
SD:	17			15	10			7

SD Standard deviation
Mean gains quoted are derived from individual values and are not, therefore, directly calculable from this table
Levels of significance:
+ P<0.05 in comparison with control (Students 't' test)
** P<0.01 in comparison with control (Williams' test)

Table A6.4.1(1)-2 13 week rat dietary toxicity study with Mancozeb
Water consumption – group mean values (g/rat/day)

Week	Group and dosage (ppm)							
	1♂ Control	2♂ 28	3♂ 113	4♂ 454	1♀ Control	2♀ 28	3♀ 113	4♀ 454
Dosing								
11.1	40.4	44.0	41.6	51.5	34.3	36.3	30.1	35.3
11.2	39.5	43.4	43.6	43.4	29.3	30.5	26.8	33.8
11.3	42.6	41.9	43.7	44.5	28.9	32.8	25.4	32.4
11.4	40.7	43.7	47.7	52.3	29.8	34.2	24.5	33.6
11.5	44.9	40.5	39.4	44.0	27.9	35.2	26.8	30.9
11.6	40.8	37.7	41.7	46.1	30.9	30.7	28.4	31.0
12	40.1	43.0	41.4	45.3	30.3	33.7	28.2	32.3
#Total mean intake								
Week 12:	289	294	299	327	211	233	190	229
SD:	41	4	34	22	25	24	15	15
% of control:	-	102	103	113	-	110	90	109

SD Standard deviation
\# Total mean intakes quoted are derived from individual cage values and are not, therefore, directly calculable from this table
No significant differences from control: P>0.05 (Williams' test)

Section A6.4.1(2) **Subchronic oral toxicity**

Annex Point IIA6.4 **13 week rat dietary toxicity study with Mancozeb and ETU**

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Official use only

	1 REFERENCE	
1.1	**Reference**	[redacted] (1986) Mancozeb: Three-Month Dietary Toxicity Study in Rats. Rohm and Haas Company. Report No. 85R-167. 27 February 1986. (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Rohm and Haas Company.
1.2.2	Companies with letter of access	Cerexagri SA.
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	This study has been performed according to the E.P.A. guidelines. These guidelines are scientifically recognised in several countries. There is no major difference with the required EU guidelines. At the time the study was performed no particular guideline was required for EC registration.
2.2	**GLP**	Yes, the test was conducted in compliance with the EPA GLP Guidelines.
2.3	**Deviations**	No
	3 MATERIALS AND METHODS	
3.1	**Test material**	
3.1.1	Test material 1	Mancozeb (Dithane M-45)
3.1.1.1	Lot/Batch number	Lot No. 43339; TD N° 85-15.
3.1.1.2	Specification	Deviating from specification given in section 2 as follows.
3.1.1.3	Description	A yellow powder.
3.1.1.4	Purity	84%
3.1.1.5	Stability	Not provided.
3.1.2	Test material 2	Ethylene thiourea (ETU).
3.1.2.1	Lot/Batch number	Product No. IX0010; TD No. 85-55.
3.1.2.2	Specification	Deviating from specification given in section 2 as follows.
3.1.2.3	Description	A white crystalline solid.
3.1.2.4	Purity	99.8%
3.1.2.5	Stability	Not provided.
3.2	**Test Animals**	*Non-entry field*
3.2.1	Species	Rat
3.2.2	Strain	Crl: CD(SD)
3.2.3	Source	Charles River Breeding Laboratories, [redacted]

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3.2.4	Sex	Male and female.
3.2.5	Age/weight at study initiation	ca. 6 weeks old. Group mean body weights on day 0 were 140.9 to 144.9 g for males and 112.1 to 114.5 g for females.
3.2.6	Number of animals per group	14 rats/group/sex (see Table A6.4.1(2)-1).
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	13 weeks.
3.3.2	Frequency of exposure	Daily via the diet.
3.3.3	Postexposure period	None.
3.3.4	**Oral**	
3.3.4.1	Type	Via the diet.
3.3.4.2	Concentration	

Group/ Treatment		Dose Level (ppm)			Dose level (mg/kg bw/day)	
		Wk 1-2	Wk 3-4	Wk 5-13	Males	Females
1	Control	0	0	0	0	0
2	Mancozeb	15	21	30	1.78	2.20
3	Mancozeb	30	42	60	3.49	4.38
4	Mancozeb	62.5	87.5	125	7.42	9.24
5	Mancozeb	125	175	250	14.98	17.82
6	Mancozeb	500	700	1000	57.34	74.64
7	ETU	125	175	250	14.28	17.81

Accuracy, homogeneity and stability of diet preparations were analysed and found to be acceptable.

