Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

mancozeb (ISO); manganese ethylenebis(dithiocarbamate) (polymeric) complex with zinc salt

EC Number:-

CAS Number: 8018-01-7

CLH-O-0000001412-86-263/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

15 March 2019

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

International Chemical Identification: Mancozeb

EC Number: **Not listed**

CAS Number: **8018-01-7**

Index Number: **006-076-00-1**

Contact details for dossier submitter: UK Competent Authority
Chemicals Regulation Directorate
Health and Safety Executive
United Kingdom

Version number: 2 **Date: December 2017**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON MANCOZEB (ISO); MANGANESE ETHYLENEBIS(DITHIOCARBAMATE) (POLYMERIC) COMPLEX WITH ZINC SALT

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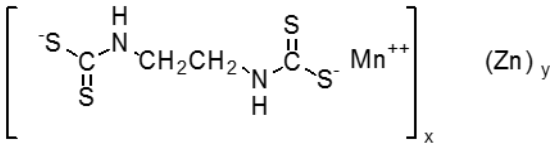
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON MANCOZEB (ISO); MANGANESE ETHYLENEBIS(DITHIOCARBAMATE) (POLYMERIC) COMPLEX WITH ZINC SALT

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	manganese ethylenebis(dithiocarbamate) (polymeric) complex with zinc salt
Other names (usual name, trade name, abbreviation)	Mancozeb, Dithane M45
ISO common name (if available and appropriate)	Mancozeb
EC number (if available and appropriate)	Not listed
EC name (if available and appropriate)	Not listed
CAS number (if available)	8018-01-7
Other identity code (if available)	CIPAC-34
Molecular formula	$(C_4H_6MnN_2S_4)x(Zn)y$
Structural formula	 Mancozeb is a polymeric complex of the monomer illustrated.
SMILES notation (if available)	
Molecular weight or molecular weight range	271.3 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not relevant
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not relevant
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 85%

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Mancozeb Mancozeb is a coordination complex of manganese ethylenebis(dithiocarbamate) and zinc	≥ 85.0%	Skin Sens 1; H317; Repr. 2; H361d***; Aquatic Acute 1; H400 (M = 10)	Skin Sens 1; H317; Repr. 2; H361d; Aquatic Acute 1; H400 (M = 10)

Further information on the substance identity can be found in the confidential attachments (Volume 4 of the Renewal Assessment Report) which are provided with the IUCLID.

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Ethylenethiourea (ETU) (2- Imidazolidinethione) CAS No. 96-45-7	Max 0.09%	Acute Tox 4*: H302; Repr. 1B: H360D***		No (It is present at less than 0.1% by weight, of mancozeb)

The only process impurity of potential toxicological and environmental significance is ethylenethiourea (ETU), which is classified as shown in table 3 above. The content of this impurity is less than or equal to 0.09% (which is less than the relevant generic concentration limit of 0.3% for classification as Repr 1B) and therefore does not impact on the classification proposed in this dossier. Further information on the impurities is considered to be confidential but full details are provided in the technical dossier.

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Hexamethylene tetramine CAS No. 100-97-0	Stabiliser	Max 2.5%	Flam. Sol. 2: H228 Skin Sens. 1: H317		No

The purity of mancozeb (the coordination complex) tested in the studies ranged from 80% - 92.3%. Information on the actual purity used is provided in the relevant summaries and tables of this report.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	006-076-00-1	manganese ethylenebis (dithiocarbamate) (polymeric) complex with zinc salt	-	8018-01-7	Skin Sens 1	H317	GHS09 GHS08 GHS07 Wng	H317			
					Repr. 2	H361d***		H361d***			
					Aquatic Acute 1	H400		H400		M = 10	
Dossier submitters proposal	006-076-00-1	manganese ethylenebis (dithiocarbamate) (polymeric) complex with zinc salt	-	8018-01-7	**Confirm** Skin Sens 1	**Confirm** H317	GHS09 GHS08 GHS07 Wng	**Confirm** H317			
					Remove Repr. 2	**Remove** H361d		**Remove** H361d			
					Add STOT RE 2	**Add** H373 (thyroid, nervous system)(oral)		**Add** H373 (thyroid, nervous system) (oral)			
					Confirm Aquatic Acute 1	**Confirm** H400		**Confirm** H400		**Confirm** M = 10	

					Add Aquatic chronic 1	**Add** H410		**Add** H410		**Add** M = 10	
Resulting Annex VI entry if agreed by RAC and COM	006-076-00-1	manganese ethylenebis (dithiocarbamate) (polymeric) complex with zinc salt	-	8018-01-7	Skin Sens 1	H317	GHS09 GHS08 GHS07 Wng	H317			
					STOT RE 2	H373 (thyroid, nervous system)(oral)		H373 (thyroid, nervous system) (oral)			
					Aquatic Acute 1	H400		H400		M = 10	
					Aquatic chronic 1	H410		H410		M = 10	

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not addressed in this proposal	No
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	Hazard class not applicable	No
Flammable solids	Hazard class not addressed in this proposal	No
Self-reactive substances	Hazard class not addressed in this proposal	No
Pyrophoric liquids	Hazard class not applicable	No
Pyrophoric solids	Hazard class not addressed in this proposal	No
Self-heating substances	Hazard class not addressed in this proposal	No
Substances which in contact with water emit flammable gases	Hazard class not addressed in this proposal	No
Oxidising liquids	Hazard class not applicable	No
Oxidising solids	Hazard class not addressed in this proposal	No
Organic peroxides	Hazard class not applicable	No
Corrosive to metals	Hazard class not addressed in this proposal	No
Acute toxicity via oral route	Hazard class not addressed in this proposal	No
Acute toxicity via dermal route	Hazard class not addressed in this proposal	No
Acute toxicity via inhalation route	Hazard class not addressed in this proposal	No
Skin corrosion/irritation	Hazard class not addressed in this proposal	No
Serious eye damage/eye irritation	Hazard class not addressed in this proposal	No
Respiratory sensitisation	Hazard class not addressed in this proposal	No
Skin sensitisation	**Harmonised classification proposed**	**Yes**
Germ cell mutagenicity	**Data conclusive but not sufficient for classification**	**Yes**
Carcinogenicity	**Data conclusive but not sufficient for classification**	**Yes**
Reproductive toxicity	**Data conclusive but not sufficient for classification**	**Yes**
Specific target organ toxicity-single exposure	Hazard class not addressed in this proposal	No
Specific target organ toxicity-repeated exposure	**Data conclusive but not sufficient for classification**	**Yes**
Aspiration hazard	Hazard class not addressed in this proposal	No
Hazardous to the aquatic environment	**Harmonised classification proposed**	**Yes**
Hazardous to the ozone layer	Hazard class not addressed in this proposal	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Mancozeb is a pesticidal active substance in the scope of Directive 91/414/EEC (and now Regulation 1107/2009). It was included in Annex I of Council Directive 91/414/EEC (Commission Directive 2005/72/EC) and is an approved active substance under Regulation (EC) 1107/2009 as specified in Commission Implementing Regulation (EU) No. 540/2011 of 25 May 2011. The Review Report for mancozeb (SANCO/4058/2001 – rev. 4.4 July 2009) provides conclusions and endpoints agreed in the original EU review for Annex I inclusion. Supplementary dossiers, recently submitted in support of the application for renewal of the approval of mancozeb under Commission Implementing Regulation (EU) 844/2012 of 18 September 2012, are under review by the UK.

The harmonised classification and labelling of mancozeb was originally considered in November 1993 by the Commission Working Group on the Classification and Labelling of Dangerous Substances and then again in 2003-2006 by the Technical Committee on Classification and Labelling (TC C&L). On both occasions, the Commission Working Group of Specialised Experts (SE) in the field of reproductive toxicity was consulted. As a consequence of these earlier reviews, the current Annex VI entry for mancozeb includes the following classification: Skin Sens. 1 (H317: May cause an allergic skin reaction); Repr. 2 (H361d: Suspected of damaging the unborn child) and Aquatic Acute 1 (H400: Very toxic to aquatic life) with acute M factor = 10.

Concern for developmental toxicity was raised due to the observation of malformations in a rat study with mancozeb (Anonymous, 1980). However, these effects only occurred at a dose causing severe maternal toxicity (death, paralysis, suffering, total litter loss). In 1993 it was concluded that the malformations were attributed to the formation of ETU, but as ETU levels produced by the parent compound would not reach the threshold for teratogenic effects, classification was not justified.

The classification was considered again between 2003-2006 by the Technical Committee on Classification and Labelling (TC C&L). It was agreed that classification for carcinogenicity was not warranted but there was no consensus regarding the classification for developmental toxicity and it was referred to the SE group.

The conclusion of the SE group form February 2005 is reproduced below.

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"Mancozeb[1] has been shown to cause a high frequency of a specific pattern of major malformations in the rat at dose levels that cause marked maternal toxicity. There is sufficient evidence from metabolic studies with these compounds and from developmental toxicity studies with ETU to assume that the malformations induced by mancozeb are directly caused by the metabolite ETU. Mancozeb causes a similar spectrum of malformations to ETU, which is classified as a Cat.2 (DSD)[2] developmental toxicant. In addition, ETU does not cause maternal toxicity at developmentally toxic dose levels. Therefore the developmental toxicity of mancozeb is not a secondary nonspecific consequence of maternal toxicity. **As a consequence, the SE agreed unanimously that classification of mancozeb for developmental toxicity was warranted.**

The above conclusion in itself points to a Cat.2 (DSD) classification for developmental toxicity. However, there was no consensus among the SE about Cat.2 versus Cat.3 classification. Additional arguments were put forward considering either a Cat.2 or a Cat.3 classification.

The developmental effects were observed only at dose levels with severe maternal toxicity, which suggests that mancozeb may be of less concern than substances that show developmental toxicity in the presence of no or slight maternal toxicity.

There is uncertainty about the dose-response relationship for maternal toxicity and developmental toxicity between 100 and 500 mg/kg/day in rat developmental studies. It is not therefore known if maternal or developmental toxicity is the most sensitive effect.

The critical short- and long term general toxicological target of Mancozeb relates to inhibition of thyroid hormone synthesis. The effect is mediated by the inhibition of thyroid peroxidase by ETU. Thyroid hormone is crucial for brain development in mammals. Recent studies have suggested that transient impairment of maternal thyroid hormone levels in the rat and in man may affect neural brain organization and behaviour. Therefore there is a concern that Mancozeb and other EBDC may cause developmental neurotoxicity, which would argue for a Cat.2 classification.

There is a species difference in kinetics and metabolism of Mancozeb including the formation of ETU as shown between rats and mice, which may partially explain the higher susceptibility of rats to ETU developmental toxicity. There is no information on the comparative kinetics and metabolism in man.

In conclusion, there was consensus among the SE about the need for classification of Mancozeb for developmental toxicity. There was an equal distribution of opinions among the SE present towards classification into Repr. Cat. 2, R 61 or Repr. Cat. 3, R 63 for both substances.

The SE had the following additional remarks:

It is advisable to study the developmental neurotoxicity (e.g. according to OECD 426) of EDBC's to address the concern about thyroid effects and brain development.

The severe maternal toxicity at the developmentally toxic doses sheds doubt about the acceptability of these studies especially with regard to the ethical aspects. However, the developmental toxicity studies with Mancozeb provided clear evidence of the mechanistic link to ETU and were therefore an important contribution to the assessment of classification of Mancozeb."

[1.] Note that mancozeb and maneb were discussed together in the meeting. The references to maneb have been removed.
[2.] Classification in accordance with DSD (Dir 67/548/EEC). Category 2 DSD = Category 1B (CLP). Category 3 (DSD) = Category 2 (CLP).

As noted in the above conclusion, the opinion of the SE group was that classification of mancozeb for developmental toxicity was warranted, but there was no consensus whether this should be Repr. Cat. 2; R61 or Repr Cat 3; R63 (equivalent to Repr. 1B; H360 and Repr 2; H361 respectively under CLP). At the TC C&L in Arona (21st-24th March 2006) it was agreed to classify mancozeb with Repr. Cat. 3; R63. This was published in the 31st ATP to DSD (2009) and the classification translated to Repr. 2; H361d - Suspected of damaging the unborn child, under CLP.

RAC general comment

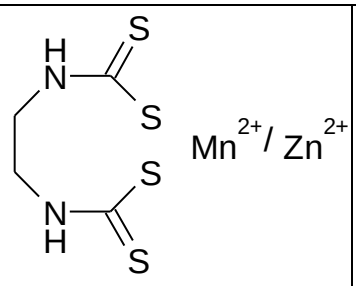
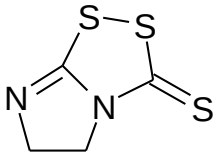
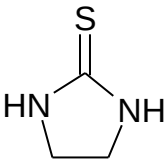
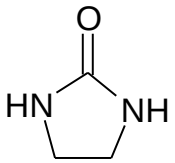
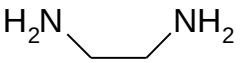
Mancozeb is an active substance used in plant protection products approved in the EU as a fungicide. It belongs to the ethylene bis(dithiocarbamate) (EBDC) family of pesticides. Structurally, it is a polymeric coordination complex of zinc and manganese ethylene bis(dithiocarbamate) containing ca. 20% manganese and 2.5% zinc. Mancozeb has an existing entry in Regulation 1272/2008/EC (the CLP Regulation) as Repr. 2 (H361d***), Skin Sens. 1 and Aquatic Acute 1 with an M factor of 10. The present classification proposal has been submitted in parallel with the Draft (Renewal) Assessment Report (RAR, 2017).

The substance as manufactured contains additives and production-related impurities. Typical purity of the currently produced material is 86–93%. The purity of mancozeb tested in the available toxicology studies ranged between 80 and 92%. The analysed batches of currently produced mancozeb did not contain more than 0.09% ethylene thiourea (ETU).

Mancozeb is of low solubility in water and most organic solvents. In contact with water mancozeb undergoes a relatively rapid abiotic hydrolysis with a half-life in the order of hours, giving rise to the degradation products ethylenethiourea (ETU), ethyleneurea (EU) and ethylenebisisothiocyanate sulfide (EBIS).

The *in vivo* metabolism of mancozeb in mammals is relatively complex. The main metabolite in rats and mice is ETU; further metabolites include EU, EDA (ethylene diamine), N-acetyl EDA, and EBIS. ETU is also formed in humans. The structural formulas of mancozeb and its main metabolites are shown in the table below.

Structure of mancozeb and its main metabolites and degradation products

mancozeb	EBIS	ETU	EU	EDA
				

ETU is an industrial chemical registered under Regulation 1907/2006/EC (the REACH Regulation). It has an existing entry in Annex VI of CLP as Repr. 1B (H360D) and Acute Tox. 4* (H302).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Reason for a need for action at Community level:

Change in existing entry due to new data

Change in existing entry due to new interpretation/evaluation of existing data

Mancozeb is a pesticidal active substance in the scope of Directive 91/414/EEC (and now Regulation 1107/2009). Supplementary dossiers, supporting the application for renewal under (EU) 844/2012, are under consideration by the UK. New data are available; including two developmental neurotoxicity (DNT) studies with mancozeb (to address the concerns raised by the SE group in the 2005 conclusion – see above) and several studies to investigate the potential of mancozeb to cause developmental toxicity, the role of metabolism to ETU and species differences in metabolism of ETU when considering human relevance.

Whilst mancozeb already has a harmonised classification, these new data support a review and revision of the current entry.

It is noted that this proposal is targeted and only addresses those hazard classes and differentiations for which there is new data or potential for a new interpretation of existing data.

5 IDENTIFIED USES

Mancozeb is used as a fungicide within the EU.

6 DATA SOURCES

The data sources used to complete this CLH report are the regulatory reports and published literature used in the original and supplementary dossiers under Council Directive 91/414/EEC (Commission Directive 2005/72/EC) and Regulation (EC) 1107/2009. Supplementary dossiers, recently submitted in support of the application for renewal under Commission Implementing Regulation (EU) 844/2012 of 18 September 2012 are under review by the UK.

At the time of submission, mancozeb is not registered under REACH.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Yellow powder (Mancozeb technical is yellow powder with musty odour)	Bos MWS, 2014 Study DL 14-006 Wasser, 1995b	Visual Purity; 92.3% Mancozeb technical; 80%
Melting/freezing point	189 °C	Bos MWS, 2014 Study DL 14-006	EC A2 (DSC) Purity; 92.3%
Boiling point	Mancozeb decomposes at 210 °C before boiling	Bos MWS, 2014 Study DL 14-006	EC A2 (DSC) Purity; 92.3%
Relative density	2.11	Bos MWS, 2014 Study DL 14-006	CIPAC MT 3.2.1
Vapour pressure	< 5.6 x 10^{-5} Pa at 25 °C	Diepenhorst PC, 1994 Study DL 93-029	EC A.4 OECD 104 (estimation, modified Watson correlation) Purity; 89%
Surface tension	Not required - water solubility < 1mg/L		
Water solubility	At 20 °C 0.2 mg/L (pH 4-5) 0.2mg/L (pH 6-8) 0.3 mg/L (pH9-10)	Bos MWS, 2014 Study DL 14-006	EC A6 Purity; 92.3%
Partition coefficient n-octanol/water	At 20 – 25 °C Log P_{ow} = 2.3 (pH 6 – 8) Log P_{ow} = 2.3 (pH 9 - 10) Log P_{ow} = ∞ (pH 4 - 5)	Bos MWS, 2014 Study DL 14-006	OECD 107 (shake flask method) Purity; 92.3%
Flash point	Not applicable, substance is a solid with melting point of 189 °C	-	-
Flammability	Not highly flammable in the sense of EC A.10	Maarsingh P, 2005 Study DL 04-092	EC A10 Purity; 91%
Explosive properties	Mancozeb does not contain structural groups indicative of explosive properties.		

Property	Value	Reference	Comment (e.g. measured or estimated)
Self-ignition temperature	157 °C	Maarsingh P, 2005 Study DL 04-092	EC A16 Purity; 91%
Oxidising properties	No oxidising properties	Maarsingh P, 2005 Study DL 04-092	EC A17 Purity; 91%
Granulometry	No data provided		
Stability in organic solvents and identity of relevant degradation products	Stable in organic solvents		
Dissociation constant	$K = 8.2 \times 10^{-13}$ (theoretical determination from water solubility) Mancozeb has no affinity to water and the solubility is low. It will not dissociate in water. This has been confirmed using conductometric method.	Bos MWS, 2014 Study DL 14-006	OECD 112
Viscosity	Substance is a solid.		

8 EVALUATION OF PHYSICAL HAZARDS

Not considered further in this proposal.

9 TOXICOKINETICS (ABSORPTION, DISTRIBUTION, METABOLISM AND ELIMINATION)

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

The absorption, distribution, metabolism and excretion (ADME) properties of mancozeb in mammals (rats, mice and monkeys) were evaluated previously (DAR – Draft Assessment Report, 2000) under Directive 91/414/EEC in studies (both regulatory and from the open literature) that were considered to be acceptable. The only new information available is a comparative interspecies *in vitro* metabolism study (Foster, 2015), a new data requirement under Regulation 1107/2009.

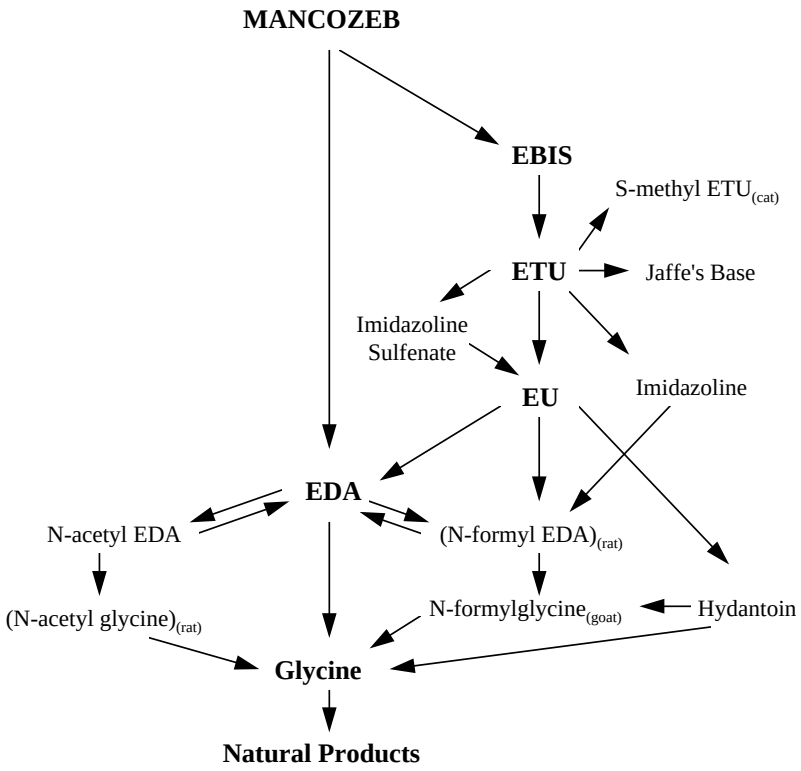
In studies in laboratory animals, mancozeb is only partially absorbed but rapidly excreted. Rats given single oral doses of ^{14}C-labelled mancozeb absorbed about 50% of the dose (calculated by adding the radioactivity present in urine, bile and tissues). Most of the dose was excreted within 24 hours with about half eliminated in the urine and half in the faeces. Less than 4% was found in the tissues after 96 hours, with the thyroid containing the highest residual levels. Most of the ^{14}C dose in faeces was unabsorbed, since only 2-8% of the dose was found in bile.

Ethylenethiourea (ETU) was the major metabolite. The other metabolites identified were ethyleneurea (EU), N-acetylethylenediamine (N-acetyl-EDA), ethylenediamine (EDA), ethylenebisisothiocyanatesulfide (EBIS) and N-formylethylenediamine (N-formyl-EDA), N-acetylglycine, and glycine. The bioconversion of mancozeb to ETU in the rat was 6.8% on a weight basis and ~20% on a mole/mole basis; the average bioconversion factor for all the EBDCs was 7.5%. The bioconversion of mancozeb to ETU was determined in the study of Anonymous, 1986g ("Metabolism of 14C-mancozeb in the rat)". This is reported in the RAR, Vol3_ CA_B6 (2017). In this study the conversion was calculated based on the recovery of ETU from urinary and bile samples (18.2% of administered dose) following an oral dose of mancozeb. The bioconversion of mancozeb to ETU on weight percentage basis was 6.8% (average 18.2% of the administered 14C mancozeb dose recovered as ETU in urine and bile x 102 g ETU/mole/271 g mancozeb/mole = 6.8%).

This was compared with the conversion factor for other EBDCs where an overall EBDC bioconversion factor of approximately 7.5% in rats was determined (Kocialski, 1989 & 1994; in the JMPR 1993 evaluation of mancozeb). ETU is further broken down to moieties that are incorporated into natural compounds such as oxalic acid, glycine, urea and lactose. Metabolism of ETU in humans, dog, rabbit and mouse appears more efficient than in rats. In monkeys, oral doses of mancozeb were very poorly absorbed, with faeces being the predominant route of excretion. Exposure during field application of mancozeb revealed an elimination half time of ETU in the urine of about 100 hours in some workers indicating slow dermal absorbtion (JMPR 1993 in the RAR, 2017).

Elimination half times of ETU in humans have been estimated to be 19-23 hours (Aprea et al, 1996, Lund et al 2008 in the RAR, 2017), while in the rat most of the ETU is excreted within 24 hours (RAR, 2017).

The spectrum of metabolites produced was similar in laboratory and farm animals, pointing to two common metabolic pathways which both lead ultimately to the formation of glycine and incorporation of the metabolites into natural products. In the quantitatively predominant pathway, the dithiocarbamate linkages in mancozeb are hydrolysed to produce EDA directly, and EDA is oxidised to glycine, joining the intermediary metabolic pool at this point. The other involves oxidation to EBIS and then to ETU, various derivatives of ETU, and EU before re-joining the main pathway with conversion to EDA, glycine, and incorporation into other products.



10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity

Not addressed in this dossier.

10.2 Skin corrosion/irritation

Not addressed in this dossier.

10.3 Serious eye damage/eye irritation

Not addressed in this dossier.

10.4 Respiratory sensitisation

Not addressed in this dossier.

10.5 Skin sensitisation

The skin sensitisation potential of mancozeb has been investigated in three Buehler tests and three guinea-pig maximisation tests.

Table 8: Summary table of animal studies on skin sensitisation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th></tr>
<tr><td>Modified Buehler method
OECD 406 (1992)
GLP
Anonymous, 1988a</td><td>Guinea pigs, Hartley, males,
20 in test groups & 10/ group in negative and positive control groups.</td><td><u>Induction:</u> 0.4 mL 50.0% w/v in distilled water, three 6 hour topical applications (1/week for 3 weeks)
<u>Challenge:</u> 0.4 mL 50% w/v in distilled water, topical
Dithane® M-45
Purity 80% minimum</td><td>Negative
<table><tr><th></th><th>24h</th><th>48h</th><th>Positive reaction</th></tr><tr><td>Test</td><td>1/20</td><td>1/20</td><td>5%</td></tr></table>
All positive responses consisted of grade 1 erythema</td></tr>
<tr><td>Buehler method
OECD 406 (1992)
GLP
Anonymous, 1986a</td><td>Guinea pigs, Dunkin-Hartley, females, 20</td><td><u>Induction:</u> 0.5 mL, 50% w/w in distilled water, 9 topical applications (3/week for 3 weeks)
<u>Challenge:</u> 0.5 mL 50% w/w in distilled water, topical
Mancozeb technical
Purity 83.4%</td><td>Positive
2/10 (20%) animals positive response</td></tr>
<tr><td>Buehler method
OECD 406 (1992)
GLP
Anonymous, 2007a</td><td>Guinea pigs, Hartley, males, 20</td><td><u>Induction:</u> 0.5g in 0.7ml corn oil, 3 topical applications (1/week for 3 weeks)
<u>Challenge:</u> 0.5g in 0.7ml corn oil, topical</td><td>Negative
No skin reactions observed following challenge in test group or negative controls.</td></tr>
<tr><td>Guinea pig maximisation test (GPMT)
OECD 406 (1992)
GLP
Anonymous, 1994</td><td>Guinea pigs, Dunkin-Hartley, males, 20 in test groups & 10 in control groups</td><td><u>Intradermal induction:</u> 0.1ml Freund’s complete adjuvant (FCA) 50/50 in physiological saline, 0.1ml test substance 50% with distilled water, & 0.1ml test substance 50% with 50/50 FCA and distilled water (pairs).
<u>Topical induction</u>: irritation induced with 0.5 mL of 10 % sodium lauryl sulphate (days 5-7) then 0.2 ml of test substance 50% with distilled water for 48 hours (days 6-8).
<u>Challenge:</u> 0.1 mL 50% test substance for 24 hours, topical
Mancozeb technical
Purity not reported</td><td>Positive
7/20 (35%) test animals</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th></tr>
<tr><td>Guinea pig maximisation test (GPMT)
OECD 406 (1992)
GLP
Anonymous, 1997a</td><td>Guinea pigs, Hartley, males and females, 20 in test groups & 10 in control groups.</td><td><u>Intradermal Induction:</u> 0.1mL Fruend’s complete adjuvant (FCA) 50/50 in distilled water, 0.1ml 14.28% test substance in distilled water & 14.28% test substance in 50/50 FCA and distilled water (pairs).
<u>Topical Induction:</u> irritation induced with 0.5ml of 10% sodium laurel sulphate in Vaseline (day 6) then 200 mg of test substance moistened with distilled water for 48 hours.
<u>Challenge:</u> 200 mg of test substance moistened with distilled water for 24 hours, topical.
Mancozeb technical
Purity 85% minimum</td><td>Negative<table><tr><th></th><th>24h</th><th>48h</th><th>Positive response</th></tr><tr><td>Control</td><td>0/10</td><td>0/10</td><td>0%</td></tr><tr><td>Test</td><td>3/20</td><td>3/20</td><td>15%</td></tr></table>All positive responses consisted of grade 1 erythema.</td></tr>
<tr><td>Guinea pig maximisation test (GPMT)
Guideline not stated
GLP not stated
Matshushita et al 1976</td><td>Guinea pigs, Hartley, female, 10/group</td><td><u>Intradermal induction:</u> 5% mancozeb
<u>Topical induction:</u> 25% mancozeb
<u>Challenge</u>: 2% or 0.5% mancozeb
Topical</td><td>Positive
100% of mancozeb treated animals responded positively after 24 and 48 hours.</td></tr>
</table>

Table 9: Summary table of human data on skin sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations
Worker exposure study (EUROPIT) Swaen *et al* 2008	EBDC pesticides	Overall there were 248 EBDC exposed and 231 non-occupationally exposed subjects in four countries (the Netherlands, Finland, Italy and Bulgaria) who were examined to evaluate the possible association between occupational exposure to EBDCs and allergy. A self-administered questionnaire was used to collect information on skin irritation, contact dermatitis, allergic rhinitis and food allergy.	No association was found between exposure status, EBDC levels and allergic contact dermatitis, allergic rhinitis, food allergy, and atopy.
Human studies of manufacturing workers have detected sporadic reports of contact allergic hypersensitivity.			

10.5.1 Short summary and overall relevance of the provided information on skin sensitisation

The original DAR (2000) under Directive 91/414 describes two Buehler tests (Anonymous, 1988; Anonymous, 1986) conducted in guinea-pigs and three guinea-pig maximisation studies (Matshushita et al., 1976; Anonymous, 1994; Anonymous, 1997). The results of the majority of these studies (Anonymous., 1986; Matshushita et al., 1976; Anonymous, 1994c) show that mancozeb is a skin sensitiser. Since then, another skin sensitisation study (Anonymous, 2007) has become available. This study conformed to GLP and OECD test guideline 406. Mancozeb (88.6% pure) was tested in male guinea pigs according to the Buehler method. Mancozeb was negative in this study. However, it is noted that the Buehler method is less sensitive than the M+K method. Overall, therefore, the results of this study are not inconsistent with the studies in the original DAR (2000) under Directive 91/414.

In the Buehler assay by Anonymous (1988), grade 1 erythema was noted in 1/20 mancozeb-treated animals 48 hours after challenge, and in 1/20 mancozeb-treated animals 24 hours after re-challenge. However, this was not the same animal that exhibited erythema during the challenge phase. Appropriate responses were seen with positive and sham controls. In this study mancozeb did not induce a positive response.

In the second Buehler study (Anonymous, 1986) 2/10 animals had slight to well-defined erythema with or without slight or well-defined oedema at 24 and 48 hours following challenge with 50% mancozeb. Similar reactions were not seen in negative control animals; therefore it was concluded that under the test conditions mancozeb is a skin sensitiser.

Guinea pig maximisation studies were conducted by Anonymous (1994) and Anonymous (1997a). In the study by Anonymous (1994) none of the control animals reacted positively to the dermal challenge. However, seven animals of the test group showed discrete to patchy erythema during the topical challenge, after both 24 and 48 hours. As 35% (7/20) of animals gave a positive response in this adjuvant test it is concluded that a skin sensitising potential was demonstrated under the conditions of this study.

In the second guinea pig maximisation test conducted by Anonymous (1997a) 3/20 animals exhibited grade 1 erythema at both 24 and 48 hours following challenge. As a 15% positive response was observed in this second adjuvant test it was concluded that mancozeb was not a skin sensitiser under the conditions of this study.

10.5.2 Comparison with the CLP criteria

On the basis of the positive results obtained in 3 out of the 6 available studies, the current classification of mancozeb for skin sensitisation in category 1 is confirmed. This endpoint has however been addressed in this targeted proposal to consider whether sub-categorisation in category 1A or 1B in accordance with the provisions of the 2nd ATP to the CLP Regulation might be appropriate.

The sub-categorisation of skin sensitisers on the basis of the guinea pig maximisation test according to the provisions of the 2nd ATP to the CLP Regulation is shown below.

Table 10: Potency on basis of the guinea pig maximisation test

Concentration for intradermal induction (% w/v)	Incidence sensitised guinea pigs (%)	Potency	Predicted subcategory
≤ 0.1	≥ 60	Extreme	1A
≤ 0.1	≥ 30 - < 60	Strong	1A
>0.1 - ≤ 1.0	≥ 60	Strong	1A
>0.1 - ≤ 1.0	≥ 30 - < 60	Moderate	1B
> 1.0	≥ 30	Moderate	1B

Three studies using this method are available. Of these, two were positive (Matshushita et al., 1976; Anonymous, 1994). In the study by Matshushita et al. (1976), a 5% intradermal induction gave a positive response in 100% of the animals. In the study by Anonymous (1994) a 50% intradermal injection gave a positive response in 7/20 test animals (35%). Both studies indicate that mancozeb should be classified for skin sensitisation in at least sub-category 1B. However since a concentration below 1% was not tested then the exclusion of sub-category 1A based on the results of the guinea pig maximisation tests is not possible.

The sub-categorisation of skin sensitisers on the basis of the Buehler assay according to the provisions of the 2nd ATP to the CLP Regulation is shown below.

Table 11: Potency based on the Buehler assay

Concentration for intradermal induction (% w/v)	Incidence sensitised guinea pigs (%)	Potency	Predicted subcategory
≤ 0.2	≥ 60	Extreme	1A
≤ 0.2	≥ 15 - < 60	Strong	1A
>0.2 - ≤ 20	≥ 60	Strong	1A
>0.2 - ≤ 20	≥ 15 - < 60	Moderate	1B
> 20	≥ 15	Moderate	1B

Three studies using this method are available. Of these, one was positive (Anonymous, 1986) as 20% of test animals responded at an induction dose of 50%. On this basis, classification in category 1B is warranted. However as a concentration below 20% was not tested it was not possible to exclude sub-categorisation into category 1A on the basis of the Buehler assay.

In situations where it is not possible to exclude category 1A for skin sensitisation the guidance on the application of the CLP criteria recommends that the default position of category 1 (i.e. without sub-categorisation) should be adopted. Therefore based upon the GPMT that gave a positive response in 35% of animals at a 50% induction dose and the Buehler assay that gave a positive response in 20% of animals at a 50% challenge concentration it is proposed that mancozeb be classified in category 1 for skin sensitisation. This is supported by some negative studies, which would seem to indicate that mancozeb is not a strong sensitiser.

Although human studies of workers have indicated no evidence of skin sensitisation (Swaen *et al*, 2008), sporadic reports of contact allergic hypersensitivity have occurred in manufacturing workers. This further supports the current classification of mancozeb with Skin Sens 1.

10.5.3 Conclusion on classification and labelling for skin sensitisation

Skin Sens 1; H317 – May cause an allergic skin reaction

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Mancozeb is currently classified as Skin Sens. 1. Upon re-examination of the data, the dossier submitter (DS) concluded that classification for skin sensitisation is warranted on the basis of positive results in 3 out of the 6 available animal studies (GPMTs and Buehler assays) with supporting evidence from human data.

The DS also examined the possibility of sub-categorisation in category 1A or 1B. Although the majority of the animal studies did not indicate a strong potency, the DS concluded that category 1A could not be excluded because concentrations below 1% and 20% were not tested in the GPMT and the Buehler tests, respectively. Consequently, the DS proposed to retain the current classification with Skin Sens. 1 without sub-categorisation.

Comments received during public consultation

Four Member States Competent Authorities (MSCAs) supported the DS's proposal to retain the existing classification as Skin Sens. 1.

Assessment and comparison with the classification criteria

Information on the available animal studies on skin sensitisation as provided in the CLH report and RAR is summarised in the following table.