3.3.4.3	Vehicle	None
3.3.4.4	Concentration in vehicle	No vehicle used. Test material mixed directly in the diet.
3.3.4.5	Total volume applied	Not applicable.
3.3.4.6	Controls	Yes, received control diet.
3.4	**Examinations**	
3.4.1	Observations	

Section A6.4.1(2) **Subchronic oral toxicity**

Annex Point IIA6.4 **13 week rat dietary toxicity study with Mancozeb and ETU**

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3.4.1.1	Clinical signs	Animal were examined daily for signs of ill health or reaction to treatment. Physical examinations were performed on all rats weekly beginning 1 week prior to the initiation of treatment. The physical examination included evaluation of external structure, behaviour, posture, and gait. Any noticeable irregularities of respiration, body temperature, or colour and consistency of excreta were recorded.
3.4.1.2	Mortality	Animals were checked at daily.
3.4.2	Body weight	Recorded weekly beginning 1 week prior to treatment.
3.4.3	Food consumption	Recorded weekly beginning 1 week prior to treatment.
3.4.4	Water consumption	Not recorded.
3.4.5	Ophthalmoscopic examination	An indirect ophthalmoscopic examination was performed on each animal once prior to the initiation of dosing and during the 13th week of treatment.
3.4.6	Haematology	Animals examined and frequency: 10 animals/sex/group after 13 weeks. Parameters: haematocrit, red blood cell count, haemoglobin, white blood cell count (total and differential), platelets, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular hemoglobin concentration, red blood cell morphology.
3.4.7	Clinical Chemistry	Animals examined and frequency: 10 animals/sex/group after 13 weeks. Parameters: glutamic pyruvic transaminase, cholesterol, urea nitrogen, glucose, alkaline phosphatase, total protein, creatinine, glutamic oxaloacetic tranaminase, gamma glutamyl transpeptidase, albumin, globulin, A/G ratio, bilirubin, calcium, inorganic phosphorous, triglycerides.
3.4.8	Urinalysis	None.
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	The following organ weights were recorded from 10 animals/sex/group: adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, thyroid/parathyroid.
3.5.2	Gross and histopathology	Gross necropsy All surviving animals were necropsied. Each necropsy included examination of all organs, tissues and body cavities. Gross abnormalities were recorded. Histopathology The following tissues were examined from 10 males and 10 females from the control group, the highest dose Mancozeb group and the ETU group: Adrenals, bone with marrow, brain, epididymides, oesophagus, eyes, gonads, heart, caecum, colon, duodenum, ileum, jejunum, rectum, kidney, liver, lungs, lymph nodes, mammary glands, muscle skeletal, peripheral nerve, pancreas, pituitary gland, prostate, salivary gland, seminal vesicles, skin, spinal cord, spleen, stomach, trachea, thymus, thyroid/parathyroid, urinary bladder, uterus, all gross lesions.