Skin sensitization studies		
Type of study; Reference	**Method**	**Observations**
Buehler test Anon. 1988a	OECD 406 GLP No. of animals: 20 treated, 10 negative controls, 10 positive controls Induction: 50% w/v in water Challenge: 50% w/v in water	**Negative** 1/20 treated animals showed positive response at 24 h and 1/20 treated animals (not the same one) at 48 h after challenge Appropriate responses were seen with positive and negative controls

Buehler test Anon. 1986a	OECD 406 GLP No. of animals: 10 treated, 10 controls Induction: 50% w/w in water Challenge: 50% w/w in water Deviation: only 10 animals in the treated group	**Positive** 2/10 treated animals showed positive response No positive response in controls
Buehler test Anon. 2007a	OECD 406 GLP No. of animals: 20 Induction: ca. 44% w/w in corn oil Challenge: ca. 44% w/w in corn oil	**Negative** No skin reactions following challenge in the treated group or negative controls
GPMT Anon. 1994a	OECD 406 GLP No. of animals: 20 treated, 10 controls Intradermal induction: 50% in water Topical induction: 50% in water; irritation induced by pre-treatment with SLS Challenge: 50% in water	**Positive** 7/20 animals showed positive response at both 24 h and 48 h after challenge No positive response in controls
GPMT Anon. 1997a	OECD 406 GLP No. of animals: 20 treated, 10 controls Intradermal induction: 14% in water Topical induction: substance moistened with water; irritation induced by pre-treatment with SLS Challenge: substance moistened with water	**Negative** 3/20 animals showed positive response at 24 h and 3/20 animals showed positive response at 48 h after challenge No positive response in controls
GPMT Matshushita *et al.* 1976	Guideline not stated GLP not stated No. of animals: 10 treated Intradermal induction: 5% in water Topical induction: 25% in water Challenge: 2% or 0.5% in water	**Positive** Positive response in all treated animals at 24 h and 48 h after treatment

The cut-off values to consider an assay positive are 15% and 30% for the Buehler test and GPMT, respectively. One Buehler assay was positive (Anon., 1986a) with 2/10 treated animals showing skin reaction, whereas the other two Buehler assays were negative. The two GLP- and guideline-compliant GPMTs, Anon. (1994a) and Anon. (1997a), showed a positive (35%) and a negative (15%) response respectively.

The published study by Matshushita *et al.* (1976) is given less weight by RAC as it is not confirmed to be guideline-compliant and its result (positive response in 100% treated animals) markedly differs from those of the rest of the regulatory studies.

Swaen *et al.* (2008) found no association between occupational exposure to EBDC pesticides and allergic contact dermatitis, allergic rhinitis, food allergy, and atopy. In the CLH report it was stated that "human studies of manufacturing workers have detected sporadic reports of contact allergic hypersensitivity", however, no reference to the source of this information was included. Thus, this statement cannot be used to support classification.

RAC notes that other EBDC fungicides (e.g. maneb, zineb, nabam) are classified with Skin Sens. 1 in Annex VI of CLP.

Overall, the animal data on mancozeb indicate a weak sensitising potential, with at least one fully guideline-compliant study (Anon., 1994a) being clearly positive. Although the majority of the data does not indicate strong potency, Category 1A cannot be excluded as concentrations below 1% and 20% were not tested in the GPMT or the Buehler test, respectively. Therefore, RAC agrees with the dossier submitter's proposal to retain the existing classification of mancozeb as **Skin Sens. 1 without subcategorisation**.

10.6 Germ cell mutagenicity

The genotoxic potential of mancozeb has been investigated in a series of *in vitro* and *in vivo studies*. The original DAR (2000) under Directive 91/414 provided the following preliminary statement regarding the use of polar solvents as vehicles in genotoxicity studies of mancozeb:

"A critical issue in the evaluation of genotoxicity studies of mancozeb is the effect of using DMSO and other highly polar, reactive solvents as vehicles. While DMSO is commonly used in genetic toxicity testing, the use of DMSO invalidates genotoxicity tests of mancozeb because it is rapidly degraded in this medium, with concomitant liberation of metal ions which are known to affect DNA maintenance and replication *in vitro*. The half-life for ^{14}C-mancozeb in DMSO is only 36 minutes (Schweitser, 1990) in comparison to 5.8 to 55 hours in aqueous media (Lawrence, 1979; 2.5.1/02). As examples of the types of genotoxic effects that can result from prematurely rapid release of metal ions into cell culture, Thompson *et al* (1989) showed that inorganic zinc salts can cause mutations in mouse lymphoma L5178Y cells and chromosomal aberrations in CHO cells. Similarly, Zakour *et al*. (1981) have shown that manganese (II) ions are among the metal ions which decrease the fidelity of DNA synthesis *in vitro*, and the U.S. National Toxicology Program has recently reported both sister chromatid exchanges (SCEs) and chromosomal aberrations with manganese (II) sulfate in CHO cells (NTP, 1992). It is recognized that such effects will not occur *in vivo* because absorption and metabolism of these essential nutrients is tightly regulated in mammalian organisms. Consequently, studies in which DMSO and other polar, reactive solvents have been used as a vehicle must be regarded as scientifically invalid as descriptions of the genotoxic potential of mancozeb."

New studies have been submitted. These include an *in vitro* micronucleus study and an *in vitro* photogenotoxicity test as a modified reverse mutation test in bacteria. In addition, an Ames test which used DMSO as a solvent was recently submitted; this was considered invalid and so has not been evaluated.

10.6.1 *In vitro*

The potential of mancozeb to induce gene mutations in bacterial cells, gene mutation/clastogenicity in mammalian cells and clastogenicity/aneuploidy in mammalian cells has been investigated in a series of *in vitro* studies. In order to satisfy new data requirements under Regulation 1107/2009, a photogenotoxicity test as a modified reverse mutation test in bacteria has also become available. In addition, an *in vitro* micronucleus study has been conducted to strengthen the robustness of the *in vitro* dataset.

Table 12: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test system (Organisms, strain)	Test substance	Concentrations tested	Result		Remarks and information on cytotoxicity
				-S9	+S9	
1.Gene mutation tests **1.A. Bacterial gene mutation tests**						
Reverse mutation in bacteria (AMES) OECD 471 (1997) GLP Chism 1984	*Salmonella typhimurium* TA 1535, TA 1537, TA 98 and TA 100	Mancozeb 88% Vehicle: Distilled water	2.5, 7.5, 25, 75 and 250 µg / plate +/- metabolic activation	neg	neg	Toxicity at 75µg/plate and 250µg/plate
Reverse mutation in bacteria (AMES) OECD 471 (1997) GLP	*Salmonella typhimurium* TA 98 and TA 100	Mancozeb 89.8% Vehicle: Not reported	65.2 to 8000 mg/L +/- metabolic activation	neg	neg	Toxicity not reported

Method, guideline, deviations if any	Test system (Organisms, strain)	Test substance	Concentrations tested	Result		Remarks and information on cytotoxicity
				-S9	+S9	
Slabbert 1994						
Reverse mutation in bacteria (AMES) OECD 471 (1997) GLP Wilmer 1982	*Salmonella typhimurium* TA 1535, TA 1537, TA 1538, TA 98 TA 100	Mancozeb 87.3% Vehicle: Not reported	0.62, 1.85, 5.56, 16.67 and 50 µg/plate. +/- metabolic activation	neg	neg	Toxicity not reported
Reverse mutation in bacteria (AMES) OECD 471 (1997) GLP Prabhu 1999	*Salmonella typhimurium* TA 1535, TA 1537, TA 98, TA 100, TA 102	Mancozeb technical purity not reported Vehicle: Not reported	1.95 to 31.25 µg/plate (-S9) & 15.62 to 250µg/plate (+S9)	neg	neg	Toxicity at 312.5 to 5000 µg/plate (+S9) & 156.25 to 5000µg/plate (-S9)
Host mediated assay in mice Derived from OECD 471 GLP McCarroll 1985	*S.typhimurium* TA 1530 (2ml intraperitoneal injection) to mice,B6C3F1, male, 10/group killed 2 or 4 hours after inoculation	Mancozeb 88% Vehicle: corn oil	Orally treated with 0 (control), 0.5, 2.0 or 5.0 g/kg bw	NA	neg	Host provides metabolic activation
1.B *In vitro* mammalian gene mutation assays						
Gene mutation assay in mammalian cells OECD 476 GLP Foxall & Byers 1984	Chinese Hamster Ovary cells	Mancozeb 88% Vehicle: distilled water	0.25 to 45 µg/ml +/- metabolic activation	neg	neg	Adequate cytotoxicity levels were achieved Positive controls included
Mouse lymphoma mutation assay EEC guideline compliant GLP Riach 1996	Mouse lymphoma L5178Y	Mancozeb purity not reported Vehicle: not reported	0.005, 0.1, 0.2, 0.4, 0.8, 1.6 & 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 µg/ml (+S9) 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75 & 0.9, 1.2, 1.5, 1.8, 2.1, 2.4, 2.7 µg/ml (-S9)	Equivocal +/- S9		Equivocal Positive controls included
2. Chromosomal damage and aneugenicity						
Chromosomal aberration assay	Chinese hamster ovary cells, CHO	Mancozeb purity 89.1	Test 1: 1.6,3,6 µg/ml (+S9) &	Pos +/- S9 but invalid because of		Toxicity at 6µg/ml (+S9) & 5µg/ml (-S9) clastogenic in CHO

Method, guideline, deviations if any	Test system (Organisms, strain)	Test substance	Concentrations tested	Result		Remarks and information on cytotoxicity
				-S9	+S9	
EEC guidelines GLP Innes 1995		Vehicle: DMSO	1.25,2.5,5 µg/ml (-S9)	use of DMSO		cell at 6-8µg/ml (+S9) & 5µg/ml (-S9)
Micronucleus test OECD 487 (2016) GLP Gilby, 2017	Human lymphocytes	Mancozeb Purity: 86.1% Vehicle: ethanol	2-10 µg/ml (± S9)	Negative		No precipitation and cytoxicity up to limit concentration of 10 µg/ml (± S9, 3 hr) No precipitation but cytoxicity at top concentration of 10 µg/ml (- S9, 20 hr)
3. DNA damage **3.A Unscheduled DNA synthesis in mammalian cells**						
DNA damage and repair, unscheduled DNA synthesis in mammalian cells OECD 482 GLP O'Neil & Frank 1988	Rat F344 hepatocytes, male, adult	Mancozeb Purity 82.4% Vehicle: not reported	Concentrations of mancozeb from 0.1 to 10 µg/mL scored	neg	neg	Toxicity at 2 to 10µg/ml Precipitation at 100 to 1000µg/ml Positive control included
3.B Sister chromatid exchange in mammalian cells						
Sister chromatid exchange assay OECD 479 GLP Ivett 1985	Chinese hamster ovary cells	Mancozeb purity 88% Vehicle: Serum free culture medium	1 to 20 µg / ml	pos	neg	Toxicity at 15µg/ml (-S9) & 12.5-20µg/ml (+S9) Positive controls included
4. Photogenotoxicity						
Reverse mutation in bacteria with and without UV irradiation OECD 471 (1997) & 432 (2004) GLP Schreib 2014	*Salmonella typhimurium* TA 98, TA 1537, TA 100, TA 1535 and TA 102	Mancozeb technical Purity 86% Vehicle: not reported	0.050 to 50 µg/plate +/- metabolic activation & UV irradiation	neg	neg	Toxicity at 3.16µg/plate & 5.0µg/plate Precipitation at 25µg/plate & 50µg/plate Not photogenotoxic

The *in vitro* genotoxic potential of mancozeb has been investigated in four bacterial gene mutation assays. All of these were reliable and well conducted guideline compliant tests. There was no indication of a genotoxic response to mancozeb in any of these assays, either with or without metabolic activation. Adequate levels of cytotoxicity were reported in all but one of these tests, indicating that required concentrations had been

reached. These negative results were supported by a well conducted host mediated bacterial gene mutation test in mice (McCarrol 1985), in which mancozeb did not demonstrate a mutagenic response.

In a mammalian cell gene mutation assay (Foxhall and Byers 1984), mancozeb was not mutagenic at the HGPRT locus of Chinese hamster ovary cells up to cytotoxic concentrations (ranging from 0.25 to 45 µg/ml) either with and without metabolic activation. However, an equivocal response was observed *in vitro* in a mouse lymphoma mutation assay (Riach 1996). In subsequent ancillary studies of DNA damage in mammalian cells (in vitro UDS and SCE) mancozeb tested negative.

One unscheduled DNA synthesis assay was available (O'Neil & Frank 1998) in which concentrations of mancozeb from 0.1 to 10 µg / ml were scored. No increase in UDS or S-phase was detected at any of the test article concentration evaluated; therefore mancozeb did not induce UDS in rat hepatocytes up to concentrations causing precipitation.

In all of these assays, adequate levels of cytotoxicity were reported and the inclusion of positive controls ensured the validity of the results.

A weakly positive response was observed in a sister chromatid exchange assay in mammalian cells (Ivett 1985), which may be explained by the interference of manganese and/or zinc with DNA repair and replication enzymes. In addition a positive chromosomal damage assay result (Innes 1995) was considered invalid because of the use of DMSO as a solvent.

In a recently conducted guideline *in vitro* micronucleus test in human lymphocytes, mancozeb tested negative for clastogenicity and aneugenicity up to the limit concentration (10 µg/ml) for this assay with and without metabolic activation (Gilby, 2017).

In a recently conducted *in vitro* photogenotoxicity study in Salmonella typhimurium (Schreib, 2014), no biologically relevant increases in revertant colony numbers of any of the five tester strains were observed following treatment with mancozeb at any concentration level, neither with nor without concomitant UV irradiation. Overall, under the experimental conditions of this photogenotoxicity test, mancozeb did not cause gene mutations with or without UV irridiation. Therefore, mancozeb is considered to be non-mutagenic in this bacterial reverse mutation assay for evaluation of photogenotoxicity.

11.6.2 *In vivo*

The potential of mancozeb to induce chromosomal damage in somatic cells has been investigated in four micronucleus tests in rats and mice and two cytogenetics studies in rats and mice.

Table 13: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Species, strain, sex, No/group	Test substance	Concentrations tested	Result
In vivo cytogenetic study in rats. OECD 475 GLP Anonymous, 1984	Rats, F344, males, 30/group	Mancozeb Purity 88 % Vehicle: corn oil	0 (control), 0.44, 1.76 or 4.4 g/kg bw, Single oral (gavage) dose. Sacrificed after 6, 24 or 48 hours.	**Negative** 50 metaphases spreads examined per animal. Clinical signs of toxicity noted in the high dose groups. No increase in the incidence of chromosomal aberrations in any dose group. Positive control group included.
Micronucleus test in bone marrow of	Mice, CD1, males &	Mancozeb	2000 mg/kg bw (limit dose), oral	**Negative**

Method, guideline, deviations if any	Species, strain, sex, No/group	Test substance	Concentrations tested	Result
mice. OECD TG 474 GLP Anonymous, 1997	females, 10/sex	Purity 85%	doses at 0 and 24 hours. Bone marrow sampled at 48 hours.	No increase in the incidence of micronuclei. Positive control group included.
Micronucleus test in bone marrow of mice. OECD TG 474 GLP Anonymous, 1987a	Mice, CD1, males & females, 15/sex/dose	Mancozeb technical Purity 88.2%	0 & 1,000 mg/kg bw, oral (gavage) dose. Bone marrow sampled at 24, 48 & 72 hours.	**Negative** No increase in the incidence of micronuclei. 1000 polychromatic erythrocytes evaluated per animal. Positive control group included.
Micronucleus test in bone marrow of mice. OECD 474 GLP Anonymous, 2008a	Mice, Swiss albino, males & females 5/sex	Mancozeb Purity not reported Vehicle: corn oil	0 or 2000 mg/kg bw, oral (gavage) dose on two consecutive days.	**Negative** No clinical signs of toxicity observed & no signs of toxicity to the bone marrow. No increase in the incidence of micronuclei Positive control included.
Micronucleus test in bone marrow of mice. OECD 474 GLP Anonymous, 1999	Mice, Swiss albino, 5 sex/group	Mancozeb technical Purity 85% Vehicle: peanut oil	500, 1000 & 2000 mg/kg bw, oral dose for two consecutive days. Bone marrow sampled at 24 hours.	**Negative** Clinical signs of toxicity noted (reduced bw and lethargy). PCE/NCE ratio was similar between treated and control groups. No increase in the incidence of micronuclei. Positive control included.
Chromosome aberration study in mice. 475 GLP Anonymous,1999	Mice, Swiss albino, males & females, 5/sex/group	Mancozeb technical Purity 85% Vehicle: peanut oil	1, 500, 1000 & 2000 mg/kg bw, oral dose	**Negative** Clinical signs of toxicity noted (diarrhoea & lethargy). No increase in chromosomal aberrations in males. Slight increase in chromatid & chromosomal breaks &

Method, guideline, deviations if any	Species, strain, sex, No/group	Test substance	Concentrations tested	Result
				fragments in females in high dose group. Positive control included.

The genotoxic potential of mancozeb has been examined in four reliable and well conducted guideline bone marrow micronucleus assays in mice. Mancozeb did not induce an increase in micronuclei in any of these tests up to limit doses of 2000 mg/kg bw. Previous toxicokinetic studies have confirmed the bioavailability of mancozeb in the blood after oral administration, and due to the highly perfused nature of the bone marrow tissue it is likely that the bone marrow had been exposed to mancozeb or its metabolites.

Two *in vivo* chromosome aberration studies were carried out in mice. A well conducted study (Anonymous, 1984) observed no increase in the incidence of chromosome aberrations in the high dose group. Clinical signs of toxicity (including lethargy, piloerection and dyspnoea) observed at the high dose of 4400 mg/kg bw confirmed that adequate doses had been administered. Although the study by Anonymous (1999) observed a slight increase in chromosome and chromatid breaks in females only, in the high dose group (1.4% compared with 0.6% in the control group), these were not considered to be biologically significant owing to the marginal nature of the increase and the sex specificity of the response. No increase in aberrant cells was observed in males up to doses of 2000 mg/kg bw. Overall it was concluded that no signs of clastogenicity were observed in any of the *in vivo* tests performed on mancozeb.

10.6.3 Short summary and overall relevance of the provided information on germ cell mutagenicity

Mancozeb has been tested in a series of *in vitro* and *in vivo* genotoxicity assays. Mancozeb is not mutagenic in bacterial and host mediated bacterial gene mutation assays, or in gene mutation assays in mammalian cells in culture. An equivocal response was observed *in vitro* in a mouse lymphoma mutation assay. However, mancozeb tested negative in a mammalian cell unscheduled DNA synthesis study. Chromosomal aberrations were induced *in vitro,* but only in the presence of DMSO and therefore this result is considered invalid. A recently and well conducted *in vitro* micronucleus study gave negative results for clastogenicity and aneugenicity (Gilby, 2017). A weakly positive response in sister chromatid exchange tests *in vitro* may be explained by manganese and/or zinc ion interference with enzymes involved in DNA repair and replication. Tight metabolic regulation of these essential nutrients prevents this effect from occurring *in vivo*.

Mancozeb was tested for photomutagenicity in a bacterial reverse mutation test in the presence of UV light. Under the conditions of the test, mancozeb was found to be non-mutagenic (Schreib, 2014).

Mancozeb was shown not to increase the number of polychromatic erythrocytes containing micronuclei in mice, or to induce chromosome aberrations or cytogenetic abnormalities in mice or rats.

Based on the available data set, mancozeb is concluded not to be a mutagen in somatic cells. As there was no indication of mutagenicity in somatic cells arising from the data set, investigations into the effects of mancozeb in germ cells was not required.

10.6.4 Comparison with the CLP criteria

Mancozeb was not mutagenic in valid *in vivo* somatic cell mutagenicity tests and so according to the guidance on the application of the CLP criteria no classification is warranted. The overall body of toxicological data from a number of *in vitro* and *in vivo* assays indicates that mancozeb is of no genotoxic concern. Therefore no classification for mutagenicity under the CLP regulation is required.

10.6.5 Conclusion on classification and labelling for germ cell mutagenicity

Not classified (conclusive but not sufficient for classification)

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS concluded that mancozeb was negative for mutagenicity *in vitro* except for an equivocal mouse lymphoma assay (Riach, 1996) and a positive chromosomal aberration assay (Innes, 1995). However, the DS did not consider the latter result valid due to the use of DMSO as a solvent in the test. All *in vivo* chromosomal aberration and micronucleus tests were negative according to the DS. Therefore, no classification for mutagenicity was proposed by the DS on the basis of the overall *in vivo* and *in vitro* evidence.

Comments received during public consultation

No comments were received on this hazard class.

Additional key elements

Numerical results of the in vitro chromosomal aberration assay by Innes (1995)

Chinese hamster ovary cells were treated for 6 h in the presence of the S9 mix and for 22 h in the absence of S9 mix, and harvested at 24 h in both cases. Two tests were conducted for each treatment type and each treatment group was run in two replicates (100 cells per replicate); values from both replicates are presented in the tables below.

–S9, Test 1

Treatment	Conc. (µg.ml^{-1})	% aberrant cells (excl. chromatid gaps)	Result when compared to HCD[#]	Cell count (% of control)
DMSO	1%	0	–	122
		0	–	78
Mancozeb	1.25	0	–	115
		0	–	124
	2.5	0	–	101
		1	–	111
	5	12	+	37^{m}
		11	+	28^{m}
Methyl methane-sulphonate	30	14	+	n.d.
	40	26	+	n.d.

[#] Negative result: < 95% confidence limit of the historical control data (here 2%); equivocal (+/-): between 95% and 99% confidence limit (here 2–5%); positive: > 99% confidence limit (here > 5%)

[m] Changes in cell morphology were also observed

n.d. = not determined; HCD = historical control data

–S9, Test 2

Treatment	**Conc. (µg.ml^{-1})**	**% aberrant cells (excl. chromatid gaps)**	**Result when compared to HCD[#]**	**Cell count (% of control)**
DMSO	1%	0 0	– –	100 100
Mancozeb	2	0 0	– –	98 95
	4	3 12	+/– +	45[m] 45[m]
	6	42 31	+ +	8[m] 9[m]
Methyl methane-sulphonate	20	7	+	n.d.
	30	11	+	n.d.

[#] Negative result: < 95% confidence limit of the historical control data (here 2%); equivocal (+/-): between 95% and 99% confidence limit (here 2–5%); positive: > 99% confidence limit (here > 5%)

[m] Changes in cell morphology were also observed

n.d. = not determined; HCD = historical control data

+S9, Test 1

Treatment	**Conc. (µg.ml^{-1})**	**% aberrant cells (excl. chromatid gaps)**	**Result when compared to HCD[#]**	**Cell count (% of control)**
DMSO	1%	1 0	– –	97 103
Mancozeb	1.6	3 2	+/– –	117 121
	3	12 1	+ –	98 107
	6	17 14	+ +	34[m] 24[m]
Cyclophosphamide	20	12	+	n.d.
	30	17	+	n.d.

[#] Negative result: < 95% confidence limit of the historical control data (here 2%); equivocal (+/-): between 95% and 99% confidence limit (here 2–5%); positive: > 99% confidence limit (here > 5%)

[m] Changes in cell morphology were also observed

n.d. = not determined; HCD = historical control data

+S9, Test 2

Treatment	**Conc. (µg.ml^{-1})**	**% aberrant cells (excl. chromatid gaps)**	**Result when compared to HCD#**	**Cell count (% of control)**
DMSO	1%	0 0	– –	100 100
Mancozeb	4	2 0	– –	68 68
	6	9 9	+ +	47[m] 42[m]
	8	20 9	+ +	30[m] 45[m]
Cyclophosphamide	20	5	+/–	n.d.
	30	8	+	n.d.

Negative result: < 95% confidence limit of the historical control data (here 2%); equivocal (+/-): between 95% and 99% confidence limit (here 2–5%); positive: > 99% confidence limit (here > 5%)

[m] Changes in cell morphology were also observed

n.d. = not determined; HCD = historical control data

Numerical results of the mouse lymphoma assay by Riach (1996)

Two independent mutation assays were performed both in the absence (Assay 1, Assay 3) and in the presence (Assay 2, Assay 4) of S9 mix. Each experiment consisted of vehicle control (4 cultures), several concentrations of mancozeb (2 cultures per concentration) and positive control (2 cultures). The laboratory considered a response at a single dose positive if the mean mutant fraction was at least 1.7-fold higher than the mean control value. An experiment was considered positive if there was a positive response at least at the highest acceptable dose and at the same time an increase in mutant frequency or an upward trend in the remaining doses.

–S9, Assay 1

Treatment	**Dose (µg.ml^{-1})**	**Mutant fraction x 10^{-6}**	**Fold increase over control**	**% small colonies**	**Relative total growth (%)**
DMSO	(100 µL)	77 56 84 89	–	39 n.d. n.d. n.d.	84 118 97 101
Mancozeb	0.2	74 63	1.0 0.8	n.d.	84 84
	0.4	103 98	1.3 1.3	n.d.	59 56
	0.8	136	1.8	43	28

		74	1.0	n.d.	44
	1.6	334 489	4.4 6.4	85 n.d.	7 5
Ethyl methane-sulphonate	250	313 324	4.1 4.2	n.d. 8	69 64
Methyl methane-sulphonate	15	307 313	4.0 4.1	n.d. 69	19 19

n.d. = not determined

–S9, Assay 3

Treatment	**Dose (µg.ml^{-1})**	**Mutant fraction x 10^{-6}**	**Fold increase over control**	**% small colonies**	**Relative total growth (%)**
DMSO	(100 µl)	78 73 89 75	–	n.a. n.d. n.d. n.d.	88 91 115 107
Mancozeb	0.5	94 88	1.2 1.1	n.d.	41 46
	0.75	125 131	1.6 1.7	n.d.	18 21
	1	176 224	2.2 2.8	n.d. 76	13 11
	1.25	352 337	4.5 4.3	n.d.	7 6
Ethyl methane-sulphonate	250	248 313	3.1 4.0	n.d. 12	55 55
Methyl methane-sulphonate	15	271 345	3.4 4.4	n.d. 61	20 19

n.d. = not determined; n.a. = not available

+S9, Assay 2

Treatment	**Dose (µg.ml^{-1})**	**Mutant fraction x 10^{-6}**	**Fold increase over control**	**% small colonies**	**Relative total growth (%)**
DMSO	(100 µl)	57 58 79 46	–	n.d. 43 n.d. n.d.	101 97 101 101

Mancozeb	0.2	58 53	1.0 0.9	n.d.	88 93
	0.4	53 46	0.9 0.8	n.d.	77 77
	0.8	56 39	0.9 0.7	n.d.	81 81
	1.6	70 83	1.2 1.4	n.d. 51	29 24
3-methylcholanthrene	2.5	347 293	5.8 4.9	57 n.d.	36 38

n.d. = not determined

+S9, Assay 4

Treatment	**Dose (µg.ml^{-1})**	**Mutant fraction x 10^{-6}**	**Fold increase over control**	**% small colonies**	**Relative total growth (%)**
DMSO	(100 µl)	69 67 70 73	–	n.d. n.d. n.a. n.d.	103 101 105 92
Mancozeb	0.9	109 126	1.6 1.8	n.d.	63 52
	1.2	150 108	2.2 1.5	n.d.	37 44
	1.5	147 166	2.1 2.4	n.d.	29 27
	1.8	251 270	3.6 3.9	n.d. 69	16 12
3-methylcholanthrene	2.5	405 367	5.8 5.3	49 n.d.	34 38

n.d. = not determined; n.a. = not available

Historical control data (within 5 years, from the same laboratory, the present experiment not included)

Metabolic activation	**Control**	**n**	**Mutant fraction x 10^{-6}**		
			Mean	**SD**	**Range**
–S9	Vehicle	87	51	21	17-124
	Ethyl methanesulphonate	87	312	74	202-631

+S9	Vehicle	81	53	19	26-111
	3-methylcholanthrene	81	465	172	164-1065

Each vehicle control value is the mean of 4 replicate cultures; each positive control value is the mean of 2 replicate cultures

Numerical results of the in vivo tests

Numerical results of the four *in vivo* micronucleus tests and two *in vivo* chromosomal aberration tests are provided below.

Micronucleus test, Anon. (1987a**)**

Time of kill	**Compound and dose**	**% micronucleated polychromatic erythrocytes**
24 h	Vehicle control	0.08
	Mancozeb 10,000 mg/kg bw	0.06
	Positive control	1.66*
48 h	Vehicle control	0.06
	Mancozeb 10,000 mg/kg bw	0.09
72 h	Vehicle control	0.08
	Mancozeb 10,000 mg/kg bw	0.06

* Statistically significant difference from vehicle control, $p \leq 0.05$

Micronucleus test, Anon. (1997b)

Treatment	**% micronucleated polychromatic erythrocytes**	
	Males	**Females**
Vehicle control	0.04	0.03
Mancozeb 2000 mg/kg bw/d	0.07	0.08
Positive control	1.78	–

Historical control data (from the same laboratory, years not provided): range 0.00 – 0.28% per 10 mice; mean 0.12%

Micronucleus test, Anon. (1999a**)**

Treatment	**% micronucleated polychromatic erythrocytes**	
	Males	**Females**
Vehicle control	0.030	0.032
Mancozeb 500 mg/kg bw/d	0.062	0.036
Mancozeb 1000 mg/kg bw/d	0.068*	0.058
Mancozeb 2000 mg/kg bw/d	0.086	0.076
Positive control	0.364*	0.350*

* Statistically significant difference from vehicle control, $p \leq 0.05$ (Student's t-test)

Micronucleus test, Anon. (2008a)

Treatment	% micronucleated polychromatic erythrocytes	
	Males	Females
Vehicle control	0.00	0.00
Mancozeb 2000 mg/kg bw	0.02	0.03
Positive control	1.68	1.72

Chromosomal aberration test, Anon. (1984a)

Group	Sacrifice after	% cells with aberrations (excluding gaps)
Vehicle control	6 h	0.4
	24 h	0.22
	48 h	1.2
Mancozeb 4.4 g/kg bw	6 h	0.42
	24 h	0.84
	48 h	1.6
Positive control	18 h	63.5*

* Statistically significant difference from vehicle control, $p \leq 0.05$

Chromosomal aberration test, JRF (1999)

Treatment	% aberrant cells	
	Males	Females
Vehicle control	0.60	0.60
Mancozeb 500 mg/kg bw/d	0.80	0.60
Mancozeb 1000 mg/kg bw/d	1.00	1.00
Mancozeb 2000 mg/kg bw/d	1.40	1.40*
Positive control	9.20*	10.40*

* Statistically significant difference from vehicle control, $p \leq 0.05$ (Student's t-test)

Assessment and comparison with the classification criteria

The genotoxic potential of mancozeb has been investigated in a series of *in vitro* and *in vivo* studies. The descriptions of some of the studies in the background document and the draft revised assessment report (RAR) were insufficient for an independent evaluation of this hazard class. In July 2018, following comments received on the draft RAR during the public consultation, EFSA requested the applicants to provide additional information on various sections. In response, the applicants provided robust study summaries for three *in vivo* micronucleus tests (Anon., 1987a; Anon., 1997b; Anon., 1999a). Additionally, RAC

requested and was provided access to original study reports to a further 7 studies (Innes, 1995; Gilby, 2017; Foxall and Byers, 1984; Riach, 1996; Anon., 1984a; Anon., 2008a; Jai Research Foundation, 1999). A brief summary of all available studies is provided in the following table but additional details can be found in the background document.

Genotoxicity studies		
Type of study; Reference	**Method**	**Observations**
In vitro		
Ames test Wilmer 1982	OECD 471 GLP *S. typhimurium* TA 1535, TA 1537, TA 1538, TA 98, TA 100 Deviation: *S. typhimurium* TA102 or *E. coli* WP2 not tested Up to 50 µg/plate Negative control: water	Negative ±S9 RAR: Toxicity at 100 µg/plate in a preliminary test
Ames test Chism 1984	OECD 471 GLP *S. typhimurium* TA 1535, TA 1537, TA 98, TA 100 Deviation: *S. typhimurium* TA102 or *E. coli* WP2 not tested Up to 250 µg/plate Vehicle: water	Negative ±S9 Inhibition of bacterial growth at 75 and 250 µg/plate
Ames test Slabbert 1994	OECD 471 GLP *S. typhimurium* TA98, TA100 Deviation: only 2 strains tested Up to 8000 mg/L Vehicle: water	Negative ±S9 Toxicity not reported
Ames test Prabhu 1999	OECD 471 GLP *S. typhimurium* TA 1535, TA 1537, TA 98, TA 100, TA 102 –S9: up to 31.25 µg/plate +S9: up to 250 µg/plate Vehicle: DMSO	Negative ±S9 Toxicity at 156.25 µg/plate (–S9) and 312.5 µg/plate (+S9)
Ames test Nagane 2008	OECD 471 GLP	Negative ±S9 Toxicity at 78 µg/plate (–S9) and 39 µg/plate (+S9)

(only in the RAR, not in the CLH report)	*S. typhimurium* TA 1535, TA 1537, TA 98, TA 100, TA 102 –S9: up to 40 µg/plate +S9: up to 20 µg/plate Vehicle: DMSO	
Photogenotoxicity (based on the Ames test) Schreib 2014	OECD 471 & 432 GLP *S. typhimurium* TA 1535, TA 1537, TA 98, TA 100, TA 102 Up to 50 µg/plate ±S9, ±UV irradiation Vehicle: not reported	Negative ±S9, ±UV Toxicity from 3.16-5.0 µg/plate Precipitation from 25-50 µg/plate
Host mediated assay (derived from the Ames test, host provides metabolic activation) McCarroll 1985	Requested by US-EPA Derived from OECD 471 GLP Male mice, 10/group Mancozeb in corn oil administered via gavage at 0, 0.5, 2.0 or 5 g/kg bw *S. typhimurium* TA 1530 via i.p. injection, animals killed 2 or 4 hours after inoculation The bacteria recovered from peritoneum were plated and incubated	Negative
Chromosomal aberration assay *in vitro* Innes 1995	OECD 473 GLP Chinese hamster ovary cells Up to 8 µg/mL Vehicle: DMSO	Positive ±S9, but mostly at cytotoxic concentrations (relative cell count < 50%)
Micronucleus test *in vitro* Gilby 2017	OECD 487 GLP Human lymphocytes Up to 10 µg/mL; the top dose was selected based on solubility Vehicle: ethanol	Negative ±S9 3-hour treatment (±S9): No cytotoxicity and no precipitation up to 10 µg/mL 20-hour treatment (–S9): Slight cytotoxicity (CBPI reduced by 15%, cytostasis 35%) and no precipitation at 10 µg/ml
HPGRT assay Foxall and Byers 1984	OECD 476 GLP Chinese hamster ovary cells –S9: up to 15 µg/mL +S9: up to 45 µg/mL Vehicle: water	Negative ± S9 Adequate cytotoxicity levels were achieved

Mouse lymphoma assay Riach 1996	OECD 476 GLP Mouse lymphoma L5178Y –S9: up to 1.75 µg/mL +S9: up to 3.2 µg/mL Vehicle: DMSO Agar version	Equivocal ±S9
UDS *in vitro* O'Neil and Frank 1988	OECD 482 GLP Rat hepatocytes, male, adult Up to 10 µg/mL scored Negative control: DMSO + culture medium	Negative ±S9 Toxicity at 2 to 10 µg/mL Precipitation from 100 µg/mL
SCE *in vitro* Ivett 1985	OECD 479 GLP Chinese hamster ovary cells Up to 20 µg/mL Vehicle: serum free culture medium	Positive –S9, negative +S9 Toxicity at 15 µg/mL (–S9) and 12.5-20 µg/mL (+S9)
In vivo		
Micronucleus test (bone marrow) Anon. 1987a	OECD 474 GLP Mouse, male and female 15/sex for mancozeb-treated group 15/sex for negative control 5/sex for positive control Single oral (gavage) dose; 0 and 10,000 mg/kg bw Vehicle: 1% aqueous methylcellulose Bone marrow sampled at 24, 48 or 72 hours (5/sex/dose) 1000 polychromatic erythrocytes per animal	Negative No mortalities Clinical signs: transient slight piloerection, hunched posture, ptosis No convincing evidence of bone marrow toxicity
Micronucleus test (bone marrow) Anon. 1997b	OECD 474 GLP Mouse, male and female 10/sex for the mancozeb-treated group 5/sex for negative controls 5/sex for positive controls Oral doses of 2000 mg/kg bw at 0 and 24 h; bone marrow sampled at 48 h	Negative (a 2-fold increase in micronucleated erythrocytes but well within the HCD) Clinical signs of toxicity (subdued behaviour, hunched appearance, piloerection) at 2000 mg/kg bw in the preliminary test but not in the main test No evidence of bone marrow toxicity

	2000 polychromatic erythrocytes per animal Vehicle: maize oil	
Micronucleus test (bone marrow) Anon. 1999a	OECD 474 GLP Mouse, male and female 5/sex/dose Oral doses of 500, 1000 or 2000 mg/kg bw on two consecutive days Bone marrow sampled 24 hours after the last treatment Vehicle: peanut oil 2000 polychromatic erythrocytes per animal	A dose-related, non-significant increase (2.9/2.4-fold at 2000 mg/kg bw) in micronucleated erythrocytes in both sexes Clinical signs of toxicity (lethargy) in some animals No effect on PCE/NCE ratio
Micronucleus test (bone marrow) Anon. 2008a	OECD 474 GLP Mouse, male and female 5/sex/group Oral (gavage) doses of 2000 mg/kg bw on two consecutive days Vehicle: corn oil 2000 polychromatic erythrocytes per animal	Negative No clinical signs of toxicity No evidence of bone marrow toxicity
Chromosomal aberration test (bone marrow) Anon. 1984a	OECD 475 GLP Rat, male 30/dose Single oral (gavage) dose; 0, 0.44, 1.76, 4.4 g/kg bw (low and mid dose not examined) Sacrificed after 6, 24 or 48 h (10 animals per dose and time point) 50 metaphases per animal Vehicle: corn oil	Negative Clinical signs of toxicity (lethargy, piloerection, dyspnoea) in the top dose group
Chromosomal aberration test (bone marrow) Jai Research Foundation (JRF), 1999 (reference missing in the CLH report)	OECD 475 GLP Mouse, male and female 5/sex/dose Oral dose of 0, 500, 1000 or 2000 mg/kg bw Sacrifice 24 h after treatment 50 metaphases per animal	A slight, dose-related, increase (2.3-fold at 2000 mg/kg bw) in chromosomal aberrations in both sexes, stat. sign. in females Clinical sings of toxicity (lethargy, diarrhoea)

Gene mutations

A number of Ames tests are available and all of them are negative including several OECD guideline compliant tests (Prabhu, 1999; Nagane, 2008; Schreib, 2014) and one Ames-based host-mediated assay (McCarroll, 1985), where the bacterial cells were exposed inside the peritoneal cavity of the mammalian host in order to ensure *in vivo* metabolic activation.

Two *in vitro* gene mutation assays in mammalian cells are available. The HPGRT assay by Foxall and Byers (1984) was negative. Cytotoxicity at the top concentrations was sufficient (meeting the requirements of the OECD TG) and the positive controls responded appropriately.

The mouse lymphoma assay by Riach (1996) was equivocal. The numerical data are presented in the background document under 'Additional key elements'. RAC notes that according to the OECD TG 490, positive results only found between 20 and 10% relative total growth (RTG) should be interpreted with caution. In the absence of metabolic activation, the first experiment was inconclusive (no dose tested between 10 to 20% RTG, increase in mutations below 10% RTG) and the second equivocal (a borderline increase around 20% RTG, a clear increase around 10% RTG). In the presence of metabolic activation, the first experiment was negative (RTG at the top dose 27%) and the second positive (a 2.2-fold increase at 28% RTG, above HCD, with an apparent dose-response relationship). Overall, the test is considered equivocal. The analysis of the colony size distribution has shown that small colonies prevailed, which may indicate clastogenicity rather than point mutagenicity.

To sum up, there are several negative tests on gene mutations in bacteria and one reliable negative and one equivocal test on gene mutations in mammalian cells, the latter however indicating potential clastogenicity rather than point mutagenicity. No *in vivo* study on gene mutations is available. Overall, in a weight of evidence assessment, RAC concludes that the potential of mancozeb to induce gene mutations has been sufficiently investigated and the overall result is 'negative'.

Chromosomal damage

The chromosomal aberration assay by Innes (1995) was positive but mostly at levels associated with considerable cytotoxicity (relative cell count below 50%), which reduces the concern about the positive result. The numerical data are presented in the background document under 'Additional key elements'. The mouse lymphoma assay by Riach (1996) summarised above also suggested clastogenic potential but the results were equivocal.

Both these *in vitro* studies used DMSO as a vehicle. The DS advised in the CLH report to disregard *in vitro* studies using DMSO and other highly polar, reactive solvents as vehicles, as these cause rapid degradation of mancozeb (half-life in DMSO 36 min compared to 6-55 hours in water) and a concomitant rapid release of manganese and zinc ions resulting in high concentrations of metal ions in the medium. According to the DS, such a situation is not expected to occur *in vivo* because absorption and metabolism of these essential elements is tightly regulated in mammalian organisms. As salts of both manganese and zinc are capable of producing chromosomal aberrations *in vitro*, the DS was of the opinion that the use of DMSO may have led to false positive results for mancozeb.

RAC has identified uncertainties in the DS's case for dismissing the *in vitro* mutagenicity studies with DMSO. Although manganese salts were positive in many *in vitro* genotoxicity assays, positive *in vivo* results upon oral administration have also been reported (ATSDR, 2012). In addition, although the rate of hydrolysis in water is lower than in DMSO, it is variable (cf. a half-life in water of less than 1 hour in the study by Völkel, 2001b) and non-negligible considering the duration of the tests.

The micronucleus test by Gilby (2017) was negative. Instead of using DMSO, the laboratory investigated solubility of mancozeb in several alternative solvents (acetonitrile, methanol, ethanol, DMF, acetone, water), out of which ethanol afforded the highest solubility of 1 mg/mL. Therefore, in this study, the test item was formulated as a suspension at 1 mg/mL in ethanol and dosed at 1% v/v (a concentration of an organic solvent in the medium that should not be exceeded according to OECD TG 487), leading to 10 µg/mL as the top dose in the experiment. This top dose caused no or slight cytotoxicity.

Six *in vivo* studies are available: four micronucleus tests and two chromosomal aberration assays. All were claimed to be negative by the DS. RAC however notes that increases in micronucleated erythrocytes were seen in two (Anon., 1999a; JRF, 1999) of the six studies. The significance of this finding is difficult to assess in view of the lack of information on historical controls. Moreover, RAC notes that both studies (plus the study by Anon., 2008a) were performed by Jai Research Foundation (JRF). JRF was the laboratory which conducted a prenatal developmental toxicity study of Anon. (1999b) that was considered unreliable by both the DS and RAC, which raises some doubts about the reliability of the genotoxicity studies Anon. (1999a), JRF (1999) and Anon. (2008a). When these studies are excluded from the assessment, only negative *in vivo* studies remain.

All the *in vivo* studies utilised the bone marrow as the target tissue for genetic damage. No direct evidence of bone marrow toxicity was seen in any of the genotoxicity studies. One of the studies (Anon., 1984a) was conducted in the rat. For this species the bioavailability of mancozeb and/or its metabolites in the bone marrow of both sexes after oral administration was confirmed by an ADME study (Anon., 1986f). For the mouse, availability in the bone marrow is inferred from systemic toxicity (e.g., T4 reduction in both sexes) observed in the mouse repeated dose studies, which indicates systemic availability, and from extrapolation from the rat ADME study.

Conclusion

Classification of a substance in Category 2 for germ cell mutagenicity is based on positive *in vivo* evidence or on chemical structure activity relationship to known germ cell mutagen supported by positive *in vitro* evidence. Genotoxicity of mancozeb has been sufficiently investigated both *in vitro* and *in vivo* and the available data do not meet the criteria for classification. Therefore, RAC agrees with the DS that **no classification** is warranted.

10.7 Carcinogenicity

The long-term toxicity and carcinogenic potential of mancozeb has been investigated in two combined chronic toxicity/carcinogenicity studies in rats, a carcinogenicity study in rats and two carcinogenicity studies in mice. In addition, the findings from two 2-generation reproduction toxicity studies were considered relevant to the

assessment of the carcinogenic potential of mancozeb. Three investigative studies from the open literature have also been considered. A number of epidemiological studies are also available.

Table 14: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Species, strain, sex, no/group**	**Dose levels duration of exposure**	**Results (Effects statistically significantly different unless stated otherwise)**

<table>
<tr>
<td>Two-year combined chronic toxicity/carcinogenicity
Oral (dietary)
OECD 453
GLP
Mancozeb 83.8%
Anonymous, 1990a</td>
<td>Rat, Crl: CD BR
10/sex/dose (1-yr)
62/sex /dose (2-yr)</td>
<td>0, 20, 60, 125, 750 ppm
Equivalent to:
Males: 0.769, 2.33, 4.83, 30.9 mg/kg/day
Females: 1.06, 3.06, 6.72, 40.2 mg/kg/day</td>
<td>No treatment-related effects on mortality.
No treatment-related overt clinical signs of toxicity.
<u>Non-neoplastic findings</u>
<u>750 ppm (males 30.9 mg/kg bw/day, females 40.2 mg/kg bw/day)</u>:
↓ Body weight: 10% females days 0-91
↓ Body weight gain: 7.4% males day 364
↓ Incidence of diarrhoea: 14/62 males (control 36); 4/62 females (control 12)
↓ T3: 30.0% males 3 months
↑ T3: 23.3% and 42.6% males at 6 and 24 months
↓ T4: 18.4%, 35.5% males at 6 and 18 months; 50.0%, 22.2%, 21.4% females at 3, 18 and 24 months
↑ TSH: 33.3%, 52.8% males at 12 and 18 months; 76.5% and 113.3% females at 6 and 18 months
↑ Absolute thyroid weight: 59.6% males; 43.2% females at 24 months
↑ Relative thyroid weight: 61.6% males; 47.4% females at 24 months
↑ Eye bilateral retinopathy incidence and severity: 19/61 males (4/60 control): 49/60 females (21/62 control)
↑ Thyroid follicular cell hypertrophy/hyperplasia: 34/61 males (1/60 control); 24/61 females (1/62 control)
<u>125 ppm (males 4.83 mg/kg bw/day, females 6.72 mg/kg bw/day)</u>:
No toxicologically significant treatment-related effects.
<u>60 ppm (males 2.33 mg/kg bw/day, females 3.03 mg/kg bw/day):</u>
No toxicologically significant treatment-related effects.
<u>20 ppm (males 0.769 mg/kg bw/day, females 1.06 mg/kg bw/day):</u>
No toxicologically significant treatment-related effects.

<u>Neoplastic findings</u>
Increased incidence of thyroid follicular cell tumours in both sexes at 750 ppm.
<table>
<tr><th>Tumours</th><th colspan="5">Dietary concentration (ppm)</th></tr>
<tr><th></th><th>0</th><th>20</th><th>60</th><th>125</th><th>750</th></tr>
<tr><th></th><th colspan="5">Males</th></tr>
<tr><th>Thyroid No examined</th><th>60</th><th>62</th><th>60</th><th>58</th><th>61</th></tr>
<tr><td>Follicular cell carcinomas</td><td>0</td><td>0</td><td>2</td><td>2</td><td>14*</td></tr>
<tr><td>Follicular cell adenomas</td><td>0</td><td>1</td><td>1</td><td>0</td><td>20*</td></tr>
<tr><th></th><th colspan="5">Females</th></tr>
<tr><th>Thyroid No. examined</th><th>62</th><th>60</th><th>62</th><th>61</th><th>61</th></tr>
<tr><td>Follicular cell carcinomas</td><td>0</td><td>0</td><td>0</td><td>1</td><td>4*</td></tr>
<tr><td>Follicular cell adenomas</td><td>1</td><td>1</td><td>1</td><td>1</td><td>6*</td></tr>
</table>
</td>
</tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	**Species, strain, sex, no/group**	**Dose levels duration of exposure**	**Results (Effects statistically significantly different unless stated otherwise)**
			* statistically significantly different ($P \leq 0.05$)
Two-year combined toxicity/carcinogenicity Oral (dietary) OECD 453, (1988) GLP Mancozeb 88.2 and 88.5% Anonymous, 1992a	Rats, Crl: CD SD 10/sex/dose (1-yr) 50/sex /dose (2-yr) 10/sex/dose (blood sampling)	0, 28, 113, 454 ppm Equivalent to: Males: 1.0, 4.0 and 16.8 mg/kg bw/day Females: 1.3, 5.1, 20.8 mg/kg bw/day	No treatment-related effects on mortality. No treatment-related overt clinical signs of toxicity. **Non-neoplastic findings** 454 ppm (males 16.8 mg/kg bw/day, females 20.8 mg/kg bw/day): ↓ *Body weight:* 4.4, 5.5% week 13 in males and females respectively ↓ *Body weight gain:* 6.8%, 11.4% weeks 0-13 in males and females respectively; 7.4% weeks 0-26 males ↓ *Thyroxin T4*: 15.6%, 19.2% males at 26 and 52 weeks; 20.0%, 22.7% and 38.7% females at 26, 52 and 78 weeks ↑ *Thyroid increased height of follicular epithelium*: 8/50 males (3/50 control); 5/50 females (1/50 control). The statistical significance of these findings is unclear. *Thyroid prominent micro follicles:* 5/50 males (0/50 control); 1/50 males (0/50 control) 113 ppm (males 4.0 mg/kg bw/day, females 5.1 mg/kg bw/day): No toxicologically significant treatment-related effects. 28 ppm (males 1.0 mg/kg bw/day, females 1.3 mg/kg bw/day): No toxicologically significant treatment-related effects. NOAEL for non-neoplastic changes 113 ppm (equivalent to 4.0 and 5.1 mg/kg bw/day in males and females respectively) based on effects on body weight and functional and morphological thyroid effects at 454 ppm. **Neoplastic findings** No treatment-related changes in neoplastic findings at any dose level.

<table>
<tr>
<td>104 week Carcinogenicity study
Oral (dietary)
Non guideline
Not GLP
Mancozeb
Belpoggi et al 2002 (from the open literature)</td>
<td>Rat: Sprague-Dawley
75/sex/group</td>
<td>0, 10, 100, 500, 1000 ppm
Equivalent to: 0.5, 5, 25 & 50 mg/kg bw/day. (based on default assumptions)
Treated for 104 weeks but observed until spontaneous death.</td>
<td>No treatment-related effects on mortality.
No treatment-related overt clinical signs of toxicity
<u>Non-neoplastic findings</u>
No toxicologically significant treatment-related effects at any dose level.
<u>Neoplastic findings</u>
Statistically significant increase in thyroid follicular cell carcinomas at both sexes at 1000ppm.
<table>
<tr><th>Thyroid gland tumours</th><th colspan="5">Dietary concentration (ppm)</th></tr>
<tr><td></td><td>0</td><td>10</td><td>100</td><td>500</td><td>1000</td></tr>
<tr><td></td><td colspan="5">Males</td></tr>
<tr><td>Follicular cell carcinomas</td><td>0</td><td>0</td><td>0</td><td>2.7</td><td>8*</td></tr>
<tr><td>C-cell carcinomas</td><td>0</td><td>0</td><td>1.3</td><td>0</td><td>2.7</td></tr>
<tr><td></td><td colspan="5">Females</td></tr>
<tr><td>Follicular cell carcinomas</td><td>0</td><td>1.3</td><td>0</td><td>0</td><td>16**</td></tr>
<tr><td>C-cell carcinomas</td><td>0</td><td>0</td><td>1.3</td><td>1.3</td><td>2.7</td></tr>
</table>
* p<0.05 and **p<0.01
Increased occurrence of benign and malignant tumours in a number of organs at all doses.</td>
</tr>
<tr>
<td>18 month carcinogenicity
Oral (dietary)
OECD 451
87/302/EEC B.32 (1988)
GLP
Mancozeb 82.8%
Anonymous, 1991a</td>
<td>Mice, CD-1
20/sex/dose (1-yr)
70/sex/dose (1.5-yrs)</td>
<td>0, 30, 100, 1000 ppm
Equivalent to:
Males: 3.75, 12.52, 130.60 mg/kg bw/day
Females: 5.18, 17.81, 179.74 mg/kg bw/day</td>
<td>No treatment-related effects on mortality.
No treatment-related overt clinical signs of toxicity
<u>Non-neoplastic findings</u>
<u>1000 ppm (males 130.60 mg/kg bw/day, females 179.74 mg/kg bw/day)</u>:
↓ Body weight gain: 13% males, 10% females at 18 months
↓ T4: 56% and 24.9% in males at 12 and 18 months respectively.
↓ T4: 76.3% and 61.5% in females at 12 and 18 months respectively.
↑ T3: 45.0% males 18 months
↓ T3: 31.3% females 18 months
Pathology: no treatment-related changes in any of the tissues examined
<u>100 ppm (males 12.52 mg/kg bw/day, females 17.81 mg/kg bw/day)</u>:
No toxicologically significant treatment-related effects.
<u>30 ppm (males 3.75 mg/kg bw/day, females 5.18 mg/kg bw/day)</u>:
No toxicologically significant treatment-related effects.
<u>Neoplastic findings</u>
No treatment-related changes in neoplastic findings at any dose level.</td>
</tr>
</table>

<table>
<tr>
<td>78 week carcinogenicity
Oral (dietary)
Mancozeb 88.6%
Anonymous, 1992b</td>
<td>Mice, CD1
60/sex/dose</td>
<td>0,25,100,1000 ppm
Equivalent to 4.25, 17 & 170 mg/kg bw/day</td>
<td>No treatment-related effects on mortality.
No treatment-related overt clinical signs of toxicity
<u>Non-neoplastic findings</u>
<u>1000ppm</u>
↓ Body weight gain: 6% males & 10% females at 52 weeks, 8% males & 14% females at 78 weeks.
<u>100ppm</u>
No toxicologically significant treatment-related effects.
<u>25ppm</u>
No toxicologically significant treatment-related effects.
<u>Neoplastic findings</u>
<table>
<tr><td></td><td>0 ppm</td><td>100ppm</td><td>1000ppm</td></tr>
<tr><td>Liver tumours</td><td>10/50</td><td>7/50</td><td>17/50</td></tr>
<tr><td>Adenomas</td><td>8/50</td><td>5/50</td><td>17/50</td></tr>
<tr><td>Carcinomas</td><td>2/50</td><td>2/50</td><td>0/50</td></tr>
</table>
No statistically significant differences or trends</td>
</tr>
</table>

Table 15: Summary table of human data on carcinogenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations
Mortality study of workers exposed to Dithane from 1948 to 1975 Anonymous, 1976b	Mancozeb	Mortality from workers exposed to mancozeb (1948 to 1975) at the Rohm and Haas Philadelphia Plant were compared to comparable mortality data from the city of Philadelphia. The exposed cohort was divided into three groups according to exposure and an evaluation system to rank relative exposure was used.	The incidences of total deaths and deaths due to cancers among Rohm and Haas workers involved in the production of mancozeb were similar to those of the control group. No thyroid cancer was found in this study.
Retrospective study of women who were employed at a rubber manufacturer using ETU. Smith 1976	ETU	A total of 1929 workers engaged in the production or manufacture of ETU were surveyed retrospectively for thyroid cancer, and compared with the thyroid cancer list of the Birmingham (England) Cancer Registry from 1957-1971.	Exposure to ETU was not associated with thyroid cancer
Epidemiology study Dennis *et al* 2010	Maneb/mancozeb	The study examined cutaneous melanoma incidence and dose–response relationships for 50 agricultural pesticides	A significant association between cutaneous melanoma and maneb/mancozeb (≥ 63 exposure days) was found. It was noted that the possibility that pesticide-specific results were driven by sun exposure could not be ruled out. It was noted that no other studies have reported an association between maneb/mancozeb and melanoma.

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations
Case control study (Agricultural Health Study) Lee *et al* 2007	Mancozeb	The relationship between agricultural pesticides and colorectal cancer incidence was analysed in 56,813 pesticide applicators with no prior history of colorectal cancer. Self-administered questionnaires were used to collect detailed pesticide exposure and other information.	No evidence that mancozeb exposure is associated with colorectal cancer incidence.
Case-control study Mills *et al* 2005 Anonymous, 2007b	Mancozeb	The study examined leukaemia and stomach cancers incidence in farm workers in California.	No association was found between occupational exposure to mancozeb and stomach cancer. An increased risk was reported for leukaemia. The significance of this association is questionable, especially considering that such findings are not supported by the animal data or by any other epidemiology study and can be explained by confounding.
Case-Control study Band *et al* 2011	Mancozeb	Occupational risk factors were investigated among 1,516 prostate cancer patients and 4,994 age-matched internal controls consisting of all other cancer sites excluding lung cancer and cancers of unknown primary site	No association between mancozeb exposure and prostate cancer.
Case control study (Agricultural Health Study) Koutros *et al* 2011	Mancozeb (life-time exposure days)	The study investigated the relationship between pesticide use and single nucleotide polymorphisms (SNPs) in xenobiotic metabolic enzyme (XME) genes and risk of prostate cancer	There was no evidence of an association between maneb/mancozeb and prostate cancer.
Epidemiology study Nordby *et al* 2005	Mancozeb (estimated from agricultural censuses, and meteorologically based fungal forecasts)	Farm holders and their spouses and children, were cross-linked with national agricultural censuses, and the national population register. Thyroid cancer (319 cases) was identified in the national cancer register.	There is no evidence that mancozeb exposure is associated with thyroid cancer in this population.

Table 16: Summary table of other studies relevant for carcinogenicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations (Effects statistically significantly different unless stated otherwise)
Two Generation (two litter) Oral (dietary) OECD 416 (1983) GLP Rat, CRl:CD(SD)BR 25/sex/group Anonymous, 1988	Mancozeb Purity 84.0% 0, 30, 120 or 1200 ppm	Study provides additional information relevant for carcinogenicity. Relevant effects for carcinogenicity in F0 and F1 parents shown only. These groups were treated for approximately 25 weeks.	*Parental toxicity* <u>1200 ppm (68.9 mg/kg bw/day)</u> F0 generation: t**hyroid effects** ↑ thyroid weight (39% males, 36% females); ↑ thyroid follicular hyperplasia (25/25 males, 22/25 females, none in controls); ↑ **follicular adenoma** (3/25 males, 0/25 females, none in controls); F0 generation: Other effects ↑ relative liver weight (16.5% males, 12.5% females); ↑ relative kidney weight (14% females);↑ pigment in lumen of proximal renal tubules (9/9 males, 25/25 females, none in controls); ↑ moderate hypertrophy/vacuolation pituitary adenohypophysis (3/25 males, only minimal/mild severity) F_1 generation: t**hyroid effects** ↑ thyroid weight (48% males, 35% females); ↑ thyroid follicular hyperplasia (24/24 males, 24/24 females, 2/25 in control males, none in control females); ↑**follicular adenoma** (4/24 males, 0/25 females, none in controls); F_1 generation: Other effects ↑ relative liver weight (14% males, 15.5% females); ↑ relative kidney weight (11% females); ↑ pigment in lumen of proximal renal tubules (24/24 males, 25/25 females, none in controls); ↑ hypertrophy / vacuolation pituitary adenohypophysis (minimal/moderate in males) <u>120 ppm (6.95 mg/kg bw/day)</u> No effects relevant for carcinogenicity. <u>30 ppm (1.73 mg/kg bw/day)</u> No effects

Two Generation (one litter) Oral (Dietary) OECD 416 (1983) Limited endpoints compared to current OECD guideline. GLP Rat, CRl:CD(SD)BR 25/sex/group Anonymous, 1992c	Penncozeb (mancozeb technical) Purity 88.4% 0, 25, 150 or 1100 ppm	Study provides additional information relevant for carcinogenicity. Relevant effects for carcinogenicity in F0 and F1 parents shown only. These groups were treated for approximately 25 weeks. Mean dosages were not provided therefore values were estimated for combined sexes.	*Parental toxicity* <u>1100 ppm (74 mg/kg bw/day)</u> F0 parents t**hyroid effects**: ↑ Thyroid weight (61% males, 25% females); ↑ thyroid follicular hypertrophy & hyperplasia (25/25 males, 24/25 females, none in controls); **follicular adenoma** (5/25 males, 1/25 females, none in controls). F1 parents **thyroid effects**: ↑ Thyroid weight (34% males & females); ↑ thyroid follicular hypertrophy & hyperplasia (23/25 males, 25/25 females, none in controls); **follicular adenoma** (11/25 males, 0/25 females, none in controls). <u>150 ppm (10 mg/kg bw/day) and 25 ppm (1.7 mg/kg bw/day) (F0 and F1)</u> No effects relevant for carcinogenicity. No histopathology of thyroid undertaken.
Investigative study (From literature review) Non guideline Not GLP Rat: F344 5-9 males/group Perez-Carreon *et al* 2009	Mancozeb (analytical standard of "high" purity") dosed in a pesticide mixture of 12 pesticides. Mancozeb included at 0.02 and 0.10 mg/mL Gavage 5 times/week for 8 weeks Vehicle: dissolved in DMSO and then suspended in 1% CMC	The study used four protocols to investigate co-carcinogenic effects and tumour promoting activity using various doses of DEN and 2AAF.	Mancozeb (in a pesticide mixture) does not have tumour promoting properties or co-carcinogenic activity in rat medium term liver carcinogenesis. However, the validity of these findings is questioned by the use of DMSO as vehicle.
Investigative study (From literature review) Non-guideline Oral (dietary) Not GLP Rat: Wistar 19 pregnant females; male and female offspring Valentich *et al* 2006	Mancozeb 100 ppm Duration: dams: GD 10 – LD 3; Offspring: 8 weeks from day 30 Purity 80%	The study investigated the effect of co-administration of mancozeb and phenobarbital on nitrosomethylurea-induced pancreatic tumours in rats.	No clear effects of mancozeb and phenobarbital were seen.

Neoplastic alterations in mouse skin (From literature review) Non-guideline Not GLP Mice 4 (sex not specified) Shilpa *et al* 2011	Mancozeb 200 mg/kg bw in DMSO Purity not reported	This study aimed to establish the early proteomic signature of mancozeb exposure in a short-term assay in mouse skin and *in vitro* models using human skin cell line.	Two proteins, S100A6 (Calcyclin) and S100A9 (Calgranulin-B) were significantly up-regulated. These proteins are markers of keratinocyte differentiation and proliferation and the authors suggested that they may play a role in mancozeb-induced neoplastic alterations. However the small group size and the fact that DMSO was used as a vehicle question the validity of these findings.

10.7.1 Chronic/carcinogenicity studies in rats

The chronic toxicity/carcinogenicity of mancozeb was investigated in three life time feeding studies in rats.

Anonymous (1990)

In a combined chronic toxicity study/carcinogenicity study there were no treatment related effects on mortality. A low incidence of diarrhoea among male rats fed the dose of 750 ppm mancozeb was the only clinical sign attributed to compound intake in rats in this study. The initial lower mean body weight gain of males (9%) and females (16%) in the 750 ppm group was thought to be compound related. There were no treatment-related effects on ophthalmoscopic examinations, haematology, clinical-chemistry or urinalysis parameters. In the 750 ppm males and females, T4 levels were lower (up to 52%) compared to controls and TSH levels were higher (up to 113%).

There were no compound-related effects at 12 months on any organ weight. At 24-months, male and female rats fed 750 ppm mancozeb had elevated mean absolute (up to 60%) and relative (up to 62%) thyroid/parathyroid weights.

Gross changes observed at necropsy during the second year of the study included enlarged thyroids in both males and females fed 750 ppm diets and thyroid masses in the male rats in this dietary group. Male and female rats fed 750 ppm diets had increased incidences of thyroid nodular hyperplasia and hypertrophy/hyperplasia. Increased incidences and severity of bilateral retinopathy were also noted for both male and female rats fed 750 ppm diets.

Intergroup comparison of microscopic non-neoplastic findings day 366 to 737

Finding		**Males**					**Females**				
Dietary concentrations (ppm)		**0**	**20**	**60**	**125**	**750**	**0**	**20**	**60**	**125**	**750**
Number examined		**60**	**62**	**61**	**58**	**61**	**62**	**60**	**62**	**61**	**61**
Thyroid follicular cell	Hyperplasia nodular	0	1	3	2	15*	1	2	2	0	11*
	Hyperplasia/hypertrophy	1	1	2	1	34*	1	0	1	0	24*
Eye	Bilateral retinopathy	4	2	1	3	19*	21	28	24	31*	49*

* Statistically significant difference from control group mean, $p<0.05$ Fischer's

Male and female rats fed 750 ppm diets had increased incidences of thyroid follicular cell carcinomas (14/61 vs 0/60 in males and 4/61 vs 0/62 in females) and adenomas (20/61 vs 0/60 in males and 6/61 vs 1/61 in females). The incidences of rats with carcinomas or adenomas were higher for males than for females.

Intergroup comparison of microscopic neoplastic findings day 366 to 737

Finding		Males					Females				
Dietary concentration (ppm)		**0**	**20**	**60**	**125**	**750**	**0**	**20**	**60**	**125**	**750**
Number examined		**60**	**62**	**61**	**58**	**61**	**62**	**60**	**62**	**61**	**61**
Thyroid follicular cell	Adenoma	0	1	1	0	20*	1	1	1	1	6
	Carcinoma	0	0	2	2	14*	0	0	0	1	4

* Statistically significant difference from control group mean, p<0.05 Fischer's

The thyroid changes observed in this study were considered to be related to mancozeb's main metabolite, ethylene thiourea (ETU), a substance which causes thyroid tumours in rats and mice. ETU belongs to a class of compounds that inhibits synthesis of thyroid hormone and induces release of high levels of TSH by the pituitary.

Anonymous (1992)a

In another combined chronic toxicity study/carcinogenicity study there were no treatment related effects on mortality or clinical signs of toxicity up to the top dose of 454 ppm. Statistically lower absolute body weights (up to 5% of control) and statistically lower body weight gains (up to 7% of control) were observed in both sexes in the top dose group. There were no treatment-related effects on ophthalmoscopic examinations, organ weights, haematology, clinical-chemistry and urinalysis parameters. Statistically significantly slightly lower thyroxine (T4) concentrations were evident in the high dosage groups at weeks 26 and 52, and in the high dosage females at weeks 63 and 78.

Treatment-related non-neoplastic findings were confined to the thyroid of males and females receiving 454 ppm, and consisted of a marginally higher incidence of increased height of follicular epithelium, generally with prominent microfollicles.

A higher incidence of pituitary tumours, particularly adenomas, was observed in males receiving 113 or 454 ppm. However, statistical significance was not demonstrated and the incidence was considered comparable with the upper limit of the laboratory historical control range. Therefore, this finding was considered unlikely to be related to treatment. No treatment-related effect was seen in the incidence of follicular cell tumours of the thyroid in male or female rats. There was a higher incidence of parafollicular cell carcinoma of the thyroid in all treated males. As this incidence fell within the laboratory historical control range, this tumour was not considered to be treatment-related.

Intergroup comparison of microscopic neoplastic findings

Finding		Dietary Concentration (ppm)							
		Males				Females			
		0	**28**	**113**	**454**	**0**	**28**	**113**	**454**
Number examined		**50**	**50a**	**50b**	**50**	**50**	**50**	**50**	**50**
Pituitary pars anterior	Adenoma	18	22	28	27	31	31	28	34
	Adenocarcinoma	3	2	2	1	7	5	4	6
Thyroid follicular cells	Adenoma	6	2	2	6	0	0	2	2
	Adenocarcinoma	2	0	1	3	0	0	0	1
Thyroid parafollicular cells	Adenoma	0	0	0	1	0	0	0	0
	Carcinoma	1	6	6	6	3	3	3	3

Belpoggi *et al* (1992)

A publication of a long-term study of mancozeb conducted at the Cancer Research Center of the Ramazzini Foundation is available (Belpoggi *et al*., 2002). Groups of 75 male and female Sprague-Dawley rats, 8 weeks old at the start of the treatment, were administered mancozeb at the concentration of 1000, 500, 100, 10, and 0 ppm in feed supplied *ad libitum* for 104 weeks. At the end of the treatment, animals were kept under controlled conditions until spontaneous death. There were no differences between treated groups and controls in food or water consumption, body weight, survival or behaviour up to the top dose of 1000 ppm. At post mortem, there were no treatment related effects in non-neoplastic histopathological findings. Increases in the occurrence of benign and malignant tumours compared to controls were observed in a number of organs. Total

malignant mammary tumours were increased in all mancozeb treated females; zymbals gland and ear duct carcinomas were increased at 1000 ppm in males and overall, the incidence of head and neck carcinomas was increased in a dose-related manner in males and in females of all treated groups. Hepatocellular carcinomas were increased for males at 1000 ppm and the incidence of pancreatic malignant tumours was increased in males and females treated with 100, 500 or 1000 ppm mancozeb. Malignant thyroid gland tumours were increased in males at 500 and 1000 ppm, and in females at 10, 100 and 1000 ppm mancozeb. Increases in haemolympho-reticular neoplasia were also observed in treated groups.

Incidence of Animals with Neoplasms (%) – Males

	0 ppm	**10 ppm**	**100 ppm**	**500 ppm**	**1000 ppm**
Zymbal gland carcinomas	1.3	1.3	5.3	8.0	16.0**
Ear duct carcinoma	2.7	10.7	6.7	9.3	13.3*
Overall total carcinomas of head and neck	6.7	16##	16##	25.3**##	33.3**##
Hepatocarcinomas	0	0	1.3	1.3	5.3
Pancreas – exocrine adenocarcinoma - Islet cell carcinoma	0 0	0 0	0 1.3	0 2.7	1.3 4.0
Thyroid gland – follicular carcinoma - C-cell carcinoma	0 0	0 0	0 1.3	2.7 0	8.0* 2.7
Osteosarcoma in bones of the head	2.7	0	0	1.3	1.3
Haemolympho-reticular neoplasia	21.3	29.3	42.7**	46.7**	40.0*

* p<0.05 and **p<0.01 using χ^2 test. ## p<0.01 using Cochrane-Armitage test for dose response relationship

Incidence of Animals with Neoplasms (%) – Females

	0 ppm	**10 ppm**	**100 ppm**	**500 ppm**	**1000 ppm**
Mammary tumours - Fibroma and fibroadenoma - Adenocarcinoma - Fibrosarcoma - Liposarcoma	 46.7 4.0 0 0	 54.7 6.7 0 0	 40.0 12.0 0 0	 42.7 10.7 0 0	 57.3 6.7 2.7 2.7
Overall total carcinomas of head and neck	9.3	32.0**	17.3	25.3*	28.0**
Pancreas – exocrine adenocarcinoma - Islet cell carcinoma	0 0	0 1.3	0 4.0	0 2.7	1.3 0
Thyroid gland – follicular carcinoma - C-cell carcinoma	0 0	1.3 0	0 1.3	0 1.3	16.0** 2.7
Osteosarcoma in bones of the head	0	1.3	0	0	1.3
Haemolympho-reticular neoplasia	14.7	26.7	36.0**	28.0	21.3

* p<0.05 and **p<0.01 using χ^2 test

Overall, rats treated with mancozeb for 104 weeks and then fed control diets until spontaneous death showed a significant increase in the total tumours and in certain specific tissue/tumour types compared to controls, in some cases even from the lowest dose of 10 ppm. The paper concludes that mancozeb should be considered as a multi-potent carcinogenic agent capable of producing tumours of many types in various sites in treated animals. Most of the tumours in this study arose after 112 weeks of age (animals were 8 weeks old at the start of dosing) and the authors state it is unlikely they would have observed the multi-potent carcinogenic activity of mancozeb if the study had ceased after 104 weeks of treatment. Therefore, the unusual design of the study and absence of any historical control data mean that it is not possible to interpret the dose response and hence the biological significance of these tumours. The conclusion of the authors that mancozeb should be considered as a multi-potent carcinogen is inconsistent with the findings from the available regulatory carcinogenicity studies on mancozeb. In addition, it is noted that no information on the purity of the test material was reported, further reducing the validity and reliability of the findings from this publication. This study was available at the time of the first review of mancozeb under Dir 91/414 and did not lead to classification for carcinogenicity.

10.7.2 Carcinogenicity studies in mice

The carcinogenicity of mancozeb was investigated in two 18 month carcinogenicity studies in mice.

Anonymous (1991)

In an 18 month carcinogenicity study in mice there were no abnormal clinical signs throughout the study up to the top dose of 1000 ppm. Survival of all mancozeb-treated groups was comparable to that of controls, except for a higher survival observed in the mid-dose males (100 ppm). Mean weekly body weights for males and females of the 1,000 ppm dose group were consistently lower and statistically different from controls. Food consumption was not affected by treatment at any concentration levels. Some light, but statistically significant differences in red blood cell parameters at 12 months were not apparent at 18 months, and were therefore not considered to be associated with treatment. Thyroid function assays showed significant decreases in mean T4 levels in the high dose males and females at 12 months and in females at 18 months. Mean T3 levels in the high dose males were significantly increased at 18 months and significantly decreased in females at 18 months. The depression in T4 values was considered to be related to mancozeb treatment. Changes in T3 values could not be unequivocally related to treatment. TSH levels were not affected. Necropsy revealed no consistent gross lesions that could be attributed to the test substance, and no consistent differences were demonstrated in organ weights. Microscopic examination of the tissues revealed no treatment-related lesions. There were no statistically significant differences in the incidences of tumours lesions between treated and control animals.