		In addition, all gross lesions, thyroid, kidneys, liver and adrenals were evaluated in all the remaining dose groups.
3.5.3	Other examinations	Thyroid hormone assays Animals examined and frequency: 10 animals/sex/group after 13 weeks. Parameters: thyroxine (T4), triiodothyonine (T3), thyroid stimulating hormone (TSH). Hepatic mixed function oxidase assay (MFO) At necropsy, representative sections of liver were taken from 6 rats/sex/group (randomly selected from the animals which were bled for clinical chemistry and haematology). Samples were processed to obtain microsomal suspensions and analyzed for hepatic mixed function oxidase (MFO) activity by both the aniline hydroxylation (AH) and aminopyrine (AP) N-demethylation methods. Residues Urine and blood samples, livers and thyroids were collected from 4 animals/sex/group for analysis of ethylenebisdithiocarbamate (EBDC) and ethylenethiourea (ETU) residues.
3.5.4	Statistics	All parameters were analysed with recognised statistical techniques.
3.6	**Further remarks**	None.
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	No treatment-related sign of toxicity was noted in any group throughout the 13 weeks dosing period.
4.1.2	Mortality	No treatment-related death occurred.
4.2	**Body weight gain**	Body weight Body weights of male rats fed diets containing Mancozeb at 1000 ppm were significantly ($p < 0.05$) decreased (3-8%) at study weeks 3 through 13. Body weights of females at this dose were decreased (3-14%) at weeks 2 through 13, but the change was statistically significant only at weeks 7 through 10. Male rats fed diets containing ETU at 250 ppm displayed decreased (3-7%) body weight beginning with week 2 and throughout the testing period. These decreases were statistically significant at weeks 2 through 4, 6 through 10, and at week 12. Female body weights of ETU treated animals were decreased (6-9%) throughout the dosing period however, the difference was statistically significant only at week 2 of the study. Body weight gain Body weight gains of males and females fed diets containing Mancozeb at 1000 ppm were 12 and 13% lower than their respective controls. Body weight gains of males and females fed diets containing ETU at 250 ppm were 6 and 13% lower than their respective controls.
4.3	**Food consumption and compound intake**	Feed consumption in male rats fed diets containing Mancozeb at 1000 ppm was decreased during weeks 3 through 13 of treatment. Feed consumption of females at this dose was only slightly decreased,

		sporadically throughout the 13 weeks of treatment. Significant decreases in feed consumption in males fed a diet containing ETU at 250 ppm were displayed during weeks 3 through 13. Females in this group showed slight decreases in feed consumption throughout most of the treatment period with significant decreases seen only at weeks 1, 3, and 6. See point 3.4.4.2 for compound intake in mg/kg bw/day.
4.4	**Water consumption**	Not investigated.
4.5	**Ophthalmoscopic examination**	There was no indication of treatment related ocular effects in rats fed diets containing Mancozeb at doses up to and including 1000 ppm or in rats fed ETU at 250 ppm for 13 weeks.
4.6	**Blood and urine analysis**	
4.6.1	Haematology	No treatment related change was seen in any of the rats fed diet containing Mancozeb. Male rats fed ETU at 250 ppm had significantly decreased platelet counts (19% lower than control, $P<0.05$).
4.6.2	Clinical chemistry	No treatment related change was seen in any of the rats fed diet containing Mancozeb. Rats fed 250 ppm ETU in the diet displayed significant increases in serum cholesterol levels (males: 69% higher than controls, $P<0.05$; females: 30% higher than control, $P<0.05$).
4.6.3	Urinalysis	Not investigated.
4.7	**Sacrifice and pathology**	
4.7.1	Organ weights	Following treatment with Mancozeb at 1000 ppm, liver and thyroid weights (absolute and relative) were increased in both male and female rats. The change in absolute liver weight was not statistically significant in either sex. The absolute weight increase for thyroid was not statistically significant in females. Spleen weights (absolute and relative) were increased in females at 1000 ppm, however, only the relative change was statistically significant. ETU increased liver and thyroid weights (absolute and relative) in male and female rats.
4.7.2	Gross and histopathology	Macroscopic findings Three of 10 male rats fed ETU at 250 ppm for 13 weeks exhibited enlarged or swollen livers on gross observation. Microscopic findings Follicular epithelial hyperplasia of the thyroid gland was observed in males and females of Group 6 (1000 ppm Mancozeb). In the thyroid of one Group 6 male, there was a small, well-defined basophilic focus of hyperplastic follicular epithelial cells. Microscopic examination of the pituitary revealed an increased amount of large, hypertrophied cells with a basophilic tinctorial appearance in the anterior lobe of the Group 6 (1000 ppm) male rats.

The kidneys of male and female rats fed 125 ppm Mancozeb and above had minimal to moderate amounts of a yellow-brown, granular pigment in the lumen of the cortical tubules. The pigment most likely represents elimination of a coloured metabolite and is not considered a manifestation of toxicity.