Anonymous (1992b)

In another 18 month carcinogenicity study, there were no compound-related mortality or clinical signs up to the top dose of 1000 ppm. Reduced body weights and lower body weight gains in males and females of the high dose group were not statistically significant. Food consumption and differential blood counts were comparable between treated and control groups. There were no remarkable intergroup differences for organ weights. There were no macroscopic or microscopic findings that could be attributed to compound administration. Under the conditions of this study, dietary administration of 1000 ppm mancozeb (88.6% pure) to Charles River CD-1 mice for 78 weeks resulted in minimal signs of systemic toxicity and no mortality or evidence of carcinogenicity.

10.7.3 Other studies relevant to carcinogenicity

Two 2-generational studies were carried out. These provide additional information on the carcinogenic potential of mancozeb. Further details of these studies are available in the RAR (renewal assessment report), section B6 produced by the UK for the purposes of renewal under Regulation 844/2012.

Anonymous (1988b)

In a two-generation study in rats, there were no treatment-related clinical signs or deaths in the adults of either generation. At the top dose of 1200 ppm, mean body weights for male and female parental rats of both generations were significantly below controls throughout the pre-mating period, and body weights remained depressed among first and second-generation females throughout the gestation and lactation periods. Mean food consumption at this level was also significantly decreased throughout the pre-mating period in male and female parents of the first but not the second generation. Thyroid follicular adenomas were seen in the top dose males of both generations.

Anonymous (1992c)

In a two-generation study in rats there were no compound-related deaths or clinical signs of toxicity evident for either generation up to the top dose of 1100 ppm. Body weights and body weight gains were significantly reduced in males and females of the high-dose group. Food consumption was significantly decreased for both sexes during the premating period of both generations at the high dose. Food consumption for F_1 females was also significantly decreased at the high dose during the period of gestation and lactation. At 1100 ppm mean thyroid weight was markedly increased in the males of both generation and moderately increased in females of both generations. Histopathology, however, revealed thyroid hyperplasia and hypertrophy in nearly all parental animals of the F_0 and F_1 generation at the high dose. Thyroid follicular cell adenomas were also found

in five males of the F_0 generation and in 11 males of the F_1 generation at the high dose. Overall thyroid follicular adenomas were seen in the top dose males of both generations.

Additional investigative studies from the literature review

A long term study into the effects of a mixture of pesticides was carried out by Isael Perez-Carreon *et al* 2009. Mancozeb in a mixture of 12 pesticides were administered by oral gavage at a dose of 25 mg/kg bw, on three consecutive days. Four protocols were followed:

A) gavage five times a week during 8 weeks with 0.5 ml of pesticide or control suspensions, week 4 single dose DEN, week 5 days 1 to 3 three daily doses 2AAF, week 5 day 4 partial hepatectomy;
B) gavage five times a week during 8 weeks with 0.5 ml of pesticide or control suspensions, week 4 single dose DEN, week 5 day 4 partial hepatectomy;
C) gavage five times a week during 8 weeks with 0.5 ml of pesticide or control suspensions, week 5 days 1 to 3 three daily doses 2AAF, week 5 day 4 partial hepatectomy;
D) gavage five times a week during 8 weeks with 0.5 ml of pesticide or control suspensions, week 5 day 4 partial hepatectomy.

No significant differences in tumour promoting activity between animals treated with the pesticide mixture (including mancozeb) and controls were identified, as evaluated using gamma-glutamyl transpeptidase (GGT) positive altered hepatocyte foci, as well, protein and mRNA levels of glutathione S-transferase P (GSTP) in liver extracts. Overall, the pesticide mixture (including mancozeb) evaluated in this report did not have tumour promoting activity in this rat medium-term liver carcinogenesis assay.

A study by Valentich *et al* 2006 investigated the effect of co-administration of mancozeb and phenobarbital on nitrosomethylurea-induced pancreatic tumours in rats. No clear effects of mancozeb and phenobarbital were seen; however the findings were inconclusive and no information on systemic toxicity was reported.

A study by Shilpa *et al* 2011 aimed to establish the early proteomic signature of mancozeb exposure in a short-term assay in mouse skin and *in vitro* models using human skin cell line. In skin samples taken from mice exposed to acetone (control) or mancozeb (200 mg/kg bw) the authors found that two proteins, namely, S100A6 (Calcyclin) and S100A9 (Calgranulin-B) were significantly up-regulated. These proteins are markers of keratinocyte differentiation and proliferation and the authors suggested that they may play a role in mancozeb-induced neoplastic alterations. It is noted that the group size for this study was small i.e. only 4 mice. The *in vitro* part of this study used HaCaT cells as a model for human skin keratinocyte carcinogenesis but was considered inappropriate due to the use of DMSO as a vehicle.

10.7.4 Short summary and overall relevance of the provided information on carcinogenicity

The carcinogenicity of mancozeb has been investigated in five life-time feeding studies, three in rats and two in mice. The majority of these studies are adequate to assess the carcinogenic potential of mancozeb in rodents. At the top doses administered (up to 31/40 (M/F) mg/kg bw/day in rats and 130/180 (M/F) mg/kg bw/day in mice), systemic toxicity was demonstrated with decreases in body weights and body weight gains.

Table 17: Thyroid follicular tumour incidences in rats administered mancozeb

Study/ Reference	Dose mancozeb ppm (mg/kg bw/day; males/females)	Tumour incidence (% tumour bearing animals at study termination)	
		Males	Females
Carcinogenicity Anonymous (1990)	0	0A, 0C [a]	1.6A, 0C
	20 (0.7/1.1)	1.6A, 0C	1.7A, 0C
	60 (2.3/3.1)	1.7A, 3.3C	1.6A, 0C
	125 (4.8/6.7)	0A, 3.4C	1.6A, 1.6C
	750 (30.9/40.2)	32.8A*, 23.0C*	9.8C*, 6.6C*
Carcinogenicity Anonymous (1992a)	0 to 454 (0-16.8/20.8)	No tumours observed at any doses	
2-Generation Anonymous (1988b)	0	No tumours (F0 & F1)	No tumours (F0 & F1)
	30 (1.73-2.49)	No tumours (F0 & F1)	No tumours (F0 & F1)
	120 (6.95-10.52)	No tumours (F0 & F1)	No tumours (F0 & F1)
	1200 (68.9-114.26)	12.0A (F0), 16.6A (F1)	No tumours (F0 & F1)
2-Generation Anonymous (1992c)	0	No tumours (F0 & F1)	No tumours (F0 & F1)
	1100 (74)	20.0A (F0), 44.0A (F1	4.0A (F0), 0A (F1)

[a]A=adenomas, C=carcinomas
*Statistically significant ($P \leq 0.05$ as described in the study report). Note that the statistical significance of histopathological findings was not reported in Anonymous (1992c).

Three literature studies have also investigated the carcinogenicity or co-carcinogenicity of mancozeb (Belpoggi *et al*, 2002; Perez-Carreon *et al*, 2009; Valentich *et al*, 2006). However, the poor reporting and the lack of information on the purity and stability of mancozeb and the lack of historical control data means that no clear conclusions can be drawn from these studies.

Overall, the main effect of mancozeb chronic exposure in rats and mice was thyroid toxicity. Rats appeared to be more sensitive to these effects than mice. Thyroid effects were observed from a dose of 16.8/20.8 mg/kg bw/d in rats and from a dose of 130/180 mg/kg bw/d in mice. Thyroid follicular tumours (carcinomas and adenomas) were also seen in male and female rats at the top dose of 31/40 mg/kg bw/d (Anonymous, 1990), but not in mice up to doses (130/180 mg /kg bw/d) causing generalised and thyroid toxicity (decreases in T4). The tumorigenic effect of mancozeb in rats is attributable to the metabolite ETU, a well-established thyroid toxicant. ETU inhibits the synthesis of thyroid hormones in the thyroid follicular cells, reducing blood levels of T4 and thereby stimulating the release of increased TSH levels by the pituitary as part of the endogenous feedback-control mechanism. Thyroid tumours occur in the rat when the threshold for pituitary-thyroid feedback is exceeded on a chronic basis resulting in an over-stimulation of the thyroid and subsequent development of proliferative lesions.

The association between exposure to mancozeb and endocrine-related outcomes has been investigated both in medical and epidemiology studies of workers handling mancozeb and of the general public who may be exposed to it. Many studies have not distinguished between EBDC pesticides in general and mancozeb. ETU in urine or blood has also been used as a biomarker of EBDC exposure. The DAR (2000) under Directive 91/414 contains five medical studies where data from workers in mancozeb/EBDC manufacturing plants were analysed to determine whether there was any evidence of thyroid dysfunction. None of these studies found any such evidence. More recent epidemiology studies have also examined the possible association between mancozeb (EBDC) exposure and effects on the thyroid. The quality of these studies varies widely and several are contradictory. The overall conclusion from these epidemiology and medical studies is that environmental or workplace exposure to mancozeb does not disrupt the thyroid hormonal system in humans and is not associated with thyroid tumours in humans.

Two studies have examined the association between prostate cancer and exposure to mancozeb and found no increased risk. This is in concordance with the absence of effects on the androgen hormonal system in experimental animals and *in vitro*.

The possible association of mancozeb exposure with various types of cancer (melanoma, colorectal, leukaemia and gastric) has also been investigated in humans. Overall, no association was found between occupational exposure to mancozeb and colorectal or gastric cancer. An increased risk was reported for melanoma and leukaemia. The significance of this association is questionable, especially considering that such findings are not supported by the animal data or by any other epidemiology study and can be explained by confounding.

10.7.5 Comparison with the CLP criteria

Overall, thyroid follicular tumours (carcinomas and adenomas) were seen in male and female rats at the top dose of 31/40 mg/kg bw/d (Anonymous, 1990), but not in mice up to doses (130/180 mg/kg bw/d) causing generalised and thyroid toxicity (decreases in T4). Male rats appeared to be more sensitive than female rats, with carcinomas being seen in 23% of the males (vs 0% in controls) and in 7% of the females (vs 0% in controls) and with adenomas being seen in 33% of the males (vs 0% in controls) and in 10% of the females (vs 1.6% in controls). Thyroid tumours in rats were associated with thyroid toxicity (effects on thyroid hormones, thyroid hypertrophy and hyperplasia) also occurring at the top dose of 31/40 mg/kg bw/d. Thyroid follicular adenomas were also seen in two rat multi-generation studies mainly in adult males of the F0 and F1 generations.

Evidence from mutagenicity studies indicates that a genotoxic mode of action can be excluded for these thyroid tumours in rats. Also, there is no evidence from human medical surveillance or epidemiology studies that mancozeb exposure causes cancer in humans, although exposure levels in human epidemiology studies are intrinsically lower than those in experimental animals.

A clear mode of action (MoA) involving the disturbance of the HPT (hypothalamus-pituitary-thyroid) axis via the metabolite ETU has been identified for these tumours (for further details of studies supporting this MoA, refer to the RAR, section B6, produced by the UK for the purposes of renewal under Regulation 844/2012). Mancozeb is metabolised to ETU in all mammals by approximately 7% by weight. ETU inhibits the peroxidase activity of TPO (thyroid peroxidase), preventing iodide oxidation and thus reducing the formation of thyroid hormone pre-cursors. The resulting reduction in circulating T4 (and T3) is detected by the hypothalamus which reacts by increasing TSH levels via the pituitary in an attempt to increase the production of T4. The action of TSH on the thyroid follicular cells leads to hypertrophy (with associated increased thyroid gland weight). Prolonged exposure can eventually result in thyroid follicular cell hyperplasia and development of tumours of the thyroid gland (adenomas and carcinomas).

The HTP axis operates in a similar way in both humans and experimental animals, so a similar mode of action would occur in humans. Accordingly, the relevance to humans of the postulated MoA cannot be excluded according to qualitative differences between species. However, there are important quantitative differences between the physiology of the thyroid and the biochemical response to TSH between rats and humans. The rodent thyroid is far more dynamic than that of humans. The follicular cells appear to be in active synthesis compared to humans where they appear to be quiescent. T4 has a much shorter half-life than in humans (12 h compared to 5–9 days), and constitutive levels of TSH are about 25-fold higher in rats compared to humans. The shorter half-life of T4 is thought to be related to the absence of thyroid binding globulin (TBG) in adult rats, which leads to a higher turnover rate of thyroid hormones. In adult humans, thyroid hormones are tightly bound to TBG in blood. In humans, follicular cell hypertrophy, which may allow the release of hormone from thyroglobulin, has been observed, but hyperplasia is rare (Hill et al, 1998; McClain and Rice, 1999). Vickers et al (2012) compared the effect of PTU (an irreversible TPO inhibitor used to treat hyperthyroidism in humans) on rat and human thyroid slices/lobes *in vitro*. TPO was inhibited in both species with similar IC50s (approximately 5 µM). However, gene expression in the thyroids, following exposure to PTU was different between rats and humans. The expression of many thyroid-specific genes was significantly altered in rats whilst in humans very few thyroid-specific genes were altered. The consequence of these differences is that the thyroid of rats is more responsive than that of humans.

The potency of ETU to the thyroid of experimental animals and humans also depends upon absorption, distribution, metabolism and excretion in those species. The metabolism of ETU seems to be least efficient in rats and more efficient in higher mammals including dogs and humans. Ruddick et al (1977) showed that pregnant rats were less able to metabolise ETU than pregnant mice (approximately 2- fold difference). *In vitro* metabolism studies in several species show that the metabolism of ETU in hepatocytes increases in the following order: rats < mice < humans, with rabbits and dogs being similar to humans (Daston, 1990; Saghir et al, 2005; Zhu, 2015).

Thyroid tumours are a relatively common finding in rat long-term studies, whilst the only known human thyroid carcinogen is ionizing radiation. In addition, there is no clear evidence that hypothyroidism (goitre) in humans progresses to neoplasia and whilst thyroid hypertrophy has been observed in humans, thyroid hyperplasia is rare. Several medical and epidemiology studies have investigated whether there is any association between EBDC exposure and thyroid cancer but have found none (Anonymous, 1976b; Anonymous, 1985a; Anonymous, 1990b; Nordby et al, 2005). In addition, kinetic differences in the metabolism of ETU between rodents and humans strongly indicate that humans are more effective at metabolising ETU than rodents. The company's analysis of this MoA according to the WHO MoA and human relevance framework is presented in Annex 1.

Overall, therefore, the thyroid follicular tumours seen in rats with mancozeb could in theory occur in humans; the question is whether the quantitative differences between rats and humans in critical dynamic and kinetic elements underpinning the thyroid carcinogenic response to mancozeb are so large that the thyroid tumours induced by mancozeb in rats would occur in humans only at very high, unrealistic dose levels, irrelevant for classification. This depends on the carcinogenic potency of mancozeb in rats. Carcinogenic potency is evaluated in the EU by estimating the T25 (the chronic daily dose which will give 25% of the animals tumours at a specific site, after correction for spontaneous incidence, within the standard life-span of that species). There is also a C&L guidance document on thyroid tumours (EC, 1999, ECBI49/99-Add1.Rev2) which proposes that carcinogens are subdivided into three potency groups of high, medium and low potency according to the criteria described below and that substances producing thyroid tumours in rodents with low or medium potency by a clearly established perturbation of the thyroid hormone axis, in general, do not need to be classified for carcinogenicity under the CLP Regulation:

High potency carcinogen - T25 ≤1 mg/kg bw/d;

Medium potency carcinogen - 1 mg/kg bw/d < T25 ≤100 mg/kg bw/d,

Low potency carcinogen - T25 >100 mg/kg bw/d.

The T25 value for the thyroid follicular tumours seen in rats with mancozeb can be calculated from the study by Anonymous (1990a). The lowest dose of mancozeb producing the highest tumour incidence is the top dose in males, where 31 mg/kg bw/d produced adenomas in 33% of rats and carcinomas in 23% of rats. No adjustment for dosing duration is required because dosing was continuous for the lifetime of the rats (104 weeks) and started at 37 days of age. No subtraction for spontaneous tumour incidence is required because the control tumour incidence in males was 0 and no laboratory historical control tumour data are available. Accordingly, the T25 value for mancozeb and adenomas is 23 mg/kg bw/d whilst that for mancozeb and carcinomas is 34 mg/kg bw/d. In both cases, the T25 value places mancozeb clearly into the medium potency carcinogen group. Therefore, mancozeb is a medium potency rat thyroid carcinogen with a clearly established non-genotoxic MoA. As such, the thyroid tumours induced by mancozeb in rats would in theory occur in humans only at very high, unrealistic dose levels, irrelevant for classification. It is actually most likely that these tumours would never develop in humans as there is no clear evidence that hypothyroidism (goitre) in humans progresses to neoplasia and because whilst thyroid hypertrophy has been observed in humans, thyroid hyperplasia is rare. Therefore, mancozeb does not meet the criteria for classification even in the lowest category. Hence, classification for carcinogenicity is not required. This is consistent with the current harmonised classification of mancozeb. It should be also noted that ETU causes thyroid tumours in rats and mice, but it is not classified for carcinogenicity in its harmonised entry.

10.7.6 Conclusion on classification and labelling for carcinogenicity

Not classified (conclusive but not sufficient for classification)

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Three rat and two mouse dietary carcinogenicity studies were summarised in the CLH report. One of the rat studies, the published study by Belpoggi *et al.* (2002) was considered unreliable by the DS due to a non-standard design and lack of information on the purity of the test substance.

Thyroid follicular adenomas and carcinomas were seen in both sexes in the rat study by Anon. (1990a), which was compliant with OECD TG 453 at 31/40 mg/kg bw/d (m/f). These were associated with reduced T4 levels, increased TSH levels and thyroid hypertrophy and hyperplasia. Thyroid follicular adenomas were also seen in two rat two-generation studies. No thyroid neoplasms were observed in mice up to 130/180 mg/kg bw/d (m/f) despite a T4 reduction at the top dose level.

Epidemiological studies did not provide convincing evidence of an association between mancozeb exposure and cancer according to the DS.

The DS elaborated extensively on the human relevance of the rat thyroid tumours induced by mancozeb and concluded that no classification is appropriate. Arguments in support of no classification can be summarised as follows:

- The tumours in rats arise via a non-genotoxic mechanism of action (MoA), namely inhibition of thyroid peroxidase (TPO) by ETU and/or mancozeb leading to disruption of the HPT axis.
- The metabolism of ETU, the metabolite responsible (at least in part) for the thyroid toxicity of mancozeb, is more efficient in humans than in rats.
- Although the mode of action (MoA) is qualitatively plausible for humans, there are large quantitative differences between rats and humans. The rodent thyroid is far more dynamic, with much higher constitutive TSH levels and a high turnover of thyroid hormones. This is related to the absence of thyroxine binding globulin (TBG) in adult rats, whereas in humans, thyroid hormones are tightly bound to TBG in blood.
- Thyroid tumours are a relatively common finding in long-term rat studies, whilst the only known human thyroid carcinogen is ionizing radiation. In addition, there is no clear evidence that human hypothyroidism (goitre) progresses to neoplasia, and whilst thyroid hypertrophy has been observed in humans, thyroid hyperplasia is rare.
- The available epidemiological studies investigating the association between EBDC exposure and thyroid cancer are negative.
- A guidance document on thyroid tumours by European Chemicals Bureau (ECB) (EU Specialized Experts, 1999) proposes that substances producing thyroid tumours in rodents with low or medium potency by a clearly established perturbation of the

thyroid hormone axis do not need to be classified for carcinogenicity. The T25 value for mancozeb-induced thyroid tumours in the rat corresponds to medium potency (RAC notes that the use of the T25 concept for determination of carcinogenic potency has been challenged and is currently being reconsidered at the EU level). The thyroid tumours induced by mancozeb in rats would occur in humans only at very high, unrealistic dose levels, irrelevant for classification.

- The current entry in Annex VI to the CLP Regulation for ETU, which causes thyroid tumours in rats and mice, does not include a classification for carcinogenicity.

Comments received during public consultation

5 MSCAs, 1 industry association and 1 individual commented on this hazard class.

1 MSCA, the industry association and the individual supported the dossier submitter's proposal of no classification for carcinogenicity. The industry association considered that ETU, for which the molecular MoA has been extensively investigated, is responsible for the actions of mancozeb on the thyroid hormonal system. The effects of ETU occur at lower doses than those of mancozeb and ETU is carcinogenic in the rat thyroid and (unlike mancozeb) also in the mouse. The species difference in carcinogenicity is likely to be, at least partly, due to differences in the metabolism of ETU between rats and mice (DAR, 2000). Industry provided supporting documents describing the key events identified as well as weight of evidence analysis considerations for mode of action. Overall, Industry was of the opinion that the significant quantitative kinetic and dynamic factors means that relevance is low and the risk to humans is negligible. The clearly established non-genotoxic MoA, the medium potency and low risk to humans mean that mancozeb should not be classified as a carcinogen, according to Industry.

The other four MSCAs did not share the DS's view on human non-relevance of the mancozeb-induced thyroid tumours in the rat and proposed classification in Category 2. The comments of these MSCAs together with the DS's responses are summarised below.

- There was general agreement that the MoA of the mancozeb-induced thyroid tumours in the rat is inhibition of TPO by ETU resulting in disruption to the HPT axis.
- Contrary to the DS's view, these four MSCAs were of the opinion that the human non-relevance of the proposed MoA has not been sufficiently demonstrated. Some MSCAs also pointed out that only rat thyroid tumours mediated by induction of Uridine 5'-diphospho-glucuronosyl transferase (UDP-glucuronosyltransferase, UGT) (UDPGT) are listed among MoAs not relevant to humans in the current CLP guidance. The DS considered the fact that the ECB document is referenced in the CLP guidance as an indication that it is still considered applicable and pointed out that the ECB's recommendation is not specific to tumours mediated by UDPGT induction. The DS also expressed an opinion that hazard classification should always represent a 'realistic hazard' to human health.
- The MSCAs emphasised that the lack of positive evidence from epidemiology studies does not imply that the animal findings should be disregarded for classification. In addition, there are epidemiological data suggesting a relationship between hypothyroidism and increased risk of thyroid cancer (European Commission, 2017).
- One MSCA reminded that the current harmonised entry of ETU has simply been translated from a previous classification under Directive 67/548/EEC. The DS replied that the carcinogenicity criteria have not changed from Directive

67/548/EEC to Regulation 1272/2008 and added that no new data relevant to the carcinogenicity of ETU are available.

- One MSCA drew attention to a positive mouse dermal carcinogenicity study (Shukla *et al.*, 1990) which was not included in the CLH report (the study is summarised in the background document under 'Additional key elements'). The DS considered the study unreliable because DMSO was used as the application vehicle. As mancozeb is unstable in DMSO, the DS suggested that the tumours might have originated as a result of exposure to breakdown products produced during the interaction of mancozeb and DMSO, and therefore no conclusions about the carcinogenicity of mancozeb can be drawn from this study.

Additional key elements

Dermal carcinogenicity study in the mouse (Shukla et al., 1990)

This literature study, not included in the CLH report or the RAR and not requested by EFSA, was mentioned in the public consultation. A brief summary is provided in the following table.

Summary of the dermal carcinogenicity study in the mouse (Shukla *et al.*, 1990)

Type of study; Reference	**Method**	**Observations**
Carcinogenicity, dermal, mouse Shukla *et al.*, 1990	Non-guideline Non-GLP Strain: Swiss Purity: mancozeb technical, min. purity 95% Groups: Group I: untreated control Group II: benzo[*a*]pyrene (BaP), 5 µg in 0.1 mL acetone Group III: mancozeb, 100 mg/kg bw/d in 0.1 mL DMSO Group IV: 0.1 mL acetone Group V: 0.1 mL DMSO Treatment 3 times per week 20 females/group (no males) Study terminated after 60 weeks due to low survival in the mancozeb group Only grossly visible tumours taken for histological examination	<u>Non-neoplastic findings</u> Mancozeb (Group III): • Reduced food consumption and body weight; bw loss from week 30 • Sluggish movement • Markedly reduced survival; at week 60 survival 6/20 vs 16-17/20 in Groups I, IV and V • Loss of fur at the treated site (in most animals), scaly skin and total baldness at the treated site (in some animals) BaP (Group II): • Survival at week 60: 12/20 vs 16-17/20 in controls <u>Neoplastic findings</u> Control groups (I, IV, V): none BaP (Group II) and Mancozeb (Group III): benign skin tumours (**squamous cell papillomas, keratoacanthomas**) at the treated site; no. of tumour bearing animals after 48 weeks: BaP 12/12, mancozeb 5/14

Assessment and comparison with the classification criteria

Rat studies

The available carcinogenicity studies in the rat are summarised in the following table.

Rat carcinogenicity studies		
Type of study; Reference	**Method**	**Observations**
2-year chronic toxicity/carcino-genicity, dietary Anon. 1990a	OECD 453 GLP Doses: 0, 20, 60, 125, 750 ppm; equivalent to 0, 0.77/1.1, 2.3/3.1, 4.8/6.7, 31/40 mg/kg bw/d (m/f) 1-year: 10/sex/dose 2-year: 62/sex/dose	Non-neoplastic findings 750 ppm (31/40 mg/kg bw/d): • Minor reductions in bw and bw gain (bw gain reduced by approx. 10%) • ↓ T4 (by approx. 20-50%) • ↑ TSH (approx. 1.4/1.7-fold m/f) • ↑ thyroid weight • Thyroid follicular cell hypertrophy/hyperplasia • Eye bilateral retinopathy ≤ 125 ppm (4.8/6.7 mg/kg bw/d): no toxicologically significant treatment-related effects Neoplastic findings 750 ppm (31/40 mg/kg bw/d): **thyroid follicular cell adenomas and carcinomas** ≤ 125 ppm (4.8/6.7 mg/kg bw/d): None Incidences of the histopathological findings in the thyroid are provided in a separate table below
2-year chronic toxicity/carcino-genicity, dietary Anon. 1992a	OECD 453 GLP Doses: 0, 28, 113, 454 ppm; equivalent to 0, 1.0/1.3, 4.0/5.1, 17/21 mg/kg bw/d (m/f) 1-year: 10/sex/dose 2-year: 50/sex/dose Blood sampling: 10/sex/dose	Non-neoplastic findings 454 ppm (17/21 mg/kg bw/d): • Minor reductions in bw and bw gain (bw reduced by approx. 5%, bw gain by approx. 7%) • ↓ T4 (by approx. 10-40%) • No stat. significant increase in TSH • Prominent microfollicles (only males, borderline stat. sign.) ≤ 113 ppm (4.0/5.1 mg/kg bw/d): • No toxicologically significant treatment-related effects Neoplastic findings None
Carcinogenicity, dietary	Non-guideline Non-GLP	Non-neoplastic findings ≤ 1000 ppm (≈ 50 mg/kg bw/d): none (thyroid function not investigated); survival not affected

Belpoggi *et al.* 2002	Animals treated for 2 years but observed until spontaneous death Doses: 0, 10, 100, 500, 1000 ppm; equivalent to approx. 0, 0.5, 25, 50 mg/kg bw/d (based on default assumptions) 75/sex/dose	Neoplastic findings According to the authors and the DS: increased incidence of benign and malignant tumours in a number of organs at all doses According to RAC: **thyroid follicular cell adenomas and carcinomas, Zymbal gland carcinomas;** incidences are provided in a separate table below

2-year chronic toxicity/carcinogenicity study, Anon. (1990a)

In this guideline study conducted in Crl:CD BR rats, a highly significant ($p < 10^{-4}$) increase in the incidence of thyroid follicular cell adenomas and carcinomas was observed in top dose males. An increase was also observed in females but it did not reach statistical significance. Historical control data were not available in the RAR. The incidences of microscopic findings in the thyroid are provided in the table below together with three sets historical control data from literature (Lang, 1992; McMartin *et al.*, 1992; Chandra *et al.*, 1992). The incidence of both benign and malignant thyroid tumours in top dose males and females exceeded the published HCD.

Incidences of the histopathological findings in the thyroid in the study Anon. (1990a)						
Dietary conc. (ppm)	**0**	**20**	**60**	**125**	**750**	**HCD# mean (range)**
Males						
Systemic dose (mg/kg bw/d)	0	0.8	2.3	4.8	31	
Number examined	60	62	61	58	61	
Thyroid follicular cell adenoma	0 (0%)	1 (1.6%)	1 (1.6%)	0 (0%)	20* (33%)	A: 5.6% (0–26%) B: 3.9% (0–8.6%) C: 0.8%
Thyroid follicular cell carcinoma	0 (0%)	0 (0%)	2 (3.3%)	2 (3.4%)	14* (23%)	A: 1.3% (0–6.0%) B: 2.2% (0–5.0%) C: 0.7%
Thyroid follicular cell hyperplasia/hypertrophy	1	1	2	1	34*	
Thyroid follicular cell hyperplasia nodular	0	1	3	2	15*	
Females						
Systemic dose (mg/kg bw/d)	0	1.1	3.1	6.7	40	
Number examined	62	60	62	61	61	
Thyroid follicular cell adenoma	1 (1.6%)	1 (1.7%)	1 (1.6%)	1 (1.6%)	6 (9.8%)	A: 2.6% (0–15%) B: 1.5% (0–3.4%)

						C: 0.4%
Thyroid follicular cell carcinoma	0 (0%)	0 (0%)	0 (0%)	1 (1.6%)	4 (6.6%)	A: 1.1% (0–5.8%) B: 1.4% (0–4.3%) C: 0.6%
Thyroid follicular cell hyperplasia/hypertrophy	1	0	1	0	24*	
Thyroid follicular cell hyperplasia nodular	1	2	2	0	11*	

* Statistically significant difference from control, $p < 0.05$ (Fisher's test)

[#] A = Lang (1992): Crl:CD BR rats, 19 studies (2-year) beginning from 1984 to 1989, 7 laboratories; since the results come from different laboratories, the criteria used for the diagnosis varied from study to study

B = McMartin *et al.* (1992): Sprague-Dawley rats from Charles River Laboratories, 9 studies (2-year) conducted between 1984 and 1991, 1 laboratory

C = Chandra *et al.* (1992): Sprague-Dawley rats from Charles River Laboratories, 17 studies (2-year) conducted "over the last 6 years" (presumably ≈ 1985-1991), 1 laboratory

The other thyroid-related findings at the top dose are consistent with thyroid-pituitary feedback homeostasis disruption. These include reduced T4 levels, increased TSH levels, thyroid follicular hypertrophy and hyperplasia, and increased thyroid weight. No such effects were present at lower doses.

General toxicity at the high dose was limited to reduced body weight gain by approx. 10%; in addition to thyroid effects, an increased incidence of retinopathy was observed at this dose. RAC is of the opinion that MTD was not reached in this study.

2-year chronic toxicity/carcinogenicity study, Anon. (1992a)

No treatment-related neoplastic effect was observed in this guideline study up to the top dose of approx. 20 mg/kg bw/d. The T4 reduction (by about 20%) at the high dose was not associated with a detectable increase in TSH or significant histopathological findings. RAC notes that general toxicity in this study was limited to slight reductions in body weight (by approx. 5%) and body weight gain (by approx. 7%), which indicates that the top dose was not sufficiently high. The positive study by Anon. (1990a) employed an approximately 2-fold higher top dose.

Carcinogenicity study, Belpoggi et al. (2002)

This study was carried out by the Ramazzini Institute (RI). The most important difference between the RI studies and other research studies is the duration of observations. In the RI cancer bioassays, a substance is administered for 2 years but animals are observed until spontaneous death.

Gift *et al.* (2013) presented a detailed analysis of the specifics of RI studies and reported the outcome of an on-site visit by an independent pathology team sponsored by US EPA and NTP. They concluded that the studies are generally well-performed although the reporting is not as detailed as required for GLP studies. However, RI data on lymphomas/leukaemias and inner ear and cranium neoplasms were considered unreliable due to confounding by end-of-life respiratory infections. As to the observation of animals until spontaneous death, Gift and

co-workers highlighted the advantage of increased sensitivity for detection of late-developing tumours and mentioned some caveats associated with this study design.

According to Belpoggi *et al.* (2002), increased tumour incidences were seen in multiple tissues in this study, in some cases already from the lowest dose level (≈ 0.5 mg/kg bw/d). Detailed results are provided in the background document under 'Supplemental information'. RAC examined the data in the publication and found a clear increase in the incidence of thyroid follicular cell tumours (adenomas and carcinomas) in both sexes and of Zymbal gland carcinomas in males at the top dose; the results are summarised in the table below. Non-significant increases in malignant tumours were seen in several other tissues (liver, mammary gland, and pancreas) but these are difficult to interpret in the absence of historical control data.

Incidences of statistically significant and dose-related neoplastic findings in the study by Belpoggi *et al.* (2002) (lymphomas and leukaemias not included)

	Males					**Females**				
Dietary conc. (ppm)	**0**	**10**	**100**	**500**	**1000**	**0**	**10**	**100**	**500**	**1000**
Systemic dose (mg/kg bw/d; based on a default conversion factor)	0	0.5	5	25	50	0	0.5	5	25	50
Number examined	75	75	75	75	75	75	75	75	75	75
Thyroid follicular cell adenoma	0	0	1	3	10**	1	2	3	0	9*
Thyroid follicular cell carcinoma	0	0	0	2	6*	0	1	0	0	12**
Zymbal gland carcinoma	1	1	4	6	12**	1	6	4	6	5

Significantly different from control: *, $p < 0.05$; **, $p < 0.01$

This study shows a significant increase in the incidence of thyroid follicular cell adenomas and carcinomas in the top dose (≈ 50 mg/kg bw/d) males and females. Both sexes were affected to a similar extent, in contrast to the study of Anon. (1990a), where the effect was more pronounced in males. No statistically significant increase was present at half of the top dose (≈ 25 mg/kg bw/d), although the incidences in males were slightly elevated above the zero incidence in controls. No non-neoplastic findings were observed according to the authors; however, non-neoplastic effects were obviously not the focus of this non-guideline study and may not have been included in the investigations.

In addition to thyroid tumours, an apparently dose-related increase in the incidence of Zymbal gland carcinomas was noted in males which was statistically significant at the top dose. The Zymbal gland has no human counterpart but tends to be a target of potent genotoxic carcinogens. The incidences in females do not show a dose-response relationship and suggest a high background incidence. As mancozeb is not considered genotoxic, the Zymbal gland carcinomas in the top dose males are of questionable toxicological significance.

Body weight and survival were not affected according to the publication.

Industry considered interpretation of the results of this study difficult due to the observation of animals until spontaneous death and lack of historical control data, and they therefore did not include this study in their MoA analysis (CLH report, Annex I).

Two-generation studies

Two 2-generation studies (Anon., 1988b; Anon., 1992c) provided additional information on the carcinogenic potential of mancozeb. At the top doses of about 70 mg/kg bw/d, thyroid follicular hyperplasia was observed in almost all parental animals, and follicular adenomas in some animals (mostly males) of both generations (incidences are provided in the table below). This is a clear evidence of reduced tumour latency.

Incidence of thyroid follicular cell hyperplasia and thyroid follicular cell tumours in the two-generation studies with mancozeb

Anon. (1988b)

		Males		**Females**	
	Dose (mg/kg bw/d)	**0**	**≈ 69**	**0**	**≈ 69**
F0	Hyperplasia	0 (0%)	25 (100%)	0 (0%)	22 (88%)
	Adenoma	0 (0%)	3 (12%)	0 (0%)	0 (0%)
F1	Hyperplasia	2 (8%)	24 (100%)	0 (0%)	24 (100%)
	Adenoma	0 (0%)	4 (17%)	0 (0%)	0 (0%)

Anon. (1992c)

		Males		**Females**	
	Dose (mg/kg bw/d)	**0**	**≈ 74**	**0**	**≈ 74**
F0	Hyperplasia/hypertrophy	0 (0%)	25 (100%)	0 (0%)	24 (96%)
	Adenoma	0 (0%)	5 (20%)	0 (0%)	1 (4%)
F1	Hyperplasia/hypertrophy	0 (0%)	23 (92%)	0 (0%)	25 (100%)
	Adenoma	0 (0%)	11 (44%)	0 (0%)	0 (0%)

Mouse studies

Two mouse dietary carcinogenicity studies are available but one of them (Anon., 1992b) only as a brief summary in a review. These studies are summarised in the table below. In addition, a dermal carcinogenicity study (Shukla *et al.*, 1990) mentioned during the public consultation of the CLH report is summarised in the background document (under 'Additional key elements').

Mouse dietary carcinogenicity studies		
Type of study; Reference	**Method**	**Observations**
18-month carcinogenicity, dietary Anon. 1991a	OECD 451 GLP Doses: 0, 30, 100, 1000 ppm; equivalent to 0, 3.8/5.2, 13/18, 131/180 mg/kg bw/d (m/f) 1-year: 20/sex/dose 1.5-years: 70/sex/dose	Non-neoplastic findings 1000 ppm (131/180 mg/kg bw/d): • Minor reductions in bw and bw gain (bw gain reduced by 13% in males and by 10% in females) • ↓ T4 (by approx. 25-75%) • No histopathological findings ≤ 100 ppm (13/18 mg/kg bw/d): • No toxicologically significant treatment-related effects Neoplastic findings None
18-month carcinogenicity, dietary Anon. 1992b Available only as a summary from a WHO/FAO review	Doses: 0, 25, 100, 1000 ppm; equivalent to 0, 4.3, 17, 170 mg/kg bw/d 60/sex/dose	Non-neoplastic findings 1000 ppm (170 mg/kg bw/d): • Minor reductions in bw and bw gain ≤ 100 ppm (17 mg/kg bw/d): • No toxicologically significant treatment-related effects Neoplastic findings • 1000 ppm (170 mg/kg bw/d): Liver adenomas (males only, adenomas 17/50 vs 8/50 in the control; adenomas + carcinomas 17/50 vs 10/50 in the control, not stat. sign.)

Dietary carcinogenicity studies, Anon. (1991a) and Anon. (1992b)

The top doses in the two oral studies ranged between 130 and 180 mg/kg bw/d. The study of Anon. (1991a) did not report any increase in neoplastic or non-neoplastic findings. Regarding thyroid function, T4 levels were reduced in this study but the T4 reduction did not lead to elevated TSH levels. General toxicity at the top dose was limited to relatively mild reductions in body weight and body weight gain.

The other study (Anon., 1992b), available only as a brief summary, reported a marginally increased incidence of liver adenomas in top dose males. This effect is not considered sufficient for classification, taking into account the lack of statistical significance, lack of an increase in females, no increase in liver tumours in another study at a comparable dose (Anon., 1991a), and the benign nature of the tumours.

However, considering (1) the very limited general toxicity at the top doses in both studies; (2) the limited general toxicity at a 10-fold higher dose in a 90-day study (Anon., 1985c); (3) the occurrence of thyroid hyperplasia in the 90-day study; and (4) the occurrence of thyroid and liver tumours in a mouse carcinogenicity study with ETU (NTP, 1992; for details

see 'Supplemental information' in the background document), RAC concludes that the carcinogenic potential of mancozeb has not been sufficiently investigated in this species and some concern is raised specifically for potential carcinogenicity in the thyroid and the liver.

Dermal carcinogenicity study, Shukla et al. (1990)

In this published non-guideline study, an increased incidence of benign skin tumours (squamous cell papillomas, keratoacanthomas) at the site of treatment was observed at a single dose level of 100 mg/kg bw mancozeb applied in DMSO 3 times a week for 60 weeks. The incidence of skin tumours was lower than in the benzo[*a*]pyrene concurrent positive control group.

General toxicity in the mancozeb group consisted of body weight loss, clinical signs (sluggish movement) and markedly reduced survival leading to termination of the study at 60 weeks.

Tyagi *et al.* (2011) (with Y. Shukla as a co-author) attempted to elucidate the mechanism of skin tumour formation and found increased expression of proteins associated with keratocyte differentiation and proliferation in mouse skin and in a human *in vitro* skin model exposed to mancozeb.

RAC acknowledges that both studies indicate a potential for induction of local benign skin tumours at the doses tested. However, the reliability of these results is difficult to assess in the absence of studies by other research groups attempting to reproduce these findings. In addition, human relevance of tumours seen at doses causing severe general toxicity including reduced survival is questionable. Thus, the skin tumours observed in the study Shukla *et al.* (1990) are not considered to support classification.

Human data on carcinogenicity

No association between thyroid cancer and exposure to mancozeb or ETU has been found in the three epidemiological studies available (Smith, 1976; Anon., 1976b; Nordby *et al.*, 2005). However, this cannot be used as an argument to negate the animal findings as the exposure levels in the exposed human subjects are likely to have been considerably lower than the dose levels causing thyroid tumours in rats. In addition, all three epidemiological studies have their limitations (e.g., relatively small sample sizes in Smith, 1976 and Anon., 1976b; crude exposure indicators in Nordby *et al.*, 2005).

As for other types of cancer, Dennis *et al.* (2010) reported an association between cutaneous melanoma and maneb/mancozeb exposure in a good-quality cohort study (Agricultural Health Study). However, the study authors acknowledged the possibility that their pesticide-specific results are driven by sun exposure because it is a strong risk factor for melanoma which is quantitatively difficult to capture via a questionnaire. Because of this limitation, RAC does not consider this study to support classification.

Mills (2005) reported an association between leukaemia and mancozeb exposure in a nested case-control study. This study, however, has several limitations including imprecise exposure indicators (estimate of the usage of a pesticide in the counties where subjects were employed as a surrogate for exposure) and lack of information on potential confounders.

The rest of the studies were negative.

Overall, RAC does not find in the available epidemiology studies with mancozeb or ETU any evidence that could either support classification or question the human relevance of the neoplastic findings seen in the animal studies.

Human relevance of the thyroid tumours in the rat

Mancozeb induced thyroid follicular adenomas and carcinomas in the rat (Anon., 1990a; Belpoggi *et al.*, 2002). Mancozeb is not considered mutagenic. Apart from genotoxicity, thyroid follicular tumours may arise in the rat via the following mechanisms (IARC, 1999):

1. Inhibition of iodine uptake at the Na^+/I^- symporter
2. Interference with TPO-stimulated organification of iodine
3. Stimulation of T4 clearance (e.g., via induction of hepatic UDPGT in rats)
4. Effect on plasma binding of thyroid hormones
5. Effect on deiodinases
6. Receptor-mediated

TPO inhibition is the MoA proposed by the DS and industry, who have provided an extensive MoA analysis (Annex I to the CLH report). Inhibition of pig and rat TPO by ETU has been demonstrated *in vitro* (Doerge and Takazawa, 1990; Freyberger and Ahr, 2006; Paul *et al.*, 2014). Both an increase in TSH and a reduction in plasma T4 have been observed in the rat at the carcinogenic dose levels, which is consistent with the proposed MoA. No induction of liver T4-UDPGT by mancozeb was observed by Flippin *et al.* (2009) in female rats up to doses causing a marked reduction in circulating T4.

RAC agrees with the DS, industry and the commenting MSCAs that TPO inhibition is likely to be the main MoA of the mancozeb-induced thyroid tumours. However, RAC notes that some additional non-genotoxic MoAs which have not been investigated may potentially contribute..

Human relevance of mancozeb-induced thyroid hyperplasia in the rat has been questioned by the DS; the presence of thyroxine-binding globulin (TBG) in humans has been mentioned as one of the arguments. Nevertheless, markedly increased TSH levels (up to 8-fold) and thyroid follicular hyperplasia (incidence 10/10 vs 0/10 in controls) have been observed in rhesus monkeys treated with approx. 19 mg/kg bw/d ETU for 6 months (Leber *et al.*, 1978; further details are provided in the background document under 'Supplemental information' in the carcinogenicity and STOT RE sections). Less marked effects were seen at approx. 6 mg/kg bw/d. Monkeys, like humans, possess TBG.

The minutes from the Specialised Experts (SE) group meeting discussing human relevance of rodent thyroid tumours (EU Specialized Experts, 1999) reported a view of some participants that an increase of TSH in humans does not pose a significant concern regarding potential thyroid carcinogenesis in humans. However, recent meta-analyses have indicated an association between increased serum TSH levels and thyroid cancer in humans (McLeod *et al.*, 2012; Zheng *et al.*, 2016). Unfortunately, because of the cross-sectional design of the meta-analysed studies they were not able to address the question whether TSH level plays a causative role in thyroid cancer pathogenesis. Studies in animal models have shown that growth stimulation by TSH is a necessary, but not a sufficient condition for cancer development; concurrent activation of different MAP kinase pathways is also required for the thyroid cancer to occur (European Commission, 2017). Although our current knowledge about the non-genotoxic mechanisms of thyroid cancer is far from complete, it does raise concern about increased TSH levels with regard to thyroid carcinogenesis in humans.

RAC further notes that unlike the conclusion by the SE group (copied in the background document under 'Supplemental information'), the current CLP guidance (version 5.0) lists only rodent thyroid tumours due to UDPGT induction as potentially not relevant to humans. This recommendation is probably based on the fact that in rodents, T4 is more loosely bound

to carrier proteins and thus is more susceptible to increased hepatic clearance than in humans where a major part of T4 is tightly bound to TBG (cf. the STOT RE section of CLP guidance). RAC also notes that the ECB document does not provide full justification for some modes of action.

To sum up, RAC does not find sufficient evidence to disregard the human relevance of the mancozeb-induced thyroid tumours in the rat. At the same time, RAC acknowledges that humans appear to be quantitatively less sensitive than rats to the induction of malignant thyroid tumours from chronic stimulation of the thyroid by elevated TSH levels (IARC, 1999).

Conclusion on classification

Category 1A is not applicable as there is no convincing evidence of carcinogenic potential in humans.

Evidence of carcinogenicity is available from two rat studies (Anon., 1990a; Belpoggi *et al*., 2002) where mancozeb caused an increased incidence of thyroid adenomas and carcinomas in both sexes. Generally, occurrence of carcinomas in two sexes of one species in two independent studies can trigger classification in Category 1B (CLP, Annex I, 3.6.2.2.3). The absence of thyroid tumours in mice in two independent dietary carcinogenicity studies (Anon., 1991a; Anon., 1992b) was associated with very limited general toxicity, making the results in this species inconclusive for the purpose of hazard assessment. The limited effects observed at the top dose of *ca*. 2000 mg/kg bw/day in the 90-day mouse study (Anon., 1985c) indicates that higher dose levels could have been tested in the mouse dietary carcinogenicity studies. Therefore, due to this selection of the top dose, the mouse studies do not adequately address the carcinogenicity concerns raised by the rat studies. However, a number of factors increasing and decreasing the concern have to be taken into account (CLP, Annex I, 3.6.2.2.6):

Factor	**Evidence**
a) tumour type and background incidence	The tumour type occurs in humans Incidence increased above HCD, background incidence not high
b) multi-site responses	No other relevant tumours in the available studies
c) progression of lesions to malignancy	Yes
d) reduced tumour latency	Yes
e) whether responses are in single or both sexes	Both sexes
f) whether responses are in a single species or several species	Single species (rat); mouse not sufficiently investigated (inconclusive results for the purpose of hazard assessment)
g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity	There is evidence that ETU increases the incidence of liver, thyroid and pituitary tumours in mice and of thyroid tumours in the rat; extrapolation to mancozeb is associated with major uncertainties
h) routes of exposure	Dietary exposure is relevant for humans

i) comparison of ADME between test animals and humans	No data on mancozeb. ETU may be metabolized slightly faster in humans than in rats
j) the possibility of a confounding effect of excessive toxicity at test doses	No
k) MoA and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity	The MoA is relevant for humans Humans are quantitatively less sensitive to the development of malignant thyroid tumours from sustained stimulation by TSH The MoA is non-genotoxic, with a threshold

Considering the factors above, RAC concludes that there is limited evidence of carcinogenicity for mancozeb and classification with **Carc. 2; H351** is appropriate. The main factors decreasing the concern, and thus supporting classification in Category 2 rather 1B, are the non-genotoxic, threshold MoA, associated with a quantitatively lower sensitivity of humans to the development of malignant thyroid tumours from sustained stimulation by TSH, and the absence of tumours at other sites than the thyroid in the available studies with mancozeb. However, RAC points out that there is currently insufficient information on the carcinogenic potential of mancozeb in the mouse and potential liver tumours at doses higher than those tested could trigger a more stringent classification.

Supplemental information - In depth analyses by RAC

Data on tumour increases in the rat carcinogenicity study by Belpoggi *et al.* (2002)

According to the authors, mancozeb produced increased incidence of many tumour types in this study:

1. Total malignant tumours in males and females
2. Malignant mammary tumours in females
3. Zymbal gland and ear duct carcinomas in males; head and neck carcinomas in males and females
4. Hepatocarcinomas in males
5. Malignant pancreatic tumours in males and females
6. Malignant thyroid tumours in males and females
7. Osteosarcomas in bones of the head in males and females
8. Haemolymphoreticular neoplasias (lymphomas and leukaemias) in males and females

Incidences of these tumours are provided in the table below.

Incidences of neoplastic findings in the study by Belpoggi *et al.* (2002)										
	Males					**Females**				
Dietary conc. (ppm)	**0**	**10**	**100**	**500**	**1000**	**0**	**10**	**100**	**500**	**1000**
Systemic dose (mg/kg bw/d; based on a default conversion factor)	0	0.5	5	25	50	0	0.5	5	25	50
Number examined	75	75	75	75	75	75	75	75	75	75
Number of animals bearing malignant tumours	29	39[r]	51[b,r]	55[b,r]	59[b,r]	31	50[b]	42	46[a]	43
Mammary gland fibroma & fibroadenoma[n]	2	4	5	3	3	35	41	30	32	43
Mammary gland lipoma[n]	3	2	2	3	2	0	0	1	0	0
Mammary gland adenocarcinoma[n]	1	0	0	0	1	3	5	9	8	5
Mammary gland fibrosarcoma[n]	0	0	1	0	1	0	0	0	0	2
Mammary gland liposarcoma[n]	2	1	1	1	1	0	0	0	0	2
Zymbal gland carcinoma	1	1	4	6	12[b]	1	6	4	6	5
Ear duct carcinoma	2	8	5	7	10[a]	6	10	7	11	11
Carcinoma of nasal cavity	0	2	1	2	1	0	0	0	2	0
Carcinoma of oral cavity, tongue, lips	2	0	1	3	2	0	3	1	0	4
Carcinoma of the pharynx	0	1	0	0	0	0	1	1	0	1
Carcinoma of the larynx	0	0	1	1	0	0	4	0	0	0
Head and neck carcinomas	5	12[r]	12[r]	19[b,r]	25[b,r]	7	24[b]	13	19[a]	21[b]
Hepatocarcinoma[n]	0	0	1	1	4	1	0	2	0	0
Pancreatic exocrine adenoma[n]	3	0	0	4	0	1	0	0	2	0
Pancreatic islet cell adenoma[n]	11	8	10	10	10	6	5	6	7	5

Pancreatic exocrine adenocarcinoma[n]	0	0	0	0	1	0	0	0	0	1
Pancreatic islet cell carcinoma[n]	0	0	1	2	3	0	1	3	2	0
Thyroid follicular cell adenoma	0	0	1	3	10[b]	1	2	3	0	9[a]
Thyroid follicular cell carcinoma	0	0	0	2	6[a]	0	1	0	0	12[b]
Thyroid C-cell adenoma	2	1	3	4	4	4	5	5	1	7
Thyroid C-cell carcinoma	0	0	1	0	2	0	0	1	0	2
Osteoma[n]	0	0	0	0	0	0	0	0	1	0
Osteosarcoma[n]	2	13	8	5	8	1	8	1	4	3
Hemolymphoreticular neoplasias	16	22	32[b]	35[b]	30[a]	11	20	27[b]	21	16

[a] $p < 0.05$ using χ^2 test; [b] $p < 0.01$ using χ^2 test; [r] $p < 0.01$ using Cochrane-Armitage test for dose-response relationship; [n] statistical analysis not provided

Head and neck carcinomas = carcinomas of Zymbal glands, ear ducts, nasal cavities, oral cavity, tongue, lips, pharynx and larynx

In the view of RAC, this study shows a clear increase in thyroid follicular cell tumours (adenomas and carcinomas) in both sexes at the top dose of 1000 ppm (≈ 50 mg/kg bw/d).

A statistically significant increase in the incidence of Zymbal gland carcinoma and ear duct carcinoma was found in males but not in females. The increase in ear duct carcinoma in males did not show a clear dose-response relationship. There was no dose-dependent increase in the incidence of other "head and neck" tumours.

The haemolymphoreticular neoplasias, increased in males, should be excluded from assessment due to possible confounding by respiratory infections in the performing laboratory (Gift *et al.*; 2013).

The osteosarcomas are unlikely to be related to treatment considering the lack of a dose-response relationship.

The remaining tumour types (mammary gland fibrosarcoma and liposarcoma in females, hepatocarcinoma in males, pancreatic exocrine and islet cell carcinoma) show increases not statistically significant on pairwise comparison (and thus possibly incidental) that are difficult to interpret in the absence of historical control data.

Thyroid follicular cell hyperplasia in the study with ETU by Leber *et al.* (1978)

The incidence and severity of follicular cell hyperplasia in this 6-month study with ETU in rhesus monkey is provided in the table below. The study was comprised of two experiments. Because of the high incidence of tuberculosis in the first experiment (phase I), the study was repeated with a new group of animals (phase II). Only the results of the second experiment (phase II) are reported here.

Incidence and severity of thyroid follicular cell hyperplasia in the study Leber *et al.* (1978)				
Dose (ppm)	**0**	**50**	**150**	**450**
Dose (mg/kg bw/d)	**0**	**≈ 1.9**	**≈ 6.3**	**≈ 19**
Number of animals examined	10	10	10	10
Thyroid follicular cell hyperplasia	0	3	5	10
– mild		3	3	
– moderate			1	4
– marked			1	2
– severe				4

The severity ratings are described in the study report as follows:

- Negative control animals: The follicular lining cells were usually flat and evenly spaced around follicles that were generally medium-to-large sized, regular in shape, and contained well-formed evenly stained colloid.
- Mild hyperplasia: These glands were characterized by medium-to-large sized follicles of generally regular shape lined by cells that were cuboidal-to-columnar in shape, increased in number, and arranged in low-to-high papillary formations which extended into the follicle lumen.
- Moderate hyperplasia: Glands classified as being moderately hyperplastic have thickening of the interfollicular space, increased numbers of columnar lining cells, and numerous papillary fronds extending into the follicular lumen which contain a light eosinophilic colloid.
- Marked hyperplasia: These glands were characterized by reduced colloid space, which generally contained lightly staining colloid. The follicular walls were very irregular in shape due to the formation of papillary fronds and microfollicles.
- Severe hyperplasia: Glands classified as severely hyperplastic had random, medium-sized distorted follicles surrounded by microfollicles that often appeared as sheets of follicular lining cells with a cleft-like lumen nearly devoid of colloid.

<u>Mouse carcinogenicity study with ETU (NTP, 1992)</u>

The top doses in the available mouse carcinogenicity studies with mancozeb were not sufficiently high for the purpose of hazard assessment, making the results inconclusive. Therefore, neoplastic findings from a NTP carcinogenicity study with ETU in B6C3F1 mice are provided in the table below as supporting information.

Dose (ppm)	**0**	**330**	**1000**	**HCD mean (range)**
Dose (mg/kg bw/d)	**0**	**≈ 50**	**≈ 140**	
Males				
Thyroid follicular cell adenoma	0	1	26**	1.4% (0–4.0%)

	(0%)	(2.0%)	(52%)	
Thyroid follicular cell carcinoma	1 (2.0%)	0 (0%)	5 (10%)	0.3% (0–2.0%)
Thyroid follicular cell adenoma or carcinoma	1 (2.0%)	1 (2.0%)	29** (58%)	1.7% (0–4.1%)
Hepatocellular adenoma	11 (22%)	16 (32%)	9 (18%)	38% (18–60%)
Hepatocellular carcinoma	13 (27%)	19 (38%)	45** (90%)	20% (10–40%)
Hepatocellular adenoma or carcinoma	20 (41%)	32* (64%)	46** (92%)	52% (40–68%)
Hepatoblastoma	0 (0%)	1 (2.0%)	6* (12%)	0.2% (0–2.0%)
Pituitary adenoma	0 (0%)	0 (0%)	8** (20%)	0.6% (0–6.3%)
Females				
Thyroid follicular cell adenoma	0 (0%)	2 (4.0%)	35** (70%)	1.7% (0–5.9%)
Thyroid follicular cell carcinoma	0 (0%)	0 (0%)	8** (16%)	0.2% (0–2.0%)
Thyroid follicular cell adenoma or carcinoma	0 (0%)	2 (4.0%)	38** (76%)	1.9% (0–5.9%)
Hepatocellular adenoma	2 (4.0%)	33** (66%)	14** (28%)	23% (12–50%)
Hepatocellular carcinoma	2 (4.0%)	29** (58%)	47** (94%)	11% (4.1–20%)
Hepatocellular adenoma or carcinoma	4 (8.0%)	44** (88%)	48** (96%)	31% (12–56%)
Hepatoblastoma	0 (0%)	0 (0%)	2 (4.0%)	0.2% (0–2.0%)
Pituitary adenoma	10 (22%)	19 (39%)	26** (53%)	9.8% (0–23%)

Statistically significantly different from control: *, $p \leqslant 0.05$; **, $p \leqslant 0.01$

Historical control data: NTP (2019); B6C3F1 mice, 19 dietary studies, studies starting 1984-1994; the present study started in 1982

A statistically significant increase above the HCD in the incidence of hepatocellular carcinomas was observed in lower dose females ($\approx$ 50 mg/kg bw/d). Although this mouse strain has a particularly high background incidence of hepatocellular tumours, the magnitude of the increase (58% vs 4%) is concerning. The higher dose group ($\approx$ 140) additionally showed an increase in thyroid follicular cell adenomas and carcinomas, hepatoblastomas and

pituitary adenomas. However, in the absence of information on the conversion factor from a dose of ETU to an equivalent dose of mancozeb, it is uncertain whether a corresponding dose of mancozeb would be below or above the MTD or below/above the limit dose.

Conclusion of the Specialized Experts group on non-genotoxic rodent thyroid carcinogens

The recommendation on classification of rodent thyroid carcinogens from the ECB document (EU Specialized Experts, 1999) with the accompanying flowchart is copied below. Categories 3 and 2 under DSD correspond to categories 2 and 1B respectively under CLP.

"Conclusion:

The Specialised Experts agreed that there is convincing scientific evidence that humans are considerably less sensitive than rodents (especially rats) regarding

(i) perturbation of thyroid hormone homeostasis induced by non-genotoxic xenobiotics

(ii) development of epithelial thyroid tumours after long-term exposure to such agents.

Furthermore, a majority of the Specialised Experts emphasised their view that, at least for point (i), these interspecies differences are of a quantitative rather than a qualitative nature. Some Specialised Experts underlined their opinion that the possibility of a qualitative difference rat-human still exists, since there is yet no convincing evidence that chemical-induced prolonged thyroid stimulation might cause thyroid-tumours in humans.

Consensus was reached by the Specialised Experts that classification in category 3 or even no classification at all may be appropriate, provided that detailed studies have shown, that an individual non-genotoxic substance causing tumours in rodent thyroid also induces persistent stimulation of the thyroid. For a distinction between classification in category 3 and no classification for carcinogenicity the tumourigenic/ carcinogenic potency of the agent should be considered. The human relevance of the specific mechanism(s) underlying the perturbation of the pituitary-thyroid hormone axis should also be taken into account. The Specialised Experts took note of results from recent research that enhancement of thyroid hormone clearance via induction of conjugating enzymes in the liver (e.g. UDPGT) may not give a satisfactory mechanistic explanation for the thyroid tumours induced by liver enzyme inducing agents such as phenobarbital.

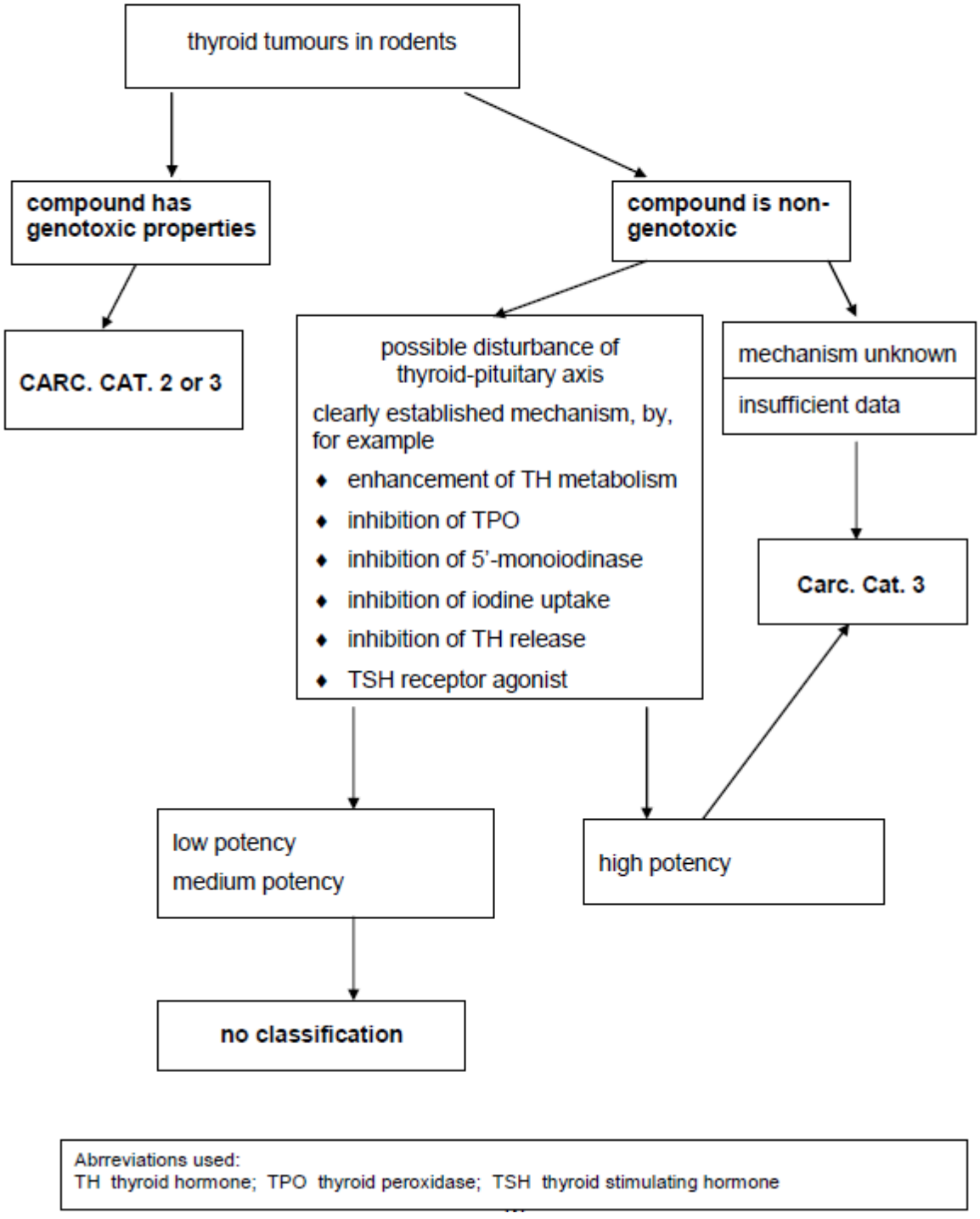
Overall, the Specialised Experts reached a consensus on how to deal with rodent thyroid tumours in the classification for carcinogenicity. A diagram is attached (Annex I), which summarizes the views of the Specialised Experts: Essentially, it was agreed that non-genotoxic carcinogenic substances producing thyroid tumours in rodents with low or medium potency by a clearly established perturbation of the thyroid hormone axis, in general, do not need to be classified. Other rodent thyroid carcinogens merit classification in either category 2 or 3.

The Specialised Experts were aware that such a general recommendation may not be applicable to every substance in question and that decisions should be made on a case by case basis."

ANNEX I

CLASSIFICATION OF SUBSTANCES CAUSING THYROID TUMOURS IN RODENTS

SPECIALISED EXPERTS recommendation



Abrreviations used:
TH thyroid hormone; TPO thyroid peroxidase; TSH thyroid stimulating hormone

10.8 Reproductive toxicity

10.8.1 Adverse effects on sexual function and fertility

Not addressed in this dossier.

10.8.2 Adverse effects on development

The developmental toxicity of mancozeb has been investigated in developmental toxicity studies in rats and rabbits. Five developmental toxicity studies (3 in the rat and 2 in the rabbit) were described in the original DAR (2000) under Directive 91/414/EEC. These studies were conducted in the 1980s and those performed in the rat used severely maternally toxic maximum dose levels (360 - 512 mg/kg bw/d). These rat studies resulted in malformations (mainly of head and neck) at high doses causing severe maternal toxicity. Mancozeb was classified as Reprotox category 2 (H361d) as a result of these studies. The evidence suggested that the malformations seen in the rat with mancozeb were due to its main metabolite, ETU. ETU is an established developmental toxicant (harmonised classification with Repr Cat 1b; H360DD) which causes malformations (mainly of head – skull, brain and spinal cord; neck – cleft plate; tail; and vertebrae) in the rat in the absence of maternal toxicity. Approximately 7% of mancozeb by weight is converted/metabolised to ETU in experimental animals. New regulatory guideline developmental toxicity studies on mancozeb (and ETU – Anonymous, 2015) have been conducted to provide data on additional endpoints and to clarify the developmental effects attributed to mancozeb. In addition, 3 developmental toxicity investigations in the rat from the open literature review are available. The availability of these new studies justifies a re-consideration of the classification of mancozeb for this endpoint.

A developmental neurotoxicity study (OECD TG 426) is also available. This study was conducted to address the concern about the potential relationship between thyroid effects and brain development. An additional open literature publication investigating the developmental neurotoxicity of mancozeb in rats has also been identified.

Table 18: Summary table of animal studies on adverse effects on development

Study, species (strain)	Dose levels and dosing period	Critical effects (Effects statistically significantly different unless stated otherwise)
Developmental toxicity studies in rats		
Developmental toxicity Oral (gavage) EPA OPPTS 870.3700 (predates current version issued 1998); reporting deficiencies Non GLP Rat, BLU (SD)BR 26 mated females/group Vehicle: corn oil purity 83.0% Anonymous, 1980	Mancozeb: 0, 2, 8, 32, 128 or 512 mg/kg bw/day on gestation days 6-15 ETU (positive control): 50 mg/kg bw/day on gestation days 6-15	**Maternal toxicity** <u>512 mg/kg bw/day</u>: 3/22 pregnant terminated prematurely and killed in extremis, , 13/22 with litters at term. Clinical signs of lethargy, scruffy coat, diarrhoea, soft faeces, bloody vaginal discharge, hunched, dehydrated; ↓ body weight gain 95%, days 6-20; ↓ food consumption (2 g, days 10-15 cf. 16 g controls) <u>128 mg/kg bw/day</u>: ↓ body weight gain 39%, days 6-20; ↓ food consumption (12 g, days 10-15 cf. 16 g controls) <u>32 mg/kg bw/day</u>: No effects

Study, species (strain)	Dose levels and dosing period	Critical effects (Effects statistically significantly different unless stated otherwise)
		ETU 50 mg/kg bw/day: No effects
		Developmental toxicity 512 mg/kg bw/day: 6/19 total resorption ↑ number of resorptions (3.77 cf. 0.81 controls); ↓ number live foetuses (7.05 cf. 10.77 controls); ↓ mean foetal weight 27% (2.80g cf. 3.82g controls); ↑ incidence malformation e.g. meningoencephalocele /exencephaly, dilated brain ventricles, cleft palate, kinked/shortened tail 128 mg/kg bw/day: No effects 32 mg/kg bw/day: No effects ETU 50 mg/kg bw/day: ↓ mean foetal weight ↑ incidence malformation e.g. meningoencephalocele /exencephaly, dilated brain ventricles, cleft palate, kinked/shortened tail Developmental NOAEL 128 mg/kg bw/day Maternal NOAEL 32 mg/kg bw/day
Developmental toxicity Oral (gavage) OECD 414 (1981) GLP Rat, CD 25 mated females/group Vehicle: 1% MC purity 88.6% Anonymous, 1988c	0, 10, 60 or 360 mg/kg bw/day on gestation days 6-15	**Maternal toxicity** 360 mg/kg bw/day: 1/25 treatment-related death (killed in extremis) preceded by clinical signs; ↑ incidence of reeling gait, partial use or slight paralysis of hind limbs (5/25 cf. 0 incidence in controls); ↓ body weight gain (25.4% days 6-20); ↓ food consumption during dosing (16.7% days 6-8, 20.8% days 9-11) 60 mg/kg bw/day: No effects **Developmental toxicity**

		<u>360 mg/kg bw/day</u>: ↑ incidence incomplete ossification of interparietal bone (90% foetuses cf. 75.8% controls); ↑ incidence incomplete ossification of thoracic vertebral centra (51.3% foetuses cf. 28.0% controls) ↑ incidence large anterior fontanelle (11.9% foetuses cf. 2.2% controls) <u>60 mg/kg bw/day</u>: No effects Developmental NOAEL 60 mg/kg bw/day Maternal NOAEL 60 mg/kg bw/day

Developmental toxicity Oral (gavage) OECD 414 (1981) GLP Rat, Wistar 24 mated females/group Vehicle: peanut oil purity 86.2% Anonymous, 1999b	0, 100, 225 or 500 mg/kg bw/day on gestation days 6-15	**Maternal toxicity** 500 mg/kg bw/day: No effects 225 mg/kg bw/day: No effects 100 mg/kg bw/day: No effects **Developmental toxicity** 500 mg/kg bw/day: ↑ macroscopic pathology in lungs, liver, kidney: ↑ lung congestion/hyperaemia; liver congestion/mottling ; kidney patchy congestion/congestion ↑ dumbbell shaped thoracic centra (15 foetuses/6 litters cf. 0 incidence in controls) 225 mg/kg bw/day: ↑ macroscopic pathology in lungs, liver, kidney: ↑ lung congestion/hyperaemia; liver congestion/mottling kidney patchy congestion/congestion) ↑ dumbbell shaped thoracic centra (16 foetuses/6 litters cf. 0 incidence in controls) 100 mg/kg bw/day: No effects Developmental NOAEL 100 mg/kg bw/day Maternal NOAEL 500 mg/kg bw/day
14 day tolerability study in non-pregnant SD rats Oral (gavage) Guideline N/A Non GLP Rat, SD 3 females/group Vehicle: 1% MC Mancozeb (86%) Anonymous, 2015b	0, 60, 120, 180, 240 or 300 mg/kg bw/day for 14 days	**General toxicity in non-pregnant females** 300mg/kg bw/day ↓ body weight (9.8% day14); 240 mg/kg bw/day: ↓ body weight (6% day14); 180 mg/kg bw/day: ↓ body weight (8.3% day14); 120 mg/kg bw/day: No effects 60 mg/kg bw/day: No effects

Preliminary range finding & TK study Oral (gavage) Non-guideline GLP Rat, Crl:CD(SD) 23 mated females/group including TK: 8 mated females/group at term Vehicle: 1% MC Mancozeb 86.0% Anonymous, 2015c	0, 80, 120 or 160 mg/kg bw/day on gestation days 6-19	**Maternal toxicity** <u>160 mg/kg bw/day</u>: ↓ body weight gain (36.8% days 9-12, 21.6% days 6-20); ↓ food consumption (10% days 6-20) <u>120 mg/kg bw/day</u>: ↓ body weight gain (31.2% days 9-12, 7.8% days 6-20) <u>80 mg/kg bw/day</u>: ↓ body weight gain (31.2% days 9-12, 6.9% days 6-20) **Developmental toxicity – limited foetal evaluation** <u>160 mg/kg bw/day</u>: No effects **Toxicokinetics** The presence of ETU in all mancozeb-treated litters demonstrated that they were exposed to mancozeb and ETU.
Developmental toxicity Oral (gavage) OECD 414 (2001) GLP Rat, Crl:CD(SD) 25 mated females/group Vehicle: 1% MC Purity 86.0% Anonymous, 2015d	0, 10, 40 or 160 mg/kg bw/day on gestation days 6-19.	**Maternal toxicity** <u>160 mg/kg bw/day</u>: ↓ body weight gain (14.2% days 6-20); ↓ food consumption (7.7% days 6-20) <u>40 mg/kg bw/day</u>: No effects **Developmental toxicity** <u>160 mg/kg bw/day</u>: No effects Developmental NOAEL 160 mg/kg bw/day Maternal NOAEL 40 mg/kg bw/day
Investigation into possible effects on sexual development Oral (gavage) Non guideline Non GLP Rats, Han-Tac:WH 10-12 dams/group Vehicle: corn oil Mancozeb (in mixture of 5 pesticides) Purity 76% Hass *et al* 2012 (from open literature)	6.25 & 25 mg/kg bw/day Pre-natal & perinatal exposure *In vitro* androgen receptor reporter gene assay, steroidogenesis assay and T-screen assay performed	No significant effects on body weight and no clinical signs of toxicity No effects on gestation length and pup body weights No effects on sexual development parameters in offspring No AR antagonism in vitro and no effect on synthesis of oestradiol, progesterone or testosterone in vitro. No effects on T-screen assay. Sexual development NOAEL > 25 mg/kg bw/day