Minimal hepatocellular hypertrophy was observed in two group 6 (1000 ppm) male rats. In one of these rats, a small amount of multifocal hepatocellular vacuolation was present.

An increased incidence of rats with hypertrophy of cells of the zona glomerulosa of the adrenal cortex occurred in the 1000 ppm Mancozeb treated male rats.

Treatment related changes similar to those seen in the rats given Mancozeb occurred in the liver and thyroid of male and female ETU treated rats and in the pituitary and adrenal glands of male ETU treated rats. The treatment related lesions in the ETU treated rats were histologically similar to those that occurred in the same tissues of the Mancozeb treated rats.

In addition to the diffuse hypertrophy and hyperplasia of the thyroid follicular epithelium, four ETU treated male rats had either a follicular adenoma (1 rat) or small, well-defined basophilic foci of hyperplastic follicular epithelium (3 rats). The ETU treated rat with the follicular adenoma also had multicentric lymphosarcoma.

See Table A6.4.1(2)-3 for a summary of key histopathological findings.

4.8 Other

Thyroid Function

Serum T4 levels were decreased at doses of 250 and 1000 ppm Mancozeb in females and at 1000 ppm Mancozeb in males. Serum TSH levels were increased in females at 250 and 1000 ppm Mancozeb and in males at 1000 ppm Mancozeb. Serum T3 levels were unchanged at doses up to and including 1000 ppm Mancozeb.

Serum T4 levels were decreased and T3 and TSH levels increased in rats fed ETU at 250 ppm for 3 months.

See Table A6.4.1(2)-2 for mean thyroid function values.

MFO

Mancozeb at 1000 ppm decreased hepatic MFO activity 31 to 35% and 34 to 40% in males and females respectively, when measured by aniline hydroxylation; the decrease was not statistically significant. AP N-demethylation activity was not affected by Mancozeb treatment.

ETU at 250 ppm also decreased MFO activity in males 29 to 37% when measured by AP N-demethylation; females were not affected. Aniline hydroxylation activity was not affected by treatment with 250 ppm ETU.

The decreased hepatic MFO activity produced by Mancozeb at 1000 ppm and by ETU (males) at 250 ppm was evident whether the hepatic MFO activity was determined on a per mg of microsomal protein basis, on a per g of liver basis or on a per total liver basis. Mancozeb, at dietary concentrations up to and including 1000 ppm, and ETU at 250 ppm, had no effect on hepatic microsomal protein concentration.

Residues

Urine, blood, and thyroid samples were analyzed for residues of EBDC,

detected as CS_2 and/or ETU.

In animals fed Mancozeb, no EBDC or ETU was detected in any of the blood samples analyzed. The urine samples from these animals showed varying levels of ETU. In general, the overall level of ETU in urine increased in a dose related manner from approximately 0.3 ppm at the 30 ppm dietary concentration to about 10 ppm in the urine from the 1000 ppm Mancozeb treated rats. In addition, residues equivalent to 0.10 to 1.1 ppm of EBDC were detected in the urine of rats fed diets at 125 to 1000 ppm Mancozeb respectively. No detectable residues of EBDC were found in urine from rats at lower dietary levels of Mancozeb. It was noted that the method of analysis measures CS_2 and therefore can not distinguish a metabolite of EBDC capable of liberating CS_2 on hydrolysis from an EBDC itself.

Due to the limited size of the thyroid samples, only thyroid samples from rats fed 1000 ppm Mancozeb were analyzed for residues of EBDC. No EBDC above the detection limit of 25 ppm was detected in the thyroid. Analyses of the remaining thyroid samples for ETU showed residues ranging from less than the detection limit of 4 ppm in animals fed 30 ppm Mancozeb to 25 ppm in animals fed 1000 ppm Mancozeb with the increase paralleling the increase in dietary concentrations.

For animals fed 250 ppm ETU, detectable levels of ETU were found in the blood, urine and thyroid of the animals. ETU levels in the blood were just slightly above the detection limit of 0.1 ppm; those found in the urine ranged from 2.9 to 63 ppm and in the thyroid ranged from 30 to 53 ppm.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

This study was conducted according to GLP and EPA Guidelines.

Mancozeb was administered in the diet to 6 groups (14/sex/group) of Crl-CD (SD) rats for 3 months at dietary concentrations of 0 (control), 30, 60, 125, 250, and 1000 ppm of active ingredient (ai). An additional group of rats (14/sex/group) was concurrently administered ethylenethiourea, (ETU) at 250 ppm ai. All rats were observed daily for signs of ill health or reaction to treatment. Body weights and feed consumption were monitored weekly beginning 1 week prior to treatment. Physical examinations were performed weekly on all animals. After 3 months of treatment, animals (10/sex/group) were bled for haematology, clinical chemistry and thyroid function analyses, killed, necropsied, selected organ weights recorded, and tissues collected for histopathologic evaluation. Liver samples were collected from 6 rats/sex/group and analyzed for mixed function oxidase (MFO) activity. Four rats/sex/group were used for analyses of ethylenebisdithiocarbamate (EBDC) as CS_2, and ETU residues in urine, blood, and thyroid.

5.2 Results and discussion

<u>Mancozeb results</u>

No treatment-related deaths occurred and no clinical signs attributed to treatment with Mancozeb were observed. Body weights of male and female rats fed diets containing 1000 ppm Mancozeb were decreased during the treatment period. Feed consumption at 1000 ppm Mancozeb was also slightly decreased in both sexes.

No treatment-related effects were seen in any of the haematology or

clinical chemistry parameters monitored at doses up to and including 1000 ppm of Mancozeb. Serum T4 levels were decreased in both sexes at 1000 ppm Mancozeb and at 250 ppm in females. Serum TSH levels were increased in both sexes at 1000 ppm and in females at 250 ppm. Serum T3 levels were unchanged at doses up to and including 1000 ppm Mancozeb.

Mancozeb at 1000 ppm decreased hepatic MFO activity in male and female rats. Mancozeb, at dietary concentrations up to and including 1000 ppm had no effect on hepatic microsomal protein concentration.

Following 3 months of treatment with Mancozeb at 1000 ppm, liver and thyroid (absolute and relative) weights were increased in both sexes and spleen weights (absolute and relative) were increased in females. No other treatment related organ weight changes were seen in either sex at doses up to and including 250 ppm Mancozeb. No significant histopathologic changes were observed in any rat at doses up to and including 60 ppm Mancozeb. A yellow-brown, granular pigment was seen in the lumen of the cortical tubules of the kidney at 125 ppm Mancozeb and higher, however, this was not considered a manifestation of toxicity. Follicular epithelial hyperplasia of the thyroid gland was observed in male and female rats fed 1000 ppm Mancozeb. Increased amount of hypertrophied cells in the anterior lobe of the pituitary, centrilobular hepatocellular hypertrophy, and an increased incidence of hypertrophy of the cells of the zona glomerulosa of the adrenal cortex were observed in male rats at 1000 ppm.

ETU findings

No deaths occurred and no clinical signs attributed to treatment with ETU were evident. ETU at 250 ppm decreased body weight and feed consumption in both sexes. Males fed ETU at 250 ppm had significantly decreased platelet counts. Serum cholesterol levels were significantly increased in both sexes with ETU at 250 ppm. Serum T4 levels were decreased and T3 and TSH levels increased in rats fed ETU at 250 ppm. ETU at 250 ppm decreased hepatic MF0 activity in males; females were not affected. ETU had no effect on hepatic microsomal protein concentration.

In 3 of the 10 ETU-treated rats gross observations of either enlarged or swollen livers were seen. ETU significantly increased liver and thyroid weights (absolute and relative) in male and female rats. No other changes in organ weights were observed. Treatment-related microscopic changes in the rats fed ETU consisted of hyperplasia of the thyroid follicular epithelium and centrilobular hepatocellular hypertrophy in both sexes, an increased amount of hypertrophied cells of the anterior lobe of the pituitary and hypertrophy of the cells of the zona glomerulosa of the adrenal cortex in male rats.