Developmental toxicity in the rat Oral Non guideline Non GLP Rats 10-22/group Vehicle: corn oil Mancozeb tested individually along with 4 other pesticides Purity not reported Jacobsen *et al* 2012 (from open literature)	6.25 &25 mg/kg bw/day From gestation day 7 to post natal day 16	No treatment related effects on body weight gain, litter size or pup mortality. No effects on hormone levels, sexual maturity, organ weights, sperm motility, mammary glands, behaviour or histopathology. No thyroid effects Post-natal development NOAEL > 25 mg/kg bw/day
Investigative developmental study Oral (gavage) Non guideline Non GLP Rat: Wistar 10-12 pregnant females/group Vehicle: corn oil Mancozeb (in a mixture of 5 pesticides) Purity 76% Overgaard *et al* 2013(from open literature)	0, 6.25, 25 mg/kg bw/day From gestation day 7 to gestation day 21 and from post natal day 1 to post natal day 16	No effect on juvenile body weight No effect on puberty onset No treatment related effects on Kiss 1 mRNA expression in the AVPC or ARC of the hypothalamus in female offspring Puberty onset NOAEL > 25 mg/kg bw/day
Developmental toxicity studies in rabbits		
Developmental toxicity Oral (gavage) OECD 414 (1981) GLP Rabbit, NZW 20 inseminated females/group Vehicle: 0.5% MC purity 83% Anonymous, 1987b	0, 10, 30 or 80 mg/kg bw/day on gestation days 7-19	**Maternal toxicity** <u>80 mg/kg bw/day</u>: 2/20 treatment-related deaths due to weight loss; ↑ clinical signs (anorexia, scant faeces, alopecia); weight loss & minimal food intake in these animals but not survivors. <u>30 mg/kg bw/day</u>: No effects **Developmental toxicity** <u>80 mg/kg bw/day</u>: 5/20 abortion (0 in controls), 2/20 total resorption (2 in controls); Developmental NOAEL 30 mg/kg bw/day Maternal NOAEL 30 mg/kg bw/day

Developmental toxicity Oral (gavage) OECD 414 (1981) GLP Rabbit, NZW 18 inseminated females/group Vehicle: 1% MC Purity 88.4% Anonymous, 1991b	0, 5, 30, 55 or 100 mg/kg bw/day on gestation days 6-18	**Maternal toxicity** 100 mg/kg bw/day: ↓ body weight gain (62% days 6-19); ↓ food consumption (37% days 6-19). 55 mg/kg bw/day: No effects **Developmental toxicity** 100 mg/kg bw/day: 5/16 abortion (2/13 in controls); ↑ post-implantation loss (26.8% cf. 21.6% controls) 55 mg/kg bw/day: No effects Developmental NOAEL 55 mg/kg bw/day Maternal NOAEL 55 mg/kg bw/day
Developmental neurotoxicity studies in rats		
Preliminary range finding & TK study Oral (dietary) OECD 426 (2007) GLP Rat, Crl:CD(SD)BR 15 mated females/group Purity 81.0% Anonymous, 2008b	Target doses 5, 30 and 60 mg/kg bw/day. From gestation day 6 to termination on post-partum day 21-28	**Maternal toxicity** 60 mg/kg bw/day: ↓ body weight gain (37% GD6-20); ↓ food consumption (16% GD6-20); ↑ serum TSH (25% GD 20, 38% PND21); ↓ serum T4 (23% GD20, 44% PND21); ↑ thyroid weight (12% GD20); ↑ thyroid follicular cell hypertrophy (6/15 cf. 3/15 controls) 30 mg/kg bw/day: ↓ body weight gain (14% GD6-20); ↓ food consumption (12% GD6-20); ↑ serum TSH (17% GD20); ↓ serum T4 (55% GD20, 24% PND21); ↑ thyroid weight (9% GD20); ↑ thyroid follicular cell hypertrophy (5/14 cf. 3/15 controls) 5 mg/kg bw/day: No effects **Developmental neurotoxicity** No developmental neurotoxicity effects seen 60 mg/kg bw/day: ↓ body weight PND 7 (males 17%, females 16%), PND 21 (males 12%, females 12.5%) 30 mg/kg bw/day: ↓ body weight PND 7 (males 22%, females 19%), PND 21 (males 13%, females 12%) 5 mg/kg bw/day: No effects

Developmental neurotoxicity Oral (dietary) Non-guideline GLP Rat, Crl:CD(SD)BR 25 mated females/group Purity 81.0% Anonymous, 2008c	Target doses 5, 15 and 30 mg/kg bw/day From gestation day (GD) 6 to GD20 or to post-natal day (PND) 21	**Maternal toxicity** <u>30 mg/kg bw/day</u>: ↓ body weight gain (26% gestation days 6-12, 5% gestation days 6-20); ↑ thyroid follicular hypertrophy (11/25 cf. 6/24 controls) <u>15 mg/kg bw/day</u>: No effects **Developmental neurotoxicity** <u>30 mg/kg bw/day</u>: No effects DNT NOAEL 30 mg/kg bw/day Maternal NOAEL 15 mg/kg bw/day
Investigative developmental neurotoxicity study Oral (gavage) Non guideline Non-GLP Rat: Wistar 9-21 time-mated females/group Axelstad *et al* 2011(from open literature)	**Mancozeb** 0, 50, 100 or 150/100 mg/kg bw/day GD 7 – PND 16 Vehicle: corn oil	**Maternal toxicity** <u>150 mg/kg bw/day</u>: Severe weight loss, mild hind limb paralysis. Dose reduced to 100 mg/kg bw/day at different timepoints. ↓ T_4 GD 15 (37%) <u>100 mg/kg bw/day</u>: ↓ body weight gain (27% days 7-21); ↓ T_4 GD 15 (27%) <u>50 mg/kg bw/day</u>: ↓ body weight gain (20% days 7-21); ↓ T_4 GD 15 (21%) **Developmental neurotoxicity** No effects seen DNT NOAEL >100/150 mg/kg bw/day Maternal LOAEL 50 mg/kg bw/day

Table 19: Summary table of recent/key developmental toxicity study conducted with ETU

Type of study/data	Test substance/dose levels	Relevant information about the study (as applicable)	Observations (Effects statistically significantly different unless stated otherwise)
Developmental toxicity Oral (gavage) OECD 414 (2001) GLP Rat, Crl:CD(SD) 24 mated females/group Anonymous, 2015a	**ETU** (>98% pure), 0, 2.5, 5, 15 or 30 mg/kg bw/day on gestation days 6-19 Vehicle: water	Study conducted to determine no effect and effect levels for the known teratogen, ETU and for direct comparison with the study on mancozeb (Anonymous, 2015d). ETU is a primary metabolite of mancozeb.	**Maternal toxicity** 30, 15, 5 and 2.5 mg/kg bw/day: No effects **Developmental toxicity** 30 mg/kg bw/day: ↓ mean foetal weight 13.5% (3.3g cf. 3.8g controls), ↑ incidence malformation and developmental variations including malformed tail, meningocele, hydrocephaly, malrotated limb, limb hyperextension, rib and vertebral anomalies, 27 presacral vertebrae and 14th rudimentary ribs, reduced ossification or unossified bones 15 mg/kg bw/day: ↑ incidence malformation (hydrocephaly in 2 litters) 5 mg/kg bw/day: no effects Developmental NOAEL 5 mg/kg bw/day Maternal NOAEL 30 mg/kg bw/day

10.8.3 Developmental toxicity in rats

The developmental toxicity of mancozeb has been investigated in four studies in rats.

Anonymous (1980)

In the study by Anonymous (1980), groups of 26 pregnant SD rats were given mancozeb by gavage from day 6 to 15 of gestation at doses of 0, 2, 8, 32, 128 or 512 mg/kg bw/day. Food consumption and body weights were decreased at 128 mg/kg bw/d and above in mancozeb treated dams. Body weights for all other mancozeb-treated groups were comparable to the control groups. Litter data indicated significant decreases in the average number of live foetuses, mean foetal weight and mean gravid uteri and increased resorptions only at 512 mg/kg bw/d.

At 512 mg/kg bw/d 6/19 litters were total resorptions. The remaining 13 litters had live foetuses of significantly lower body weight compared to the control. Adverse compound-related effects were clearly manifested at 512 mg/kg bw/d for gross abnormalities (agnathia, cleft palate, meningoencephalocele), soft tissue effects (dilated ventricles, compressed spinal cord), and skeletal tissue effects (incomplete ossification of the skull, clavicle, scapula). The effects observed at 128 mg/kg bw/d were not biologically or statistically significant.

Summary of significant foetal anomalies - external

	Corn oil (10 mL/kg/day)	Dithane M-45 (mg/kg bw/day)					ETU (mg/kg bw/day)
	Control	2	8	32	128	512	50
Number of foetuses/litters	278/23	248/22	245/23	248/23	212/20	155/13	232/21
Agnathia	0	0	0	0	0	15/1	8/1
Cleft palate	0	0	0	0	0	24/3	8/1
Cleft lip	0	0	0	0	0	15/1	8/1
Meningoencephalocele	0	0	0	0	0	27/4	181/20
Ablepharia	0	0	0	0	0	1/1	0
Urogenital cleft	0	0	0	0	0	3/1	0
No anal opening	0	0	0	0	0	0	2/2
Kinked tail	1/1	0	0	0	0	58/8	114/16
Short tail	0	0	0	0	0	15/3	141/19
Tail agenesis	0	0	0	0	0	0	59/11
Hemimelia	0	0	0	0	0	0	2/1
Clubbed limb	0	0	0	0	0	15/1	2/1
Forelimb flexure	0	0	0	0	1/1	4/2	104/15
Abnormal pelvic limb posture	0	0	0	0	1/1	0	75/15
Oligodactyly	0	0	0	0	0	0	6/2
Syndactyly	0	0	0	0	0	0	6/1
Webbed digits	0	0	0	0	0	0	8/3

Summary of significant foetal anomalies - visceral

	Corn oil (10 mL/kg/day)	Dithane M-45 (mg/kg bw/day)					ETU (mg/kg bw/day)
	Control	2	8	32	128	512	50
Number of foetuses/litters	90/23	80/22	83/23	84/23	73/20	52/13	81/21
Nasal cavity haemorrhage	0	0	0	0	0	1/1	0
Olfactory bulbs deformed	0	0	0	0	0	7/3	78/21
Eyes deformed	0	0	0	0	0	0	2/1
Exencephaly	0	0	0	0	0	5/1	5/3
Dilated brain ventricles	0	0	2/2	0	1/1	28/9	75/20
Deficiency of tissue in olfactory bulb	0	0	0	0	0	7/3	78/21
Brain tissue atrophy	0	0	0	0	0	9/2	42/13
Spinal cord haemorrhage	0	0	1/1	2/1	1/1	0	0
Spinal cord compressed	0	0	0	0	0	13/4	64/18
Fluid filled pericardial sac	9/4	3/2	11/6	9/6	9/6	10/6	14/7
Stomach reduced, thick walled	0	0	0	0	0	5/1	46/13
Adrenal agenesis	0	0	0	0	0	0	2/1
Hydroureter	23/15	35/18	28/16	20/12	27/14	18/8	76/21
Hydronephrosis	7/4	13/7	14/8	10/7	5/5	9/6	53/18
Hypoplastic kidney	0	0	2/1	0	0	0	0
Kidney pelvis haemorrhage	0	1/1	0	0	0	0	0
Ectopic kidney	0	0	0	0	0	0	6/3
Kidney agenesis	0	0	0	0	0	0	9/4
Ovary agenesis	0	0	0	0	0	0	2/1
Testis agenesis	0	0	0	0	0	0	2/1
Cryptorchidism	0	0	0	0	0	2/2	14/8
Fat pad oedema	0	0	0	0	0	0	44/13
Body oedema	0	0	0	0	0	8/2	2/2

Summary of significant foetal anomalies - skeletal

	Corn oil (10 mL/kg)	Dithane M-45 (mg/kg bw/day)					ETU (mg/kg bw/day)
	Control	2	8	32	128	512	50
Number of foetuses/litters	187/23	168/22	162/23	165/23	139/20	103/13	151/21
Skull incomplete ossification	0	0	3/2	2/2	6/2	36/5	111/19
Skull wide cranial suture	1/1	0	0	0	0	34/6	127/19
Clavicle incomplete ossification	0	0	0	0	0	6/1	0
Clavicle curved	0	0	0	0	0	5/1	24/6
Scapula incomplete ossification	0	0	0	0	0	16/2	0
Scapula misshapen	0	0	0	0	0	0	6/2
Sternebrae fused	0	0	0	0	0	9/1	6/2
Ribs incomplete ossification	0	1/1	3/2	2/1	6/2	6/1	13/6
Ribs fused and/or thickened	0	0	0	0	0	10/1	31/10
Ribs wavy	0	0	0	0	0	6/1	24/4
More than 13 ribs	11/8	8/6	6/4	11/9	9/5	12/6	7/7
Less than 13 ribs	0	0	0	0	0	0	3/2
Cervical vertebrae fused	0	0	0	0	0	0	1/1
Thoracic vertebrae fused	0	0	0	0	0	0	1/1
Lumbar vertebrae fused	0	0	0	0	0	0	6/3
Lumbar vertebrae absent	0	0	0	0	0	1/1	0
Sacral vertebrae fused	0	0	0	0	0	0	2/2
Sacral vertebrae absent	0	0	0	0	0	13/2	1/1
Caudal vertebrae fused	0	0	0	0	0	0	4/3
Caudal vertebrae absent	0	0	0	1/1	0	19/4	32/9
Kyphosis	0	0	0	0	0	3/2	20/6
Pelvic bone incomplete ossification	0	0	0	0	0	7/7	5/3
Pelvic bone misshapen	0	0	0	0	0		1/1
Pubis absent	0	0	0	0	0	10/1	1/1
Hindlimb long bones incomplete ossification/ misshapen	0	0	0	0	0	6/1	5/2
Hindlimb long bones absent	0	0	0	0	0	10/1	1/1

Anonymous, 1988c

In another developmental toxicity study (Anonymous, 1988c), mancozeb was administered by gavage to groups of 25 time-mated SD rats on days 6-15 of gestation at doses of 0, 10, 60 and 360 mg/kg bw/day. One female at 360 mg/kg/day was killed in extremis after showing marked loss of body weight, hind limb paralysis and a general loss of condition. A further four females at 360 mg/kg/day showed a reeling gait during the last days of treatment which developed into slight, transient, hind limb paralysis after dosing had been completed. Hind limb paralysis was therefore seen in 5 animals out of 25 animals at the top dose. Body weight gain of females receiving 360 mg/kg/day was reduced (by 25%) during the treatment period and, although after treatment ceased, the rate of weight gain improved to control values, the initial deficit was not recouped. Females at 10 or 60 mg/kg/day were unaffected. At 360 mg/kg/d, food intake was reduced during the treatment period. Females receiving 10 or 60 mg/kg/d were unaffected.

Litter parameters showed no treatment-related intergroup differences. Skeletal evaluation revealed a slight reduction in ossification of the interparietal bone and thoracic vertebral centra in all treated groups, which was statistically significant at 360 mg/kg/day. Morphological development was otherwise unaffected by treatment with the exception of an increased incidence of large anterior fontanelle (11.9% foetuses cf. 2.2% controls; not statistically significant). No other adverse effects upon foetal survival, growth and development in utero were observed.

Summary of significant foetal anomalies (% foetal incidence/number of litters affected)

	Mancozeb (mg/kg bw/day)			
	0	**10**	**60**	**360**
Number of foetuses/litters examined for external anomalies No significant findings	358/25	345/25	347/25	322/23
Number of foetuses/litters examined for internal anomalies No significant findings	182/25	175/25	176/25	160/23
Number of foetuses/litters examined for visceral[a] anomalies	176/25	170/25	171/25	162/23
Increased dilatation of brain ventricles	1.1%/1	0.6%/1	0.6%/1	0
Moderate internal hydrocephaly	0	0	0	0.6%/1
Haemorrhage cerebro-spinal fluid and brain ventricles	0	1.2%/2	0.6%/1	1.2%/2
Unilateral hydronephrosis	1.7%/6	1.2%/2	0	0
Bilateral hydronephrosis	1.1%/2	0	0	0.6%/1
Unilateral hydroureter	5.1%/6	5.9%/8	5.3%/8	3.7%/5
Bilateral hydroureter	1.1%/2	1.8%/3	0.6%/1	1.9%/2
Number of foetuses/litters examined for skeletal anomalies	182/25	175/25	176/25	160/23
Medium anterior fontanelle	97.8%/25	99.4%/25	96.0%/25	88.1%/23
Large anterior fontanelle	2.2%/3	0.6%/1	4.0%/4	11.9%/10
Incomplete ossification of supra-occipital bone	18.1%/14	16.0%/14	19.3%/14	20.6%/18
Incomplete ossification of interparietal bone	75.8%/25	80.0%/25	86.9%/25	90.0%*/23
Ribs 13/14	0.5%/1	1.7%/2	1.7%/2	2.5%/3
Ribs 14/14	1.1%/2	0.6%/1	1.1%/2	0
Ribs short and/or wavy	0	0	0	1.3%/1
Ribs thickened	0	0	0	1.3%/1
Incomplete ossification of thoracic vertebral centra	28.0%/20	39.4%/22	39.2%/23	51.3%/21

[a] After freehand serial sectioning

*Statistically different from control ($p<0.05$)

Overall, mancozeb administered at a dose level of 360 mg/kg/day to pregnant rats during organogenesis was associated with maternal toxicity, as evidenced by reduced body weight gain and food intake. In addition, transient slight paralysis of the hind limbs was recorded in 5 out of the 25 females, and one severely affected

animal was killed in extremis. With the exception of slight reductions in the degree of ossification of the interparietal bone and thoracic vertebral centra, there were no adverse effects upon survival, growth and development in utero.

Anonymous, 1999b

A study by Anonymous (1999b) looked at the teratogenicity of mancozeb in the Wistar rat. Groups of 24 pregnant females were administered mancozeb by gavage from day 6 to 15 of gestation at doses of 0, 100, 225 or 500 mg/kg bw/day. No mortality occurred during the course of the study. No treatment-related clinical signs were observed whatever the dose level. The pregnancy rate was 83.33% for all the dose groups without any variation among this parameter between treated and control groups. The body weight and body weight gain of the dams was unaffected by treatment. The food consumption remained normal during the study. With the exception of an incidental decrease in mean number of *corpora lutea* in the low dose group and a significant difference in sex ratio at the high dose group (incidental and not treatment-related), the reproductive parameters did not show any variations from normal. The overall incidence of major malformation, minor external, visceral and skeletal anomalies were unaffected by treatment up to a dose level of 100 mg/kg bw/day. The incidence of lung emphysema, kidney congestion and dumbbell shaped thoracic centre were significantly higher in mid dose level (225 mg/kg bw/day) and high dose level (500 mg/kg bw/day) groups. In conclusion, in this study in rats, no maternal toxicity was observed up to the highest tested dose of 500 mg/kg bw/day. Developmental toxicity (lung emphysema, kidney congestion and dumbell shaped thoracic centre) was observed from a dose of 225 mg/kg bw/day. It is noted that the findings of this study are inconsistent with those of the previous two studies.

Anonymou, 2015b; Anonymous, 2015c; Anonymous, 2015d

In order to address the issues identified with the developmental toxicity of mancozeb in the rat, 3 new developmental toxicity studies have been conducted.

The first study (Anonymous, 2015b) was a range finding study in non-pregnant females to determine dose levels of the test substance for subsequent studies in pregnant rats. In addition, an assessment of plasma levels of mancozeb and its metabolite ethylenethiourea (ETU) was performed. Mancozeb was administered orally by gavage to 5 groups of 3 non-pregnant female SD rats once daily from study days 0 through 13. Dosage levels were 60, 120, 180, 240, and 300 mg/kg bw/d. A concurrent control group composed of 3 non-pregnant females received the vehicle on a comparable regimen. All females survived to the scheduled necropsy on study day 14. Noteworthy clinical findings observed during the study were limited to the post-dosing observations, and consisted of a single occurrence of salivation for 1 female in the 300 mg/kg bw/d group approximately 48 minutes following dose administration on study day 8. Body weight losses were noted for all 3 females in the 300 mg/kg bw/d group sporadically throughout the treatment period, which resulted in a mean body weight loss of 15 g when the overall dosing period (study days 0-14) was evaluated and a mean body weight on study day 14 that was 9.8% lower than the control group value. Slight body weight losses were also noted for 2 of the 3 females in the 180 and 240 mg/kg bw/d groups when the overall dosing period was evaluated, which resulted in lower (8.3% and 6.0%) mean body weights in these groups, respectively, compared to the control group. There were no consistent trends in body weight changes in the 60 or 120 mg/kg bw/d dose groups and body weights were similar to the control group. Food consumption in the 60, 120, 180, 240, and 300 mg/kg bw/d groups was similar to that noted in the control group throughout the dosing period. At necropsy, no remarkable internal findings were noted at any dose level. Rat plasma concentrations of mancozeb ranged from below the limit of quantification (<10.0) to 213 ng/mL and plasma concentrations of ETU ranged from below the limit of quantification (<10.0) to 1950 ng/mL, 6 hours after the final dose administration. Overall, plasma mancozeb concentrations were significantly lower than those for ETU; this was the consequence of the rapid metabolism of mancozeb to ETU. A clear dose response between administered mancozeb and plasma ETU levels was seen. Doses of 60 and 120 mg/kg bw/d were considered to be well tolerated, based on the lack of toxicologically significant effects. Based on these results, dose levels of 80, 120, and 160 mg/kg bw/d were selected for a preliminary oral (gavage) study of mancozeb in pregnant SD rats.

In the subsequent study (Anonymous, 2015c), mancozeb was administered orally by gavage to groups of 23 pregnant female SD rats once daily from GD 6 through to GD 19. Dose levels were 0, 80, 120 and 160 mg/kg bw/d. No mancozeb-related clinical findings were noted. Lower mean body weight gains with corresponding

reduced mean food consumption were noted in the 160 mg/kg bw/d group compared to the control group during GD 9-12 and 15-20, as well as when the overall treatment period (GD 6-20) was evaluated, resulting in a slightly lower (4.9%) mean body weight in this group on GD 20. A lower (approximately 40%) mean net body weight gain was also noted in the 160 mg/kg bw/d group, resulting in a mean net body weight that was 6.5% lower than the control group; gravid uterine weight in this group was similar to the control group. Food consumption was also consistently reduced at 160 mg/kg bw/d. Intrauterine growth and survival in the 80, 120, and 160 mg/kg bw/d groups were similar to the control group and no external foetal malformations or developmental variations were observed at any dosage level. Mean thyroxine (T_4) levels in the 120 and 160 mg/kg bw/d groups were 23.3% to 30.5% lower than in the control group at the 4-hour post-dosing time points. Mean T_4 levels at the 2- and 6-hour post-dosing time points were either similar to the control or lower in the test substance-treated groups; however, there was no consistent dose response relationship for the decreases in mean T_4 at these time points.

All pregnant rats dosed orally with mancozeb at 80, 120, or 160 mg/kg bw/d were systemically exposed to mancozeb and its metabolite, ETU. Concentrations of mancozeb in both maternal and foetal plasma samples were much lower than those of ETU. On GD 19, exposure to mancozeb and ETU, in terms of AUC_{last}, increased as dosage increased from 80 to 160 mg/kg bw/d. Systemic exposure to mancozeb and ETU increased nearly dose-proportionally over the 80 to 160 mg/kg bw/d range. Exposure to the metabolite ETU, in terms of AUC_{last}, was 56-, 71-, and 55-fold higher than exposure to mancozeb at 80, 120, and 160 mg/kg bw/d, respectively, in maternal animals. Peak plasma mancozeb concentrations were reached at 2 or 4 hours post-dosing, indicating rapid absorption. Peak plasma ETU concentrations were reached at 6 hours post-dosing at all mancozeb dosage levels, indicating that once absorbed, mancozeb is rapidly metabolised to ETU. Oral administration of mancozeb at 80, 120, and 160 mg/kg bw/d to pregnant rats resulted in exposure of the foetuses to ETU. Similarly to maternal animals, exposure to ETU in foetuses increased nearly dose-proportionally with increasing mancozeb dose. Exposure to ETU was similar in maternal animals and foetuses, with foetal/dam ratios of approximately 0.92. Overall, in this preliminary developmental toxicity study, maternal toxicity was observed at a dosage level of 160 mg/kg bw/d as evidenced by lower mean body weight gains and corresponding reduced mean food consumption compared to the control group, which resulted in a slightly lower mean body weight on gestation day 20. There were no test substance-related effects on intrauterine growth, survival, and external foetal morphology at any dosage level. Based on these results, dosage levels of 10, 40, and 160 mg/kg/d were selected for a definitive prenatal development toxicity study of mancozeb.

In the definitive study (Anonymous, 2015d), mancozeb was administered orally by gavage to groups of 25 pregnant female SD rats once daily from GD 6 through to GD 19. Dose levels were 0, 10, 40 and 160 mg/kg bw/d. No mancozeb-related clinical findings were noted. Lower mean body weight gains were noted in the 160 mg/kg bw/d group near the end of the treatment period resulting in a lower overall mean body weight gain (14.2% for GD 6-20) and lower mean body weights at the end of the treatment period (GD 19 and 20. Mean food consumption in this group was generally lower throughout the treatment period. Intrauterine growth and survival were not affected by mancozeb at any dose level. There were no test substance-related foetal malformations or developmental variations in the 10, 40, or 160 mg/kg bw/d groups.

Incidence of foetal malformations

	Dose level of Mancozeb (mg/kg bw/day)			
	0	10	40	160
No. foetuses/litters examined – external	358/23	379/25	356/24	383/25
Microphthalmia/anophthalmia	0	1/1	0	0
Omphalocele	0	0	0	1/1
Localised foetal oedema	0	1/1	0	0
No. foetuses/litters examined - soft tissue	358/23	379/25	356/24	383/25
Hydrocephaly	0	1/1	0	0
Situs inversus	0	0	1/1	0
No. foetuses/litters examined – skeletal	358/23	379/25	356/24	383/25
Costal cartilage anomaly	0	1/1	0	0

Incidence of foetal variations

	Dose level of Mancozeb (mg/kg bw/day)			
	0	10	40	160
No. foetuses/litters examined – external	358/23	379/25	356/24	383/25
Findings - none	0	0	0	0
No. foetuses/litters examined - soft tissue	358/23	379/25	356/24	383/25
Major blood vessel variation	0	0	1/1	1/1
Spleen small	0	1/1	0	0
Renal papilla(e) not developed and/or distended ureter(s)	10/4	1/1	0	0
No. foetuses/litters examined – skeletal	358/23	379/25	356/24	383/25
Reduced ossification of the skull	1/1	0	4/4	3/3
Hyoid unossified	1/1	2/1	5/3	5/4
Reduced ossification of the vertebral arches	4/4	0	2/2	1/1
Unco-ossified vertebral centra	0	0	0	1/1
Cervical centrum 1 ossified	53/17	41/17	45/17	41/14
Extra site of ossification ventral to cervical centrum 2	0	0	1/1	0
7^{th} cervical rib(s)	2/2	1/1	3/3	2/1
Bent rib(s)	2/1	0	1/1	0
Reduced ossification of the 13^{th} ribs	2/1	2/2	2/2	0
14^{th} rudimentary rib(s)	25/11	40/15	20/10	35/18
27 presacral vertebrae	0	1/1	0	0

Incidence of foetal skeletal variations

	Dose level of Mancozeb (mg/kg bw/day)			
	0	10	40	160
No. foetuses/litters examined – skeletal	358/23	379/25	356/24	383/25
Sternebra(e) 1, 2, 3 and/or 4 unossified	4/3	0	1/1	1/1
Sternebra(e) 5 and/or 6 unossified	58/15	45/19	68/19	56/17
Sternebra(e) malaligned (slight or moderate)	4/4	3/2	1/1	5/4
Pubis unossified	2/2	0	0	0

Overall, in this modern guideline-compliant developmental toxicity study in rats, no developmental toxicity was seen up to the top dose of 160 mg/kg bw/d at which a moderate level of maternal toxicity (decreased mean body weight gain and food consumption) occurred. No kinetic measurements for mancozeb or ETU were performed in this study; however, estimates obtained from the data available in the preliminary study (Anonymous, 2015c) indicate that doses of mancozeb up to that causing maternal toxicity (160 mg/kg bw/d) in this investigation result in peak plasma concentrations of ETU lower than those seen following administration of ETU at the NOAEL (5 mg/kg bw/d), LOAEL (15 mg/kg bw/d) and effect level (30 mg/kg bw/d) for developmental toxicity in Anonymous (2015a).

Studies identified from the open literature

A study by Hass *et al* (2012) was conducted to determine whether a mixture of low doses of five environmentally relevant endocrine disrupting pesticides with dissimilar modes of action would cause adverse effects on sexual development. The five pesticides used were mancozeb (technical grade, purity 76%), epoxiconazole, prochloraz, tebuconazole and procymidone. Dams were dosed orally by gavage, from GD7 to 21 and from the day after birth to PD16. Mancozeb was administered to 10-12 dams/group at 6.25 and 25 mg/kg bw/d. Controls (22 dams) were given corn oil. The pesticides were also investigated in the androgen receptor reporter gene assay *in vitro* to determine their ability to activate the androgen receptor and to inhibit androgen-induced activation of the androgen receptor. The T-screen assay was used for in vitro detection of the agonistic and antagonistic properties of the pesticides at the level of the thyroid hormone receptor. The potential for effects on the synthesis of estradiol, progesterone and testosterone in vitro in the human adrenocortical carcinoma cell line H295R was also investigated.

There were no statistically significant effects on maternal body weight during gestation and no clinical signs of toxicity. There were no effects on gestation length and pup body weights. Nipple retention in male offspring was significantly increased at the high dose of mancozeb ($P<0.05$) however, it is most likely this was a chance finding as no anti-androgenic activity was demonstrated for mancozeb (it did not exhibit androgen receptor antagonism in vitro nor did it affect the synthesis of estradiol, progesterone or testosterone in the H295R steroid synthesis assay). In the male offspring, there was no effect of treatment on ano-genital distance. One male in

each of the mancozeb-treated groups showed malformation of the seminal vesicle which was not significant in comparison with the controls. Mancozeb did not exhibit androgen receptor antagonism in vitro. Neither did it affect the synthesis of estradiol, progesterone or testosterone in the H295R steroid synthesis assay. Triiodothyronine (T_3) induced dose-dependent proliferation of GH3 cells in the T-screen assay but no data were reported in the publication to support this conclusion.

In conclusion, in this study in rats involving pre-natal and perinatal exposure to mancozeb up to 25 mg/kg bw/d, no treatment-related effects on sexual development parameters were seen. Nipple retention was increased in male offspring at the top dose; however, it is most likely this was a chance finding as no anti-androgenic activity was demonstrated for mancozeb (it did not exhibit androgen receptor antagonism in vitro nor did it affect the synthesis of estradiol, progesterone or testosterone in the H295R steroid synthesis assay).

A similar study was conducted in the same laboratory in order to determine whether a mixture of low doses of five environmentally relevant endocrine disrupting pesticides with dissimilar modes of action would cause effects on male and female development (Jacobsen *et al* 2012). Fourteen groups of 10-22 rats were administered individual pesticides (mancozeb 6.25 or 25 mg/kg bw/day) or mixtures of all 5 pesticides in corn oil, from gestation day (GD) 7 to post natal day (PD) 16. There were no treatment related effects on dam body weight gain, litter size or pup mortality in any of the dose groups. When dosed alone none of the individual pesticides had any effects on hormone levels, sexual maturity, organ weights, sperm motility, mammary glands, behaviour or histopathology. In conclusion mancozeb at doses of 6.25 or 25 mg/kg bw/d administered orally to females during pregnancy and lactation (GD 7 to PD16) had no effect on the development of the offspring (in particular thyroid, reproductive organs and behavioural endpoints) both before and after puberty.

Another similar study was conducted in the same laboratory to determine whether a mixture of low doses of five environmentally relevant endocrine disrupting pesticides with dissimilar modes of action would cause effects on sexual development and expression of kisspeptin in the hypothalamus (Overgaard et al., 2013). Groups of 10-12 pregnant female Wistar rats were dosed by gavage from gestation day (GD) 7 to GD 21 and from the day after delivery until postnatal day (PD) 16 with 0, 6.25 or 25 mg mancozeb/kg bw/d in corn oil. There were no effects on juvenile body weight but adult body weight was higher than control in the mancozeb 25 mg/kg bw/d group. There were no treatment related effects on puberty onset. There were no treatment related effects on Kiss 1 mRNA expression in the AVPV (anteroventral periventricular nucleus) or ARC (arctuate nucleus) of the hypothalamus. In conclusion, gestational (GD 7 to GD 21) and lactational (PD 1-PD 16) exposure of rats to mancozeb at 6.25 or 25 mg/kg bw/d had no effect on puberty onset or on Kiss1 mRNA expression in the hypothalamus in female offspring.

10.8.4 Developmental toxicity in rabbits

The developmental toxicity of mancozeb has been investigated in 2 studies in rabbits.

In the first rabbit study by Anonymous (1987b), groups of 20 NZW pregnant rabbits were administered mancozeb by gavage from day 7 to 19 of gestation at doses of 0, 10, 30 or 80 mg/kg bw/d. No treatment-related deaths occurred in the control or in the 10 and 30 mg/kg bw/d dose groups. One animal died in the 30 mg/kg bw/d group but this was attributed to mis-dosing. Two does were sacrificed in a moribund condition at the high dose and the deaths considered treatment-related (one of these does was pregnant and did not abort). Clinical signs were comparable between controls and animals receiving 10 and 30 mg/kg bw/d. Animals receiving 80 mg/kg bw/day manifested significant increases in clinical signs. Alopecia. anorexia, ataxia, scant faeces and abortions were all observed at the highest dose tested. Body-weight gain and food consumption were not significantly different between controls and groups receiving 10 or 30 mg/kg bw/d. At 80 mg/kg bw/d, body-weight and food consumption were significantly decreased in does that aborted and those sacrificed moribund. Does producing at least one viable foetus had body-weight gains and food consumption values similar to controls. No treatment-related changes were evident in the incidence of gross post-mortem findings between does in the control and treated group. Reproductive parameters between controls and does receiving 10 or 30 mg/kg bw/d were comparable as measured by the number of abortions, litters produced, and the mean number per litter or corpora lutea, implantations, resorptions, dead and live foetuses or sex ratio. At 80 mg/kg

bw/d, a significant increase in does aborting (5/15) and 2 total resorptions out of 20 litters were reported with a corresponding decrease in the number of litters produced. All other parameters were comparable to the control group. Mean foetal body weight was similar between the control and treated groups. There were no significant increases in the types or incidence of malformations or developmental variations between control or treated groups. Overall, developmental toxicity (abortions and resorptions) was seen in this study at the top dose of 80 mg/kg bw/d in the presence of maternal toxicity.

In the second rabbit study by Anonymous (1991b), mancozeb was administered by gavage to groups of 18 NZW pregnant rabbits from day 6 to 18 of gestation at doses of 0, 5, 30, 55 and 100 mg/kg bw/d. No treatment-related clinical effects or treatment-related necropsy findings were detected. Two dams in the control group and five in the 100 mg/kg bw/d dose group aborted. A clear and substantial body-weight loss was observed in does at 100 mg/kg bw/d for days 6-9 post-coitum (*i.e.* days 1-3 of compound administration). Body-weight gain during days 9-15 was also substantially depressed compared to controls at the top dose. Food consumption was also markedly decreased between days 6-19 at 100 mg/kg bw/d. A slight post-implantation loss (5%) was also observed at the top dose, but given its magnitude it was not considered adverse. Overall, no developmental toxicity was seen in this study up to a dose (100 mg/kg bw/d) causing maternal toxicity.

10.8.5 Developmental neurotoxicity in rats

A developmental neurotoxicity (DNT) study in rats (OECD TG 426) is also available. This study (Anonymous, 2008b – a dose range finding study; Anonymous, 2008c – the main DNT study) was conducted to address the concern about a potential relationship between thyroid effects and brain development.

In the dose range finding study (Anonymous, 2008b), mancozeb was given on a continuous basis in the diet to groups of 15 pregnant female SD rats from gestation day 6 until euthanasia (post partum day 21). The target test substance doses were 5, 30 and 60 mg/kg bw/d. No test substance-related clinical findings were observed in parent females. At 60 mg/kg/d, body weight, body weight gain and food consumption were significantly decreased during the gestation treatment period (by 10.5%, 37% and 16%, respectively; $p<0.01$). Significant reductions in gestation body weight gain and food consumption were also evident at 30 mg/kg/d (by 14% and 12%, respectively; $p<0.01$). Subsequently, mean body weights in the 60 mg/kg/d group females remained lower during lactation, but there were no test substance-related effects on mean body weight gain or food consumption. No test substance-related effects on mean body weights, body weight gains or food consumption were noted during gestation in the 5 mg/kg/d group or during lactation in the 5 and 30 mg/kg/d groups.

An increased incidence of thyroid gland follicular cell hypertrophy was noted in the 30 and 60 mg/kg/d groups on LD 21 and was associated with a statistically significant reduction in serum T4; increased TSH at 60 mg/kg/d did not achieve statistical significance. Relative thyroid weights were increased in some of the 60 mg/kg/d group dams and associated with follicular cell hypertrophy.

Summary of thyroid effects

	Dose level of Mancozeb (mg/kg bw/day)			
	0	5	30	60
Total T_4 (µG/dL) GD 20	1.32	0.82	0.59*	1.02
TSH (ng/mL) GD 20	9.58	12.46	11.20	11.98
Total T_4 (µG/dL) LD 21	3.94	4.36	2.98*	2.22**
TSH (ng/mL) LD 21	10.82	10.19	10.75	14.91
Absolute thyroid weight (g) GD 20	0.0233	0.0213	0.0254	0.0260
Absolute thyroid weight (g) LD 21	0.0240	0.0223	0.0216	0.0232
Thyroid cyst, ultimobranchial, present GD 20	2/5	1/5	0/5	1/5
Thyroid hypertrophy, follicular cell - minimal GD 20	0/5	0/5	1/5	1/5
Thyroid cyst, ultimobranchial, present LD 21	5/10	2/10	5/9	2/10
Thyroid hypertrophy, follicular cell - minimal LD 21	2/10	0/10	4/9	5/10

Statistically significant difference from control *P<0.05
Statistically significant difference from control **P<0.01

Mean gestation length and parturition were unaffected by mancozeb. The mean number of pups born, the percentage of males at birth, live litter size on PND 0, postnatal survival and general physical condition were unaffected by maternal exposure to mancozeb. Lower mean body weight gains were observed in the male and female pups in the 30 and 60 mg/kg/d groups during PND 4-7, 7-11 and 17-21, resulting in mean body weights that were 10.9% to 21.6% lower than the control group values during PND 7-21. These body weight decreases were most marked on PND 7 and 11, but were not dose-related.

There were no macroscopic findings in the pups that were found dead, euthanized in extremis or at the scheduled necropsy that could be attributed to maternal exposure to mancozeb. Plasma and milk analyses in the high dose animals (60 mg/kg/d) showed that the pups were exposed to residues of both mancozeb and ETU. The mean concentrations in plasma for GD 20 dams and foetuses were 0.356 ppm and 0.278 ppm, respectively. For LD 4 dam and PND 4 pup plasma, the mean concentrations were 1.18 ppm and 0.037 ppm, respectively. The mean concentration in milk was 0.426 ppm in the LD 4 samples and 0.440 ppm in the LD 10. ETU residues were found in plasma and milk from all dose groups. The ETU residue levels increased with increasing dietary concentration of mancozeb. In this DNT range-finding study, dams given 30 or 60 mg/kg/d mancozeb had lower mean body weights, body weight gains and food consumption, higher mean serum concentrations of TSH, lower mean serum concentrations of total T_4 and a higher incidence of thyroid gland follicular cell hypertrophy which generally corresponded with increased relative thyroid weight at the highest dose. The offspring in the 30 and 60 mg/kg/d groups had decreased body weights and body weight gains. There were no treatment-related effects at 5 mg/kg/d. Therefore, dietary doses of 5, 15 and 30 mg/kg/d were selected for the definitive DNT study.

In the definitive DNT study (Anonymous, 2008c), mancozeb was given on a continuous basis in the diet to groups of 25 pregnant female SD rats from gestation day 6 until euthanasia (post partum days 21-28). The target test substance doses of 5, 15 and 30 mg/kg bw/d were achieved. One group of animals received the basal diet alone and served as the control group. Clinical observations including FOB, body weight and food consumption were monitored for the females during the study. Dams were allowed to litter and rear their offspring to day 21 post partum. At necropsy, thyroid weights were collected and the thyroids processed for histopathological examination. Pre-weaning developmental landmarks (pinna detachment, eye opening and surface righting response) were evaluated in offspring. On postnatal day (PND) 4, litters were culled to 8 pups/litter (4 pups/sex, when possible). If a litter failed to meet the sex ratio criteria (at least 3 pups/sex), the litter was not used for neurobehavioral or neuropathological evaluation. Following culling, a subset (Subset A) of 20 pups/sex/group was assigned to FOB (PND 4, 11, 21, 35, 45 and 60), acoustic startle response (PND 20 and 60), locomotor activity (PND 13, 17, 21 and 61) and learning and memory (PND 62). From this subset, 15 pups/sex/group were selected for brain weight evaluations on PND 72; of these, 10 pups/sex/group were selected for neuropathological and morphometric evaluations on PND 72. A second subset (Subset B) of 20 pups/sex/group was selected for learning and memory tests (PND 22). A third subset (Subset C) of 15 pups/sex/group was selected for brain weight evaluations on PND 21; of these, 10 pups/sex/group were selected for neuropathological and morphometric evaluations on PND 21. Indicators of physical development (balanopreputial separation and vaginal patency) were evaluated for all F1 animals in Subset A. All F1 animals not selected for behavioural evaluations were euthanized and necropsied on PND 21. F1 animals selected for learning and memory assessment on PND 22 were necropsied following completion of these assessments.

Dietary exposure to mancozeb from gestation day 6 through to weaning produced no test substance-related effects on clinical findings, FOB parameters, food consumption, gestation length, parturition or macroscopic findings at necropsy. Test substance-related effects were evident for maternal body weight gain, thyroid weight and thyroid histopathology at 30 mg/kg bw/d. Maternal body weight gain was reduced following the onset of treatment. The effect was statistically significant for gestation days 6–9 and 6-12. For gestation days 6-20, the decrease in body weight gain was 4.6%. However, the effect on body weight gain was influenced by an incidentally higher litter size in the 30 mg/kg bw/d group compared to the concurrent control group (16.0 vs. 14.7 pups/litter). When litter size was used as a covariate in the statistical analysis of body weight gain, the reduction at 30 mg/kg bw/d was highly significant. In addition, when the body weight gain data were normalized for litter size and gestation day 20 conceptus weights, the decrease in gain during gestation days 6-20 was 9.4%. Therefore, 30 mg/kg bw/day was shown to be an ideal high-exposure level based on the OECD test guideline criteria.

A 30 mg/kg bw/d, absolute and relative mean thyroid weights were increased by 7.5% and 9.1%, respectively, and corresponded with an increased incidence of thyroid follicular cell hypertrophy; these changes were considered to be test substance-related.

Summary of thyroid effects

	Dose level of Mancozeb (mg/kg bw/day)			
	0	5	15	30
Absolute thyroid weight (g)	0.0187	0.0192	0.0185	0.0201
Thyroid weight (g/100g final body weight)[b]	0.00638	0.007	0.006	0.00696
Thyroid hypertrophy, follicular cell - minimal	6/24	-	-	11/25

[b] Values for the 5 and 15 mg/kg bw/day groups expressed only to 3 decimal places.

There were no test substance-related effects on any of the F1 litter parameters including survival, clinical signs, FOB, growth, development, motor activity, startle response, learning and memory, brain morphometry and histopathology of the central and peripheral nervous systems. Based on these findings and on the presence of ETU in pup plasma and in milk (investigated in the preliminary study), it can be concluded that mancozeb has been adequately tested for DNT and the results show that mancozeb has no developmental neurotoxicity.

A DNT study from the open literature (Alexstad 2011) is also available. This study was conducted to investigate if perinatal mancozeb exposure would cause developmental neurotoxicity in rats. Two separate studies were conducted, a preliminary dose range-finder (6 dams/group) and a large dose response study (22 dams/group). Time-mated Wistar rats were gavaged once daily from GD7 to PND16. Dose levels for the preliminary study were 0, 200, 350 or 500 mg/kg bw/d. In the main study, dose levels were 0, 50, 100 or 150 mg/kg bw/d.

Doses of 150 mg/kg bw/d and above caused toxic effects in the dams. In the preliminary study, severe weight loss and hind limb paralysis occurred in all groups after a few days and doses were halved on GD12. All animals in the two highest dose groups and two in the low dose group were killed on GD14 due to continued toxic signs. In the main study, two high-dose dams were killed on GD16 and the high dose was lowered to 100 mg/kg bw/d. Maternal body weight and body weight gain was significantly lower than control in all dose groups throughout gestation. Body weight gain was generally higher than control in all groups throughout lactation but terminal body weight was significantly lower than control in the high-dose group.

There were no effects on gestation length, litter size, post implantation loss, neonatal death, gender distribution, AGD (ano-genital distance) or nipple retention. Offspring body weight was lower compared to controls in the high dose group at birth and on PND6, and remained slightly (but not significantly) lower than control until PND45. In dams, T_4 was dose-dependently reduced in all treated groups on GD15. There was no effect on PND 24 and thyroid weights were also unaffected. In offspring no treatment-related effects on T4, thyroid gland weight or histopathology were seen.

Effects of Mancozeb on T4 and testosterone in dams and offspring

	Dose Mancozeb (mg/kg bw/day)			
Hormone measurement (nM)	Control	50	100	150/100
No. of samples	14-15	15-20	17-22	8-10
T_4 – dams GD15	41.4	32.7*	30.15*	26.26*
T_4 – dams PND24	24.8	21.2	19.6	23.14
T_4 – pups PND16	32.5	35.8	34.6	33.1
T_4 – pups PND24	18.5	19.8	22.1	24.3
Testosterone PND16	1.05	1.58	1.54	1.65

* Significantly different from control $p < 0.0001$

There were no effects on offspring organ weights on PND16 (testes, epididymis, prostate, ovaries, liver), PND24 (testes) or in young adults (ovaries) and no histopathological effects in ovaries and testes (other organ not examined). None of the behavioural tests showed any effect of mancozeb exposure.

In conclusion, mancozeb at 150 mg/kg bw/d and above induced toxic effects in dams characterised by severe bodyweight loss and hind limb paralysis. The severity of the maternal toxicity was such that the dose had to be reduced to 100 mg/kg bw/d and some animals had to be terminated prematurely. Increased maternal T_4 levels at 50 mg/kg bw/d and above did not affect offspring T_4 levels, thyroid weights or histopathology on PND 16 and no effects on reproductive organ weights or behaviour in adult offspring. Overall, no developmental toxicity was seen in this study up to a dose of 100/150 mg/kg bw/d. Thyroid toxicity (increased T_4 levels) was observed in the maternal animals from the lowest dose of 50 mg/kg bw/d. These results indicate that in rats, maternal hypothyroxinemia during gestation does not necessarily lead to hyperactivity or reduced learning abilities in the offspring. It is noted that the maternal toxicity reported in this study was not consistent with that observed in other developmental toxicity studies. In regulatory guideline studies, pregnant rats have been shown to tolerate 160 mg/kg bw/d (Anonymous, 2015d) with only a transient effect on body weight. Only the high dose level of 360 mg/kg bw/d (Anonymous, 1988c) induced significant maternal toxicity, including transient, hindlimb paralysis.

10.8.6 Recent/key developmental toxicity study on ETU in rats

ETU is an established developmental toxicant (harmonised classification with Repr Cat 1B; H360D) which causes malformations (mainly of head and neck) in the rat in the absence of maternal toxicity. Approximately 7% of mancozeb is converted to ETU in experimental animals. The evidence suggests that the malformations (mainly of head and neck) seen in the rat with mancozeb are due to its main metabolite, ETU. A new regulatory guideline rat developmental toxicity study on ETU, including measurements of plasma levels of ETU has been conducted to demonstrate that the foetal malformations caused by mancozeb in the rat are due to the production of a teratogenic dose of ETU.

In a prenatal developmental toxicity study of ETU, the test substance was administered orally by gavage to 4 groups of 24 pregnant female SD rats once daily from GD 6 to 19 (Anonymous, 2015a). Dose levels were 2.5, 5, 15, and 30 mg/kg bw/d. No treatment-related clinical findings were noted. Mean body weight losses or lower mean body weight gains were noted in all ETU-treated groups following the initiation of dose administration (GD 6-7). Corresponding lower mean food consumption was noted during this same period in the 15 and 30 mg/kg bw/d groups. Lower mean food consumption was also noted in the 30 mg/kg bw/d group at the end of the dosing period (GD 18-20). As the effects on body weight and food consumption were minor and tended to be limited to the first days of treatment, they were not considered adverse. Mean foetal body weights in the 30 mg/kg bw/d group were up to 13.5% lower than the control group.

Higher incidences of the following foetal malformations were noted at 30 mg/kg bw/d: external (short, bent, curly, constricted, or bifurcated tail, meningocele, malrotated limb, limb hyperextension, and domed head), visceral (hydrocephaly, subcutaneous haemorrhage, and meningocele) and skeletal (skull anomaly, rib anomaly, interrupted ossification of the rib[s], vertebral anomaly with or without associated rib anomaly, vertebral centra anomaly, costal cartilage anomaly, only 12 pairs of ribs present, and small, interrupted, detached, or thin rib[s]). ETU-related increased incidences of visceral and skeletal developmental variations were also noted at 30 mg/kg bw/d. Skeletal developmental variations in this group included 27 presacral vertebrae and 14th rudimentary ribs. Skeletal developmental variations consisting of reduced ossification or unossified bones (reduced ossification of the skull, hyoid unossified, sternebra[e] nos. 1, 2, 3, and/or 4 unossified, reduced ossification of the rib[s], unco-ossified vertebral centra, pubis unossified, and reduced ossification of the vertebral arches) and visceral developmental variations (renal papilla[e] not fully developed and/or distended ureter[s]) noted at 30 mg/kg bw/d were indicative of developmental delay and considered secondary to the reduced foetal weights noted at this dosage level. At 15 mg/kg bw/da, foetal test substance-related effects were limited to an increased incidence of the malformation hydrocephaly. No ETU-related foetal malformations or developmental variations were noted in the 2.5 and 5 mg/kg bw/d groups.

Incidence of foetal malformations

	Dose level of ETU (mg/kg bw/day)				
	0	2.5	5	15	30
No. foetuses/litters examined – external	330/24	320/23	322/23	332/23	363/24
Meningocele	0	0	0	0	183/22
Domed head	0	0	0	0	192/22
Limb malrotated	0	0	0	0	46/11
Limb hyperextension	0	0	0	0	4/1
Tail constricted	0	0	0	0	1/1
Bifurcated tail	0	0	0	0	1/1
Short tail	0	0	0	0	61/12
Bent tail	0	0	0	0	200/24
Curly tail	0	0	0	0	1/1
Localised foetal oedema	0	0	0	1/1	0
	Dose level of ETU (mg/kg bw/day)				
	0	2.5	5	15	30
No. foetuses/litters examined - soft tissue	330/24	320/23	322/23	332/23	363/24
Meningocele	0	0	0	0	4/3
Hydrocephaly	0	0	0	7/2	304/24
Subcutaneous haemorrhage	0	0	0	0	61/21
Right-sided aortic arch	0	0	1/1	0	0
Interrupted aortic arch	0	0	0	1/1	0
Retroesophageal aortic arch	0	0	0	1/1	0
Lungs lobular dysgenesis	0	0	1/1	1/1	0
Situs inversus	0	0	0	1/1	0
No. foetuses/litters examined – skeletal	330/24	320/23	322/23	332/23	363/24
Skull anomaly	0	1/1	0	0	28/17
Bent limb bone(s)	0	1/1	0	0	0
Bent scapula(e)	0	1/1	0	0	0
Rib anomaly	0	0	0	0	55/18
Interrupted rib(s)	0	0	0	0	23/10
Interrupted ossification of the rib(s)	0	0	0	0	4/4
Only 12 pairs of ribs	0	0	0	0	1/1
Rib(s) small	0	0	0	0	5/4
Rib(s) detached	0	0	0	0	5/3
Rib(s) thin	0	0	0	0	1/1
Costal cartilage anomaly	0	0	0	1/1	13/9
Vertebral anomaly with/without rib anomaly	0	0	0	0	45/19
Vertebral centra anomaly	0	0	0	0	3/2

Incidence of foetal variations

	Dose level of ETU (mg/kg bw/day)				
	0	2.5	5	15	30
No. foetuses/litters examined – external	330/24	320/23	322/23	332/23	363/24
Findings	0	0	0	0	0
No. foetuses/litters examined - soft tissue	330/24	320/23	322/23	332/23	363/24
Haemorrhagic ring around the iris	1/1	1/1	0	0	0
Major blood vessel variation	0	1/1	2/2	1/1	0
Liver accessory lobule(s)	4/3	1/1	1/1	1/1	0
Renal papilla(e) not developed and/or distended ureter(s)	0	4/2	0	8/5	28/7
	Dose level of ETU (mg/kg bw/day)				
	0	2.5	5	15	30
No. foetuses/litters examined – skeletal	330/24	320/23	322/23	332/23	363/24
Reduced ossification of the skull	4/3	14/4	10/8	5/5	116/24
Hyoid unossified	4/3	6/4	3/3	2/2	40/16
Reduced ossification of the vertebral arches	0	0	0	0	5/2
Unco-ossified vertebral centra	0	0	0	0	11/8
Cervical centrum 1 ossified	55/16	52/18	81/20	110/21	82/17
7^{th} cervical rib(s)	1/1	1/1	2/2	4/2	0
Bent rib(s)	4/4	12/4	6/4	7/5	9/6
Reduced ossification of the rib(s)	0	0	1/1	0	6/3
Reduced ossification of the 13^{th} ribs	3/1	0	0	2/1	3/2
14^{th} rudimentary rib(s)	13/9	32/13	48/13	63/15	87/23
14^{th} full ribs	0	1/1	2/1	1/1	2/2
27 presacral vertebrae	0	0	6/3	2/1	23/8
Sternebra(e) 1, 2, 3 and/or 4 unossified	0	0	0	2/2	5/33
Sternebra(e) 5 and/or 6 unossified	56/14	19/7	28/12	37/11	67/19
Sternebra(e) malaligned (slight or moderate)	3/3	3/3	3/3	7/5	6/5
Pubis unossified	0	0	0	0	8/2

The mean concentrations of ETU in the blood of maternal and foetal rats on GD 20 were 124, 355, 1280, and 2940 ng/mL in the maternal animals, and 113, 299, 1170, and 2790 ng/mL in foetuses at 2.5, 5, 15, and 30 mg/kg bw/d, respectively. The concentration responses for blood ETU with dose level appeared visually to be linear and exposure in the foetuses was similar to the maternal animals, with mean maternal/foetal ratios of 1 at all dose levels.

In conclusion, in a recent guideline developmental toxicity study in rats, no maternal toxicity was seen up to the top dose of 30 mg/kg bw/d ETU. Developmental effects were noted at 30 mg/kg bw/d, as evidenced by lower mean foetal weights, foetal malformations and developmental variations. Developmental effects were also noted at 15 mg/kg bw/d, but were limited to hydrocephaly in 2 litters.

Other developmental studies on ETU

The developmental toxicity of ETU in mammals has been investigated in numerous studies in rats, mice, hamsters, rabbits, cats and guiinea pigs. These are described in more details in the RAR (2017).

Overall, on the basis of these studies, it can be concluded that ETU is unequivocally teratogenic in the rat (producing malformations predominantly of the central nervous system and head) at doses that do not induce maternal toxicity. The hamster has also been shown to be sensitive to the teratogenic potential of ETU at high doses approaching the maternotoxic range. The mouse is less sensitive to the teratogenic effects of ETU than the rat and hamster. In the guinea pig, rabbit and cat, ETU does not produce clear evidence of teratogenic potential. An overall NOAEL for developmental effects of ETU has been demonstrated in the rat, the most sensitive species, at 5 mg/kg bw/d. These data support classification of ETU with Repr Cat 1B (H360D) under the CLP Regulation. This is consistent with the current harmonised classification of ETU.

10.8.7 Other relevant information to the assessment of developmental toxicity

Two guideline 2-generation studies in the rat are available. These have been described in detail in the RAR (2017). In the first study (Anonymous et al 1988), mancozeb (84%) at dietary levels up to 1200 ppm (70 mg/kg bw/d) produced no adverse effects on reproductive capability or on the health and survival of offspring. In the second study (Anonymous, 1992), mancozeb (88.4%) at dietary levels up to 1100 ppm (65 mg/klg bw/d) produced no adverse effects on fertility and reproductive performance. However, a slight delay in eye opening, a 9% decrease in pup weights on days 14 and 21 and a 19% reduction in pup viability on day 4 were seen at the top dose of 1100 ppm. These effects were associated with parental toxicity (decreased body weight gains (by 10-24%), reduced food consumption and thyroid toxicity in males and females).

10.8.8 Short summary and overall relevance of the provided information on adverse effects on development

Five developmental toxicity studies (3 in the rat and 2 in the rabbit) were described in the original DAR (2000) under Directive 91/414. These studies were conducted in the 1980s and those performed in the rat used severely maternally toxic maximum dose levels (360 - 512 mg/kg bw/d); these maternally toxic doses resulted in malformations (mainly of head and neck) in the rat. Mancozeb was classified as Reprotox category 2 (H361d) as a result of these studies. The evidence suggested that the malformations seen in the rat with mancozeb were due to its main metabolite, ETU. ETU is an established developmental toxicant (harmonised classification with Repr Cat 1B; H360D) which causes malformations (mainly of head – skull, brain and spinal cord; neck – cleft palate; tail; and veretebrae) in the rat in the absence of maternal toxicity. Approximately 7% of mancozeb is converted to ETU in experimental animals.

The teratogenicity study by Anonymous (1980) was central to the decision to classify mancozeb. This study showed that mancozeb caused total litter loss and malformations, mainly of the head and neck, at the high dose of 512 mg/kg bw/d, at which severe maternal toxicity occurred. More recent investigations of the developmental toxicity of mancozeb and ETU in the rat (Anonymous, 2015d & a) have demonstrated that the foetal malformations observed by Anonymous (1980) were totally attributable to the production of a teratogenic dose of ETU and that this was only produced at mancozeb exposures which caused excessive maternal toxicity (death/killing in extremis, paralysis, body weight and food consumption decreases and suffering).

No developmental neurotoxicity was seen in a regulatory study in the rat up to a dose (30 mg/kg bw/d) which caused maternal toxicity (decreases in body weight gain and thyroid histopathology). Additional investigations of the developmental toxicity of mancozeb in the rat form the open literature did not identify effects on neurological endpoints, sexual behaviour, post-natal development and puberty onset. No developmental toxicity was observed in rabbits up to doses (80-100 mg/kg bw/d) causing severe maternal toxicity. The company (Mancozeb Task Force)'s assessment of developmental toxicity is provided in Annex 2. The DS agrees with the applicant assessment, which is provided mainly for completeness and further details.

10.8.9 Comparison with the CLP criteria

Five developmental toxicity studies (3 in the rat and 2 in the rabbit) were described in the original DAR (2000) under Directive 91/414. Malformations (mainly of head and neck) were seen in the rat at severely maternally toxic dose levels. Mancozeb was classified as Reprotox category 2 (H361d) as a result of these studies. The evidence suggested that the malformations seen in the rat with mancozeb were due to its main metabolite, ETU. ETU is an established developmental toxicant (harmonised classification with Repr Cat 1B; H360D) which causes malformations (mainly of head and neck) in the rat in the absence of maternal toxicity. Approximately 7% of mancozeb is converted to ETU in rats.

The rat teratogenicity study by Anonymous (1980) was central to the decision to classify mancozeb. This study showed that mancozeb caused total litter loss and malformations, mainly of the head and neck, at the high dose of 512 mg/kg bw/d, at which severe maternal toxicity occurred.

On the basis of this study, the C&L Specialised Expert Group agreed in 1993 that classification for developmental toxicity was not appropriate because the foetal malformations seen in the rat with mancozeb

were attributed to the formation of ETU, a teratogenic metabolite of mancozeb classified with Repr. Cat 1B; H360D, but that the levels of ETU produced by mancozeb would not reach the threshold for teratogenic effects. However, the same Group concluded in 2005 that classification of mancozeb with Reprotox category 2 (H361d) was warranted. The Group noted that although the malformations occurred in the presence of severe maternal toxicity (death/killing in extremis, paralysis, body weight and food consumption decreases, and suffering), they could not be considered the secondary, unspecific consequence of the observed maternal toxicity.

More recent investigations of the developmental toxicity of mancozeb and ETU in the rat, including measurements of plasma levels of ETU (Anonymous, 2015a & d) have demonstrated that the foetal malformations observed by Anonymous (1980) were totally attributable to the production of a teratogenic dose of ETU. The developmental toxicity study of ETU in the rat (Anonymous, 2015a) clearly identified 5 mg/kg bw/d as a non-teratogenic dose (NOAEL). Anonymous (2015a) also identified 15 mg/kg bw/d as a LOAEL, causing a minimal teratogenic response, whilst 30 mg/kg bw/d was well within the teratogenic dose range. The mean doses of mancozeb calculated to produce blood levels of ETU equivalent to those generated by doses of ETU of 5, 15 and 30 mg/kg bw/d were 143 (range 130-166), 429 (range 390-499) and 859 (range 780-998) mg/kg bw/d respectively. Consequently, when mancozeb was administered to rats at a dose (160 mg/kg bw/d) that was maternally toxic (decreases in body weight and food consumption) but did not caused excessive maternal toxicity (Anonymous, 2015d), insufficient ETU was generated to produce teratogenicity as ETU-driven teratogenic effects would start to appear from a dose of mancozeb of ~429 mg/kg bw/d (range 390-499 mg/kg bw/d). These dose conversions are explained more clearly below.

The doses of mancozeb required to generate equivalent ETU blood levels to those seen after oral administration of ETU at 5, 15 and 30 mg/kg bw/day in pregnant rats (Anonymous, 2015a) were calculated as follows. A developmental toxicity study in rats (Anonymous, 2015a) was conducted with ETU (at doses of 0, 2.5, 5, 15 and 30 mg/kg bw/d), This study identified the NOAEL (5 mg/kg bw/d), LOAEL (15 mg/kg bw/d) and clear-effect-level (30 mg/kg bw/d) for the teratogenicity of ETU in rats. Satellite animals for toxicokinetic analysis were also dosed in this study, such that the AUC and Cmax for ETU in maternal plasma were determined. Plasma levels of ETU (in dams) peaked at 6h after dosing and there was a linear relationship between dose of ETU and plasma ETU levels. The maternal blood levels of ETU (estimated at 24 hours after the final dose) at the ETU NOAEL, LOAEL and clear-effect-level were thus determined.

A similar study (Anonymous, 2015d) was conducted with mancozeb (at doses of 0, 80, 120 and 160 mg/kg bw/d) where AUC and Cmax for the metabolite ETU in maternal plasma were determined. Plasma levels of ETU (in dams) also peaked at 6h after dosing mancozeb, indicating that once absorbed, mancozeb is rapidly metabolised to ETU. There was also a linear relationship between dose of mancozeb and plasma ETU levels. Plasma concentration versus time data for ETU following administration of mancozeb was used to calculate the rate constants (k) for the decline in ETU plasma concentrations. At each dose level of mancozeb, the mean, minimum and maximum maternal plasma levels of ETU were determined. The maternal plasma concentrations of ETU estimated at 24 hours after the final dose of ETU to pregnant rats were used, along with the rate constants (k) for the decline in ETU concentrations after dosing of mancozeb, to estimate the maximum ETU concentrations at 6 hours after dosing with mancozeb (Tmax). Linear regression lines were fitted through the 6-hour ETU concentrations versus dose of mancozeb data to calculate the mean dose of mancozeb required to give concentrations of ETU equal to those at the ETU NOAEL (5mg/kg bw/d), LOAEL (15 mg/kg bw/d) and clear-effect-level (30 mg/kg bw/d). An estimate of the uncertainty of the value was made by estimating the maximum and minimum mancozeb dose values.

The dossier submitter is of the view that these recent investigations have demonstrated that teratogenic levels of ETU will only be generated at mancozeb doses (360-521 mg/kg bw/day) which cause excessive maternal toxicity. This is because only a small amount (approximately 7%) of mancozeb is converted to ETU in animals; in addition, the rate of this conversion is likely to be slow, such that systemic peaks of ETU are only generated at very high doses of mancozeb. The relevance to hazard identification and classification of such teratogenic effects seen only in the presence of excessive maternal toxicity (death/killing in extremis, paralysis, body weight and food consumption decreases, suffering) is questionable. On this basis, classification of mancozeb for developmental toxicity is not warranted and R2 (H361d) should be removed.

10.8.10 Adverse effects on or via lactation

Not addressed in this dossier.

10.8.11 Conclusion on classification and labelling for reproductive toxicity

Not classified (conclusive but not sufficient for classification)

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Only adverse effects on development have been addressed in the CLH dossier. Adverse effects on fertility or sexual function and on or via lactation were outside the scope of the present assessment by the DS.

The DS explained that five developmental toxicity studies (3 in the rat and 2 in the rabbit) were described in the original DAR (2000) assessed under Directive 91/414/EEC. Mancozeb received a harmonised classification as Repr. 2 (H361d) based on one of the rat studies (Anon., 1980) where malformations (including meningoencephalocele, dilated brain ventricles and tail malformations) were observed at a severely maternally toxic dose level of 512 mg/kg bw/d (maternal toxicity manifested as mortality, clinical signs and drastically reduced food consumption). The evidence suggested that the malformations were due to the main metabolite of mancozeb, ETU, which was also tested in this study and produced malformations in the absence of maternal toxicity. ETU is an established developmental toxicant with a harmonised classification as Repr. 1B (H360D).

New regulatory developmental toxicity studies on mancozeb and ETU (Anon. 2015a,c,d) have been conducted to clarify the developmental effects attributed to mancozeb. Availability of these new studies was used by the DS as a justification for reconsideration of the classification of mancozeb for developmental toxicity.

According to the DS, these new studies have demonstrated that the foetal malformations observed in the study by Anon. (1980) were attributable to the production of a teratogenic dose of ETU and that, based on extensive toxicokinetic investigations, the dose of mancozeb needed to produce malformations would be approximately 430 mg/kg bw/d. This was predicted to be a dose associated with such severe maternal toxicity that any potential developmental findings at this dose would have to be disregarded for classification purposes. The main study carried out with mancozeb itself (Anon., 2015d) tested only doses up to 160 mg/kg bw/d, which was considered by the DS an appropriate top dose causing sufficient maternal toxicity, i.e. reduced body weight gain and food consumption.

In addition, two new developmental neurotoxicity studies are available: a regulatory study by Anon. (2008c), conducted to address the concern about the potential relationship between thyroid effects and brain development, and a literature study by Axelstad *et al.* (2011). Both studies were negative regarding developmental neurotoxicity. This, according

to the DS, sufficiently alleviated any remaining concerns about the adverse impact of mancozeb-induced thyroid toxicity on brain development.

An additional recent developmental neurotoxicity study conducted with ETU (Anon., 2013) was also provided for the weight of evidence analysis. The DS concluded that ETU does not cause developmental neurotoxicity in rats at doses where thyroid hormone levels are reduced. Although ETU is teratogenic in rats, similar effects were not seen in rabbits or mice. Several lines of evidence indicate that the main morphological effects on rat foetuses are caused by a direct MoA and not via thyroid hormone reduction.

In summary, the DS proposed to remove the existing Repr. 2 (H361d) classification for mancozeb on the basis of new information (Anon., 2015d; Anon., 2008c; Axelstad *et al*., 2011) as well as mechanistic and toxicokinetic considerations on mancozeb and ETU.

Comments received during public consultation

7 MSCAs, 1 industry association and 1 individual commented on this endpoint.

The industry association and the individual supported the dossier submitter's proposal of no classification for developmental toxicity. They concluded that mancozeb is not developmentally toxic when tested in a modern regulatory guideline study in the rat and is not developmentally neurotoxic in the rat. Industry argued that, given that humans appear to more closely resemble the non-sensitive species in their ability to metabolise ETU, any risk to humans is likely to be minimal. Therefore, in line with the DS, industry concluded that mancozeb does not meet the criteria for classification according to the CLP Regulation.

6 MSCAs did not support removal of Repr. 2 for development. Their comments together with the dossier submitter's responses are summarised below.

- There was general agreement that the malformations in the study of Anon. (1980) can be attributed to the metabolite ETU. Several MSCAs suspected that the mechanism of action (MoA) behind brain malformations is mediated via thyroid hormone disruption.
- Several commenting MSCAs were of the view that the severe maternal toxicity at the highly teratogenic top dose in the study of Anon. (1980) warrants downgrading the classification to Category 2 but not to no classification. One of them pointed out that according to the CLP regulation, developmental effects occurring even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated that the developmental effects are secondary to maternal toxicity. In response, the DS insisted that maternal toxicity at the top dose was so severe that the developmental findings at this dose should be completely discounted.
- The doses in the new PNDT study by Anon. (2015d) were considered too low by some commenters. The range-finding study by Anon. (2015b) indicated that doses up to 300 mg/kg bw/d could be tested without causing severe maternal toxicity. The DS did not respond to this.
- Some MSCAs asked about the validity of the older rat PNDT study of Anon. (1999b), where no maternal toxicity was observed at doses up to 500 mg/kg bw/d. The DS replied that the reliability of this study is questionable as the developmental findings are inconsistent with those of the other available rat

studies and the lack of maternal toxicity at doses up to 500 mg/kg bw/d is not in agreement with maternal effects seen in the other studies.

- One MSCA pointed out that the methodology used to choose the top dose in the developmental neurotoxicity study of Anon. (2008c) is not in accordance with the OECD test guideline (TG) 426 (adopted on 16 Oct 2007). This TG states that if the substance has been shown to be developmentally toxic (which is the case here), the highest dose level should be the maximum dose which will not induce excessive offspring toxicity. The MSCA argued that this is not true for the top dose of 30 mg/kg bw/d as the older developmental toxicity studies indicate much higher developmental LOAELs. This MSCA also pointed out that a dose of 30 mg/kg bw/d in rats does not address the concern about developmental effects of thyroid disruption as this is a level where thyroid effects only begin to appear. In their response, the DS insisted that the choice of the top dose was adequate as it produced maternal toxicity (including effects on thyroid weight and histopathology) and measurable levels of ETU in pup plasma and milk. They also pointed out the lack of developmental neurotoxicity in the study by Axelstad *et al.* (2011) at doses up to 150 mg/kg bw/d.
- One MSCA pointed out uncertainties in relation to the negative results in the developmental neurotoxicity (DNT) studies. Pups were not exposed directly by gavage in any of the DNT studies and brain development mainly occurs postnatally in rats. Limited milk transfer leads to low exposures, which could explain the absence of neurotoxic effects. Learning and memory tests implemented in standard DNT studies may not be sensitive enough to detect effects on cognitive development. The DS acknowledged the limitations of the current testing guidelines but pointed out that this is an issue related to DNT testing in general, and is not specific to the substance in question.
- Another MSCA requested evaluation of effects on pups during lactation due to a slight delay in eye opening and reduced pup weight and viability in one of the 2-generation studies (Anon., 1988b). The DS replied that this effect was secondary to maternal toxicity and therefore classification for effects on or via lactation is not justified.

Additional key elements

Inhalation PNDT study with mancozeb in rats (Lu and Kennedy 1986)

This study from literature, not included in the CLH report but identified by EFSA in the review process, is summarised below.