Results of residue analysis

No EBDC or ETU was detected in the blood samples from animals fed Mancozeb at concentrations up to and including 1000 ppm. In the urine, the level of ETU increased in a dose related manner from approximately 0.3 ppm at 30 ppm to about 10 ppm in the 1000 ppm Mancozeb treated rats. EBDC residues in urine equivalent to 0.10 to 1.1 ppm were detected in rats fed 125 to 1000 ppm Mancozeb, respectively. No EBDC was detected in urine from rats at lower dietary levels of Mancozeb. In the thyroids from rats fed 1000 ppm Mancozeb, no

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		EBDC above the detection limit of 25 ppm was detected. ETU residues in thyroid ranged from less than the detection limit of 4 ppm in animals fed 30 ppm Mancozeb to 25 ppm in animals fed 1000 ppm Mancozeb with the increase paralleling the increase in dietary concentrations. In rats fed ETU (250 ppm), ETU levels in blood were just above the detection limit of 0.1 ppm; those in urine ranged from 2.9 to 63 ppm and in thyroid from 30 to 53 ppm. Comments The conclusions and evaluation (reliability, deficiency and guideline compliance) of this study are presented as in Annex B (and its addenda) of the PPP Monograph for Mancozeb.
5.3	**Conclusion**	There were no treatment related effects observed in the 30, 60 and 125 ppm Mancozeb dose groups. 250 ppm Mancozeb: decreased T4 levels and increased TSH levels. 1000 ppm Mancozeb: several signs of toxicity on thyroid function and on liver. 250 ppm EUT: several signs of toxicity on thyroid function and on liver. The NOEL for Mancozeb was 125 ppm in food, corresponding to 7.4 mg/kg bw/day for males and 9.2 mg/kg bw/day for females.
5.3.1	LO(A)EL	250 ppm (decreased T4 levels and increased TSH levels).
5.3.2	NO(A)EL	125 ppm (equivalent to 7.4 mg/kg bw/day for males and 9.2 mg/kg bw/day for females)
5.3.3	Other	None
5.3.4	Reliability	1
5.3.5	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	LO(A)EL: NO(A)EL: Other conclusions: *(Adopt applicant's version or include revised version)*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*

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Annex Point IIA6.4

13 week rat dietary toxicity study with Mancozeb and ETU

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Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.4.1(2)-1 13 week rat dietary toxicity study with Mancozeb and ETU Outline of study design

Group	Compound	Dietary Conc. (ppm) [b] Wk1-2	Wk3-4	Wk5-13	No. Rats[a] Init	Cl Chem, Hist[c] Hemat, Thy Tests	EBDC[d] & ETU
1	Control	0	0	0	28	20	8
2	MANCOZEB	15	21	30	28	20	8
3	MANCOZEB	30	42	60	28	20	8
4	MANCOZEB	62.5	87.5	125	28	20	8
5	MANCOZEB	125	175	250	28	20	8
6	MANCOZEB	500	700	1000	28	20	8
7	ETU	125	175	250	28	20	8

a-Number of rats equally divided between sexes.
b-Concentration of Mancozeb or ETU in ppm of active ingredient (ai).
c-Rats used for clinical chemistry, hematology, histopathology, ophthalmology, hepatic mixed function oxidase activity and thyroid functions tests.
d-Analysis of EBDC & ETU residue levels in blood, urine and thyroid.

Table A6.4.1(2)-2 13 week rat dietary toxicity study with Mancozeb and ETU Mean thyroid function values

Treatment	Dose Group	Dose level (ppm)	T3 (ng/ml)		T4 (µg/dl)		TSH (ng/ml)	
			M	F	M	F	M	F
Control	**1**	0	1.22	1.40	5.27	3.78	1.20	0.49
Mancozeb	**2**	30	1.19	1.44	5.32	3.39	1.13	0.68
	3	60	1.16	1.42	5.35	3.55	1.75	0.66
	4	125	1.30	1.35	5.65	3.20	1.58	0.39
	5	250	1.28	1.37	5.28	2.71*	1.88	0.95
	6	1000	1.31	1.35	3.49*	2.16*	4.33*	1.32*
ETU	**7**	250	1.56*	1.63*	2.62*	1.34*	6.10*	1.78*

*Significantly different from controls ($p<0.05$)