Summary of the PNDT study with mancozeb via inhalation (Lu and Kennedy, 1986)		
Type of study; Reference	**Method**	**Observations**
PNDT study, inhalation, rat	Comparable to OECD 414 Non-GLP Strain: Crl:CD Purity: 80%	**Study 1** (range-finding study) <u>Maternal toxicity</u> 1890/500 mg/m^3: • Mortality 24/38 (average time for onset of mortality: GD 14); the animals found dead or

Lu and Kennedy 1986	Whole-body exposure, GD 6-15, 6 h/day Food consumption not recorded Study 1 (range-finding study) Concentrations: 0, 110, 890, 1890/500 mg/m^3 MMAD mostly 1.4-1.9 µm 37-38 females/group The 890 mg/m^3 group was exposed for 4-5 days and then held without further exposure In the 1890/500 mg/m^3 group half of the animals were exposed to 1890 mg/m^3 for 1 day, then to 500 mg/m^3 for 5 days and then held without exposure; the second half was exposed to 500 mg/m^3 for 5 days and then held without exposure Study 2 (main study) Concentrations: 0, 1, 17, 55 mg/m^3 27 females/group	killed *in extremis* had whitish-yellow material in the trachea, congested lungs, yellowish material and sticky dark red fluid in the GIT which was filled with gas, and enlarged adrenal glands • Clinical signs: laboured breathing, rapid respiration, extreme hypoactivity, ruffled fur, emaciation, hind limb weakness 890 mg/m^3: • Mortality 30/37 (average time for onset of mortality: GD 10); gross pathology findings as in the 1890/500 mg/m^3 group (except the red fluid in the GIT) • Clinical signs: laboured breathing, rapid respiration, hypoactivity, ruffled fur, emaciation, hind limb weakness 110 mg/m^3: • Mortality 3/37 (1 on GD 15, 2 on GD 16) • Clinical signs: hind limb weakness, rapid and laboured breathing (slight) • Bw loss GD 6-15 <u>Developmental toxicity</u> 1890/500 mg/m^3: only 3 litters; no significant effects 890 mg/m^3: no litters 110 mg/m^3: reduced foetal bw (by 14%, not stat. sign.); wavy ribs **Study 2** (main study) <u>Maternal toxicity</u> 55 mg/m^3: • Transient hindlimb weakness (6/27) • Reduced bw gain, GD 6-15 (by 40%) ≤ 17 mg/m^3: no effects <u>Developmental toxicity</u> 55 mg/m^3: wavy ribs ≤ 17 mg/m^3: no effects

<u>Extended one-generation reproductive toxicity study with ETU (Anon., 2013)</u>

A developmental neurotoxicity study with mancozeb has been conducted (Anon., 2008c) to address concerns about developmental consequences of thyroid disruption. However, the top dose in the DNT study with mancozeb caused only very slight thyroid effects.

Therefore, a summary of a recent extended one-generation study with ETU (Anon., 2013), reporting more pronounced thyroid disruption, is provided here.

Summary of the EOGRTS with ETU (Anon., 2013)		
Type of study; **Reference**	**Method**	**Observations**
EOGRTS, rat Anon. 2013	OECD 443 GLP Doses: approx. 0, 0.2, 2, 10 mg/kg bw/d Strain: Crl:CD(SD) Purity: 100% P generation: 27/sex/dose F1 cohort 1A: reproductive/endocrine toxicity; 26/sex/dose F1 cohort 1B: endocrine group; 26/sex/dose F1 cohort 2A: developmental neurotoxicity; 12/sex/dose; terminated on PND 78 F1 cohort 2B: neuropathology; 10/sex/dose; terminated on PND 22 Second generation (F2) not triggered Developmental neurotoxicity investigations: acoustic startle, FOB, motor activity; histopathology, brain morphometry	<u>General toxicity</u> 10 mg/kg bw/d: • ↓ bw in P maternal animals during lactation • ↓ bw in F1 pups during lactation ≤ 2 mg/kg bw/d: no effects <u>Reproductive findings</u> No effects <u>Thyroid effects</u> P generation (effects after 11-13 weeks of dosing) 10 mg/kg bw/d: • ↓ T4 (by approx. 70%) • ↑ TSH (approx. 4-fold) • ↑ thyroid wt (abs. by 63/35% m/f) • Follicular cell hyperplasia, very slight to slight (all animals vs none in controls); follicular cell hypertrophy (very slight to slight) • Follicular cell adenoma (m 2/27 vs none in controls) • Pituitary, slight hypertrophy of pars distalis (24/27 vs none in controls) 2 mg/kg bw/d: • ↓ T4 (by approx. 30%) • Follicular cell hyperplasia, very slight to slight (m 15/27, f 7/27, none in controls); follicular cell hypertrophy (very slight to slight) • Pituitary, slight hypertrophy of pars distalis (m 5/27, f 3/27, none in controls) 0.2 mg/kg bw/d: only slight histopathological changes, no changes in thyroid wt or hormone levels F1 on PND 22, non-selected weanlings (only effects on hormone levels listed here) 10 mg/kg bw/d: • ↓ T4 (by approx. 50%) • ↑ TSH (2.8-fold)

		2 mg/kg bw/d: • ↓ T4 (f by 24%) • ↑ TSH (f 1.5-fold) 0.2 mg/kg bw/d: no effects F1 on PND 85, cohort 1A (only effects on hormone levels listed here) 10 mg/kg bw/d: • ↓ T4 (by approx. 70%) • ↑ TSH (6.0/3.3-fold m/f) 2 mg/kg bw/d: • ↓ T4 (by 14/32% m/f) 0.2 mg/kg bw/d: no effects <u>Developmental neurotoxicity</u> 10 mg/kg bw/d: • ↓ brain size in cohort 2A (brain weight reduced by 6-7%, reduced brain macroscopic and microscopic measurements by ≤ 5%); no effect in cohort 2B • ↓ bw in males (by 12%) but not in females (only by 3%, not stat. sign.) at sacrifice ≤ 2 mg/kg bw/d: no effects No effects on neurobehavioural endpoints

Assessment and comparison with the classification criteria

Developmental toxicity

<u>Developmental studies evaluated in the initial DAR (2000)</u>

The rat and rabbit prenatal developmental toxicity (PNDT) studies with mancozeb (or mancozeb <u>and</u> ETU) evaluated in the original DAR are summarised in the following table.

Developmental toxicity studies with mancozeb (or mancozeb and ETU) evaluated in the initial DAR (2000)		
Type of study; **Substance;** **Reference**	**Method**	**Observations**
Rat		
PNDT study, gavage, rat Mancozeb, ETU	EPA OPPTS 870.3700 Non-GLP	**Mancozeb** <u>Maternal toxicity</u> 512 mg/kg bw/d:

Anon. 1980	Doses mancozeb: 0, 2, 8, 32, 128, 512 mg/kg bw/d Dose ETU (positive control): 50 mg/kg bw/d Dosing GD 6-15 26 females/group	• Mortality 3 out of 22 pregnant (1 found dead on GD 18, 2 killed due to signs of abortion on GD 17 and 18) • Clinical signs: lethargy, ataxia, scruffy coat, diarrhoea or soft faeces, hunched, dehydrated • Markedly reduced food consumption (GD 10-15: 2.0 g/day vs 16.2 g/day in controls) • bw loss instead of gain GD 6-15; bw corrected for gravid uterus weight reduced by 19% 128 mg/kg bw/d: • ↓ food consumption (GD 10-15: 12.8 g/day vs 16.2 g/day in controls) • ↓ bw gain (GD 6-15 by 52%); bw corrected for gravid uterus weight reduced by 8% (not stat. sign.) ≤ 32 mg/kg bw/d: no effects <u>Developmental toxicity</u> 512 mg/kg bw/d: • Total resorption 6/19 • ↓ foetal weight (by 28%) • Malformations: meningoencephalocele, dilated brain ventricles, cleft palate, kinked/short tail • Variations: reduced ossification 128 mg/kg bw/d: single incidences of forelimb flexure, abnormal pelvic limb posture, dilated brain ventricles ≤ 32 mg/kg bw/d: no effects **ETU** (50 mg/kg bw/d) <u>Maternal toxicity</u> No effects <u>Developmental toxicity</u> • No increase in resorptions • ↓ foetal weight (by 12%) • Malformations: meningoencephalocele, exencephaly, dilated brain ventricles, brain tissue atrophy, kinked/short tail, forelimb flexure, vertebrae fused/absent, kidney agenesis, cryptorchidism • Variations: hydronephrosis, hydroureter, reduced ossification Incidences of selected foetal anomalies are presented in a separate table below
PNDT study, gavage, rat Mancozeb Anon. 1988c	OECD 414 GLP Doses: 0, 10, 60, 360 mg/kg bw/d Dosing GD 6-15 25 females/group	<u>Maternal toxicity</u> 360 mg/kg bw/d: • Mortality (killed *in extremis*) 1/25, preceded by bw loss and hind limb paralysis • Slight, transient hind limb paralysis 4/25 • ↓ bw gain (GD 6-20 by 25%) and food consumption (by approx. 20% GD 6-15)

		≤ 60 mg/kg bw/d: no effects <u>Developmental toxicity</u> 360 mg/kg bw/d: • ↑ incidence of incomplete ossification of interparietal bone and thoracic vertebral centra, large anterior fontanelle ≤ 60 mg/kg bw/d: no effects
PNDT study, gavage, rat Mancozeb Anon. 1999b	OECD 414 GLP Doses: 0, 100, 225, 500 mg/kg bw/d Dosing GD 6-15 24 females/group	<u>Maternal toxicity</u> 500 mg/kg bw/d: lung congestion/hyperaemia (24/24 vs 12/24), liver congestion/mottling (12/24 vs 2/24), kidney congestion (7/24 vs 3/24) 250 mg/kg bw/d: lung congestion/hyperaemia (20/24 vs 12/24), liver congestion/mottling (14/24 vs 2/24), kidney congestion (9/24 vs 3/24) 100 mg/kg bw/d: no effects <u>Developmental toxicity (f, foetuses; l, litters)</u> 500 mg/kg bw/d: • Reduced ossification (dumbbell shaped thoracic centra 15f/6l vs none in controls) • Lung emphysema (9f/7l vs 1f/1l), heart ventricle dilatation (5f/5l vs 1f/1l), adrenal congestion (6f/6l vs 1f/1l), congested kidney (9f/9l vs 1f/1l), hydroureter (7f/7l vs 1f/1l), convoluted ureter (5f/5l vs 1f/1l), brain dilated lateral ventricle (9f/9l vs 3f/3l) 225 mg/kg bw/d: • Reduced ossification (dumbbell shaped thoracic centra 16f/6l vs none in controls) • Lung emphysema (7f/7l vs 1f/1l), congested kidney (7f/7l vs 1f/1l), hydroureter (6f/6l vs 1f/1l) 100 mg/kg bw/d: no effects
Rabbit		
PNDT study, gavage, rabbit Mancozeb Anon. 1987b	OECD 414 GLP Doses: 0, 10, 30, 80 mg/kg bw/d Dosing GD 7-19 20 females/group	<u>Maternal toxicity</u> 80 mg/kg bw/d: • 2/20 sacrificed in moribund condition (one of them was pregnant and did not abort) • 5/20 abortion (none in controls) • Body weight and food consumption significantly decreased in does that aborted and those sacrificed moribund; does producing at least one viable foetus had bw gains and food consumption similar to controls 30 mg/kg bw/d: no effects

		Developmental toxicity 80 mg/kg bw/d: abortions 30 mg/kg bw/d: no effects
PNDT study, gavage, rabbit Mancozeb Anon. 1991b	OECD 414 GLP Doses: 0, 5, 30, 55, 100 mg/kg bw/d Dosing GD 6-18 18 females/group	Maternal toxicity 100 mg/kg bw/d: • 5/16 abortion vs 2/13 in controls • Reduced food consumption (by 37% GD 6-19) and bw gain (by 62% GD 6-19) 55 mg/kg bw/d: no effects Developmental toxicity 100 mg/kg bw/d: • Abortions • Slight increase in post-implantation loss (27% vs 22% in controls) 55 mg/kg bw/d: no effects

Prenatal developmental toxicity (PNDT) study, Anon. (1980)

Mancozeb was classified with Repr. 2 for developmental effects under the Dangerous Substance Directive (DSD) mainly based on the outcome of the Anon. (1980) study. This study is also considered by RAC to be the key study for classification of mancozeb. In this study, the top dose of 512 mg/kg bw/d was severely teratogenic with the most prevalent malformations being meningoencephalocele, dilated brain ventricles, cleft palate and tail malformations. Other effects at the top dose included reduced pup weight, delayed ossification and an increase in resorptions. Developmental toxicity at the next lower dose of 128 mg/kg bw/d was limited to single incidences of several malformations. These single occurrences are likely to indicate proximity of this dose level to the threshold dose for the induction of malformations in this study. ETU was employed as a positive control at 50 mg/kg bw/d. Incidences of the most relevant anomalies are listed in the table below. For a full list please refer to the CLH report.

Incidences of selected external and visceral malformations in the study with mancozeb by Anon. (1980)

	Corn oil (10 mL/kg/day)	Dithane M-45 (mg/kg bw/day)					ETU (mg/kg bw/day)
	Control	2	8	32	128	512	50
External							
Number of foetuses/litters	278/23	248/22	245/23	248/23	212/20	155/13	232/21
Cleft palate	0	0	0	0	0	24/3	8/1
Meningoencephalocele	0	0	0	0	0	27/4	181/20
Kinked tail	1/1	0	0	0	0	58/8	114/16

Short tail	0	0	0	0	0	15/3	141/19
Tail agenesis	0	0	0	0	0	0	59/11
Forelimb flexure	0	0	0	0	1/1	4/2	104/15
Abnormal pelvic limb posture	0	0	0	0	1/1	0	75/15
Visceral							
Number of foetuses/litters	90/23	80/22	83/23	84/23	73/20	52/13	81/21
Dilated brain ventricles	0	0	2/2	0	1/1	28/9	75/20
Brain tissue atrophy	0	0	0	0	0	9/2	42/13
Spinal cord compressed	0	0	0	0	0	13/4	64/18
Kidney agenesis	0	0	0	0	0	0	9/4
Cryptorchidism	0	0	0	0	0	2/2	14/8

The table shows that the teratogenic profile of mancozeb is similar to that of ETU. RAC considers it plausible that the malformations in the mancozeb top dose group resulted from generation of teratogenic levels of ETU.

The top dose caused pronounced maternal toxicity. Clinical signs of toxicity started to appear between GD 11 and 13 and worsened till GD 17. These included lethargy, ataxia, scruffy coat and dehydrated appearance. One dam was found dead on GD 18 and two others were sacrificed (GD 17 and 18). Food consumption was drastically reduced (GD 10-15: mean 2.0 g/day vs 16.2 g/ day in the control group) and the dams were losing weight till the end of the dosing period. Limited maternal toxicity was observed at 128 mg/kg bw/d, consisting of reduced body weight gain (by 52% GD 6-15) and reduced food consumption (12.8 g/day vs 16.2 g/day in controls GD 10-15); the terminal body weight corrected for gravid uterus weight was reduced by 8% (not statistically significant). No maternal toxicity was present in the ETU-treated group, which demonstrates that the ETU-induced malformations were not secondary to maternal toxicity. RAC further notes that occurrence of malformations in mancozeb-treated groups did not correlate with maternal toxicity at the level of individual animal data (for details see 'Supplemental information' in the background document).

RAC considers the maternal toxicity at the top dose of 512 mg/kg bw/d mancozeb to be excessive. On the other hand, RAC notes that this is a dose level associated with a relatively high incidence of malformations and the threshold for induction of malformations in this study is likely to lie close to 128 mg/kg bw/d as indicated by single occurrences of several anomalies at the latter dose. Since only limited maternal toxicity was present at 128 mg/kg bw/d, maternal toxicity does not reduce the concern about the developmental findings in mancozeb-treated groups. The 2 cases of dilated brain ventricles at 8 mg/kg bw/d are difficult to interpret in view of the steep dose-response curve seen in PNDT studies with ETU. It is also noted that developmental effects observed at maternally toxic doses are not automatically discounted under the CLP (CLP, Annex I, 3.7.2.3.4 and 3.7.2.4.3), especially

in the case of irreversible effects such as structural malformations (CLP, Annex I, 3.7.2.3.5).

PNDT study, Anon. (1988c)

The only developmental effect at the top dose of 360 mg/kg bw/d was reduced ossification of the skull and of the thoracic vertebra. Maternal toxicity in most of the top dose animals was limited to modest reductions in food consumption and body weight gain. However, 1 animal was severely affected (body weight loss and hind limb paralysis) and consequently killed *in extremis*. 4 other dams showed slight, transient hind limb paralysis at the end of the dosing period.

The skeletal variations observed in this study may reflect a general developmental delay and are not considered sufficiently adverse to contribute to classification. Nevertheless, the increased incidence of incomplete ossification of the interparietal bone might be related to meningoencephalocele observed at a higher dose in the study by Anon. (1980) (cf. Khera, 1973).

PNDT study, Anon. (1999b)

The top dose in this study was 500 mg/kg bw/d. The various developmental anomalies reported are of low incidence and/or low or questionable biological significance. Therefore, this study is considered negative regarding developmental toxicity.

As for maternal toxicity, considering the results of the other rat PNDT studies with mancozeb of similar design (Anon., 1980; Anon., 1988c; Anon., 2015d), at least a slight reduction in food consumption and body weight gain would be expected at a dose of 500 mg/kg bw/d. No effect whatsoever on these two parameters as well as a total lack of clinical sings at any time point is not considered plausible. This might be explained by a systematic error leading to a much lower exposure of the animals than stated or by incorrect description of what really occurred in the study. As both causes would invalidate the results, the study Anon. (1999b) is considered to be of low reliability.

PNDT study, Lu and Kennedy (1986)

No increase in malformations was observed in this rat inhalation study up to doses causing high maternal mortality. The only developmental effect was increased incidence of wavy ribs in the presence of maternal toxicity manifested as mild hind limb paralysis and reduced body weight gain.

PNDT studies in the rabbit

No developmental toxicity was observed in the rabbit up to maternally toxic doses (Anon., 1987b; Anon., 1991b).

<u>New developmental studies</u>

The developmental toxicity studies with mancozeb that have become available since the initial DAR (2000) are summarised in the following table.

New developmental toxicity studies with mancozeb		
Type of study; Reference	**Method**	**Observations**
14-day tolerability study in non-pregnant rats, gavage Anon. 2015b	Non-guideline Non-GLP Doses: 0, 60, 120, 180, 240, 300 mg/kg bw/d 3 females/group Toxicokinetic investigations	300 mg/kg bw/d: ↓ bw by 9.8% 240 mg/kg bw/d: ↓ bw by 6% 180 mg/kg bw/d: ↓ bw by 8.3% ≤ 120 mg/kg bw/d: no effects
PNDT range-finding study, gavage, rat Anon. 2015c	Non-guideline GLP Doses: 0, 80, 120, 160 mg/kg bw/d Dosing GD 6-19 23 females/group Toxicokinetic investigations	<u>Maternal toxicity</u> 160 mg/kg bw/d: ↓ bw gain (GD 6-20 by 22%; GD 9-12 by 37%), reduced food consumption (GD 6-20 by 10%); ↓ corrected bw by 6.5% 120 and 80 mg/kg bw/d: ↓ bw gain (GD 6-20 by approx. 7%; GD 9-12 by approx. 30%) No consistent effect on maternal T4 levels <u>Developmental toxicity</u> (limited foetal evaluation) ≤ 160 mg/kg bw/d: No effects
PNDT study, gavage, rat Anon. 2015d	OECD 414 GLP Doses: 0, 10, 40, 160 mg/kg bw/d Dosing GD 6-19 25 females/group	<u>Maternal toxicity</u> 160 mg/kg bw/d: ↓ bw gain (GD 6-20 by 14%) and food consumption (GD 6-20 by 8%); ↓ corrected bw by 6% ≤ 40 mg/kg bw/d: No effects <u>Developmental toxicity</u> ≤ 160 mg/kg bw/d: No effects
Range-finding study for a developmental neurotoxicity study, dietary, rat Anon. 2008b	Non-guideline Non-GLP Doses: 0, 5, 30, 60 mg/kg bw/d Dosing GD 6 to PND 21 15 females/group Toxicokinetic investigations No investigations into neurotoxicity	<u>Maternal toxicity</u> 60 mg/kg bw/d: • ↓ bw gain (by 37% GD 6-20) and food consumption • No significant effect on T4 or TSH on GD 20 • ↓ T4 (by 44%) and ↑ TSH (1.4-fold, not stat. sign.) on PND 21 • Follicular cell hypertrophy, minimal (5/10 vs 2/10, not stat. sign.) 30 mg/kg bw/d: • ↓ bw gain (by 14% GD 6-20) and food consumption • ↓ T4 (by 24%) on PND 21 • Follicular cell hypertrophy, minimal (4/9 vs 2/10, not stat. sign.)

		<u>Developmental toxicity</u> (no investigations into neurotoxicity) ≤ 60 mg/kg bw/d: No effects Mancozeb and ETU were detected in plasma and milk of the dams and in plasma of the foetuses and pups
Developmental neurotoxicity study, dietary, rat Anon. 2008c	OECD 426 GLP Doses: 0, 5, 15, 30 mg/kg bw/d Dosing: GD 6 to weaning (PND 21-28) 25 females/group	<u>Maternal toxicity</u> 30 mg/kg bw/d: • ↓ bw gain (by 26% GD 6-12, by 5% GD 6-20) • Thyroid follicular hypertrophy (minimal, 11/25 vs 6/24 in controls – not stat. sign.) ≤ 15 mg/kg bw/d: No effects <u>Developmental toxicity</u> ≤ 30 mg/kg bw/d: No effects
Developmental neurotoxicity study, gavage, rat Axelstad *et al.* 2011	Non-guideline Non-GLP Doses: 0, 50, 100, 150/100 mg/kg bw/d 22 females/group The top dose was reduced from 150 to 100 mg/kg bw/d due to maternal toxicity at different time points; this group had a low number of litters (n=9)	<u>Maternal toxicity</u> 150/100 mg/kg bw/d: • Severe bw loss • Mild hind limb paralysis • ↓ T4 on GD 15 (by 37%) 100 mg/kg bw/d: • ↓ bw gain (by 27% GD 7-21) • ↓ T4 on GD 15 (by 27%) 50 mg/kg bw/d: • ↓ bw gain (by 20% GD 7-21) • ↓ T4 on GD 15 (by 21%) <u>Developmental neurotoxicity</u> No effects in any dose group
Investigations into effects on behaviour and sexual development; gavage, rat Hass *et al.* 2012 Jacobsen *et al.* 2012	Non-guideline Non-GLP Doses: 0, 6.25, 25 mg/kg bw/d No. of females per group: 15, 5, 7 Dosing GD 7-21 and PND 1-16	25 and 6.25 mg/kg bw/d: • No effect on learning or memory • No effect on sexual development

PNDT study, Anon. (2015d)

No developmental effects were seen in this study. Maternal toxicity at the top dose of 160 mg/kg bw/d manifested as modest reductions in food consumption (by 8% GD 6-20) and

body weight gain (by 14% GD 6-20); the corrected terminal body weight was reduced by 6%.

In a 14-day preliminary study in non-pregnant rats (Anon. 2015b), the top dose of 300 mg/kg bw/d induced body weight loss leading to reduced body weight (by 10%) and no clinical signs of toxicity.

RAC notes that maternal toxicity at 160 mg/kg bw/d was rather limited and the tolerability study in non-pregnant animals (Anon., 2015b) indicates that a higher dose could have been tested. Therefore, due to this selection of the top dose, the Anon. (2015d) study does not address the concerns raised by the previous Anon. (1980) study.

Based on toxicokinetic investigations (Anon., 2015c) and a parallel study with ETU (Anon., 2015a), the DS estimated the LOAEL for brain malformations at approx. 430 mg/kg bw/d mancozeb. The methodology used for this extrapolation is discussed in detail under 'Supplemental information' in the background document.

Developmental neurotoxicity study, Anon. (2008c)

This study was conducted to address the concern about a potential relationship between thyroid effects and brain development. No effects on functional observational battery, motor activity, startle response, learning and memory, brain morphometry or histopathology of the CNS and PNS were observed up to the top dose of 30 mg/kg bw/d.

It is noted that the top dose did not induce sufficient thyroid toxicity in maternal animals. In a preliminary study, the only significant thyroid-related effect at 30 mg/kg bw/d was a T4 reduction by 24% on lactation day 21 (the reduction on GD 20 might have been incidental as there was no significant reduction at the next higher dose). General maternal toxicity was limited to minor reductions in body weight gain (by 5% GD 6-20). Thus, the concern about the potential impact of maternal thyroid disruption by mancozeb on brain development in the offspring has not been sufficiently addressed by this study.

Developmental neurotoxicity study, Axelstad et al. (2011)

In this study, groups of 9-21 mated rats were dosed with 0, 50, 100, or 150 mg mancozeb/kg bw/d from gestation day 7 to postnatal day 16. The top dose of 150 mg/kg bw/d had to be reduced to 100 mg/kg bw/d during the course of the study due to severe body weight loss and mild hind limb paralysis. T4 reduction by 27% was observed at 100 mg/kg bw/d. No treatment-related effect was detected in the adult offspring in a battery of behavioural tests (radial arm maze, motor activity, acoustic startle response). The relatively low threshold for maternal toxicity compared to other studies is noted but the reason for this is not known.

Extended one-generation study with ETU, Anon. (2013)

The top dose in this study, 10 mg/kg bw/d, caused a marked T4 reduction (by 70%). Slightly smaller brain (brain weight reduced by 6-7% in both sexes) was the only neurotoxicity-related finding in this study. This effect cannot be explained by lower body weights as only a slight body weight reduction (by 3% compared to controls) was observed in females (in males there was a body weight reduction by 12%). The lower brain weight might be related to brain malformations seen at higher doses in PNDT studies with ETU. Effects on learning and memory were not investigated.

Relationship between maternal hypothyroidism and developmental neurotoxicity

The importance of maternal contribution of T4 for proper *in utero* brain development is well established. In humans the consequences of maternal thyroid hormone deficiency range from decreased IQ to severe neurological damage, depending on the degree of deficiency (reviewed for example by Moog *et al.*, 2017).

Limited data on the impact of maternal hypothyroidism on neurological development are available for mancozeb and ETU. The rat EOGRTS with ETU (Anon., 2013) did not show any functional defects but effects on learning and memory were not investigated in this study. RAC also notes that the standard study design may not reveal subtle effects on cognitive development. A recent epidemiology study (van Wendel de Joode *et al.*, 2016) in children 6-9 years old reported an association of urinary ETU levels with impaired verbal learning but not with nine other neurobehavioural outcomes. These data are not considered sufficient to support classification.

Developmental toxicity of ETU

ETU is a potent rat teratogen inducing a broad spectrum of malformations in the absence of maternal toxicity. Its teratogenic properties have been investigated in a number of regulatory and published studies, including recent ones. The studies included in the CLH report or the RAR are summarised under 'Supplemental information' except for an extended one-generation reproductive toxicity study (Anon., 2013), which is summarised in the background document under 'Additional key elements'. The key information on ETU relevant for classification of mancozeb can be summed up as follows:

- The rat is the most sensitive species to induction of malformations by ETU out of those tested (rat, rabbit, mouse, hamster).
- The malformations in the rat most consistently observed across the studies are:
    - enlargement of brain ventricles due to loss of brain tissue (hydrocephalus *ex vacuo*)
    - cranial meningocele or meningoencephalocele (protrusion of meninges or meninges and brain through a defect in the skull)
    - tail malformations (short, bent, kinky, absent)
    - malrotated limb
- The dose-response curve in the rat is very steep. Only low incidence of effects or no effects at all are seen around 10 mg/kg bw/d while practically all foetuses are malformed at doses around 40 mg/kg bw/d.
- A single oral dose of 50 mg/kg bw on GD 13 may result in 100% foetuses malformed (Teramoto, 1978b). A single oral dose of 30 mg/kg bw on GD 15 can induce very severe hydrocephalus in some of the pups (Khera and Tryphonas, 1977).
- Dilation of brain ventricles induced during organogenesis tends to progress during postnatal life (Khera and Tryphonas 1977). Thus, the full extent of the structural brain damage may not be apparent in a standard PNDT study.
- The ETU-induced hydrocephalus is unlikely to be mediated by maternal hypothyroidism (Emmerling 1978; Lu and Staples 1978; Stanisstreet *et al.* 1990). Mechanistic studies indicate that cell necrosis in the central nervous system is part of the MoA (Teramoto 1978b; Khera and Tryphonas 1977; Khera 1987).

Conversion of mancozeb to ETU

According to the DS, recent toxicokinetic studies in pregnant rats (Anon., 2015a,c) showed that a gavage dose of approximately 429 mg/kg bw mancozeb is needed to produce the same peak plasma concentration of ETU as 15 mg/kg bw ETU (see the Background Document). This corresponds to a conversion factor of approx. 3.5%.

Toxicokinetic and metabolic studies with mancozeb in non-pregnant rats by Anon. (1986f) and Anon. (1986g) provided a conversion factor of approx. 7%. This factor is based on the amount of ETU recovered from urine and bile after a single oral dose of mancozeb.

A comparison of the developmental effects at the top dose in the PNDT study with mancozeb by Anon. (1980) with those in PNDT studies with ETU suggests a conversion factor of about 5–7%.

All three factors are associated with uncertainties. Derivation of these factors and the uncertainties are described under 'Supplemental information' in the background document.

The actual conversion factor for rat PNDT studies is considered likely to lie somewhere in the range of 3.5% to 7%.

Conclusion on classification

According to the CLP criteria, classification in Category 1A is based on evidence from human data. No evidence of association between reproductive toxicity and exposure to mancozeb in humans is available. Therefore, classification as Repr. 1A is not warranted.

Categories 1B and 2 are reserved for presumed and suspected human reproductive toxicants respectively, and classification in these categories is based on the presence of 'clear' (Category 1B) or 'some' (Category 2) evidence of an adverse effect on sexual function, fertility, or development. In addition, such evidence must be present in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effects on reproduction must be considered not to be a secondary non-specific consequence of the other concurrent toxic effects.

RAC concludes that mancozeb meets the criteria for classification in Category 1B due to clear developmental findings in rats considered not to be a secondary non-specific consequence of the other concurrent toxic effects. The following considerations have been summarised in the opinion:

- Mancozeb induced severe malformations in the Anon. (1980) study at a maternally toxic dose of 512 mg/kg bw/d. According to CLP criteria (3.7.2.3.5), the presence of maternal toxicity shall not be used to negate findings of embryo/foetal effects, unless it can be clearly demonstrated that the effects are secondary non-specific effects. This is especially the case when the effects in the offspring are significant, e.g. irreversible effects such as structural malformations. As summarised above, RAC considers that the developmental effects observed in Anon. (1980) are severe and irreversible. The WoE indicates that they occurred due to a direct action of mancozeb and/or its major metabolites (including ETU) on the foetuses and are not related to the excessive maternal toxicity observed at 512 mg/kg bw/d.
- In relation to the Anon. (1980) study, RAC has the following additional concerns:

  - Single occurrences of severe and rare external and visceral malformations also occurred at 128 mg/kg bw/d in the same study indicating that this dose, associated with only limited maternal toxicity, lies close to the threshold dose causing malformations;
  - ETU was used in the study as a positive control group to compare with mancozeb since embryo/foetal effects were observed in earlier independent studies with ETU in rats (e.g. Khera, 1973; Teramoto *et al*., 1978; Chernoff *et al*., 1979). The spectrum of malformations with mancozeb was similar to that in the ETU-treated group, where no maternal toxicity was observed.
- A single dose of 30 mg/kg bw/d ETU on GD 15 induced severe dilation of brain ventricles due to necrosis of brain tissue (Khera and Tryphonas, 1977). Although the study has been performed before GLP, RAC notes that the study has been well-conducted and it does not reduce the concern about developmental effects of mancozeb.
- The mode of action (MoA) of mancozeb and/or its major metabolites (including ETU) behind the hydrocephalus in rats is not fully established. The pattern of findings of embryo/foetal effects is complex. Mechanistic studies indicate that cell necrosis in the central nervous system is part of the MoA (Teramoto 1978b; Khera and Tryphonas 1977; Khera 1987).
- RAC notes the equivocal PNDT study of Anon. (1988c) with reduced ossification of the skull and of the thoracic vertebra at 360 mg/kg bw/day. The increased incidence of incomplete ossification of the interparietal bone might be related to meningoencephalocele observed at a higher dose in the study by Anon. (1980) (cf. Khera, 1973).
- RAC is of the opinion that humans resemble the rat species in their ability to metabolise mancozeb to ETU.

RAC also notes additional negative PNDT studies in rats (Anon., 1999b; Lu and Kennedy, 1986) and in rabbits (Anon., 1987b; Anon., 1991b). RAC questions the validity of the negative rat PNDT study of Anon. (1999b), where no maternal toxicity was observed at doses up to 500 mg/kg bw/d.

In the most recent PNDT study of Anon. (2015d), no developmental effects were seen. However, maternal toxicity at the top dose of 160 mg/kg bw/d was rather limited and the preliminary study in non-pregant animals (Anon., 2015b) indicated that a higher dose could have been tested. Therefore, due to this selection of the top dose, the Anon. (2015d) study does not adequately address the developmental toxicity concerns raised by the previous Anon. (1980) study.

RAC acknowledges the comments provided during the public consultation (comment No 17 in the RCOM table) referring to the doses used in the studies Anon. (2015c & d) that were probably chosen too low to cause effects on the foetal development. The comments further noted that with regard to the dose range finding study (Anon., 2015b), doses up to 240-300 mg/kg bw/d mancozeb could be possible without causing severe maternal toxicity in the animals.

In conclusion, RAC considers that the new data is not convincing enough to reduce the concern for the malformations seen in the original Anon. (1980) study. Therefore, removal of the current classification in Category 2 proposed by the DS is not considered appropriate. Moreover, the severe and irreversible developmental findings in Anon. (1980) make it difficult to argue for a category 2 classification. There is no mechanistic data available to

indicate specific maternally-mediated mechanisms that give rise to secondary developmental effects in the offspring. The lack of connection between the maternal toxicity and severe malformations in the rat study Anon. (1980) leads RAC to conclude that mancozeb meets the criteria for classification in **Category 1B** for adverse effects on development.

Supplemental information - In depth analyses by RAC

Relationship between maternal and developmental toxicity in the study with mancozeb by Anon. (1980)

Dose of 512 mg/kg bw/d mancozeb (dams with foetuses at term):

Dam no.	No. of foetuses	FC GD 6-15 (g/day)	Corrected terminal bw (g)	Gross malform.	Soft tissue malform.	Dilated brain ventricles
7032	9	2.6	244	Yes	Yes	Yes
7034	15	1.2	258	Yes	Yes	Yes[#]
7036	14	2.0	248	Yes	Yes	Yes
7039	14	4.2	281	No	No	No
7040	9	1.3	155	Yes	Yes	Yes
7041	12	3.8	244	Yes	Yes	Yes
7042	12	7.3	260	No	No	No
7043	11	3.5	208	Yes	Yes	Yes
7047	14	4.7	250	Yes	Yes	Yes
7048	15	2.3	298	Yes	Yes	Yes
7051	8	9.1	253	Yes	Yes	Yes
7053	11	6.7	231	Yes	Yes	Yes
7055	11	1.2	256	No	No	No
Control mean		12.8	300			

[#] 1 foetus (no. 3) with dilated brain ventricle and underdeveloped brain according to the raw data; this information is not included in the summary of soft tissue findings

Dose of 128 mg/kg bw/d mancozeb (litters with malformations):

Dam no.	No. of foetuses	FC GD 6-15 (g/day)	Corrected terminal bw (g)	Malformations
7011	12	12.5	285	Foetus no. 5: right hindquarter rotated inward Foetus no. 6: left forepaw limb flexure

7017	12	12.6	244	Foetus no. 5: dilated ventricles
Group mean		11.2	277	
Control mean		12.8	300	

The data in the tables indicate lack of correlation between maternal toxicity and the incidence of external and soft tissue malformations.

Overview of the developmental toxicity studies with ETU

The following table summarizes developmental toxicity studies with ETU, both standard and mechanistic, presented in the CLH report or the RAR.

Developmental studies with ETU		
Type of study; Reference	**Method**	**Observations**
Rat		
PNDT study, gavage Khera 1973 (Summary is based on the RAR)	Study from literature Purity 100% Strain: Wistar Study 1 Doses: 0, 5, 10, 20, 40 mg/kg bw/d Dosing 21-42 days before conception to GD 15 10-14 litters per dose Study 2 Doses: 0, 5, 10, 20, 40, 80 mg/kg bw/d Dosing GD 6-15 8-14 litters per dose (except the top dose) Study 3 Doses: 0, 5, 10, 20, 40 mg/kg bw/d Dosing GD 7-20 15-17 litters per dose	Maternal toxicity 80 mg/kg bw/d (Study 2): Mortality 9/11 dams by GD 13-14 ≤ 40 mg/kg bw/d: No effects Developmental toxicity (the percentages refer to incidences in the individual studies, i.e. Study 1, 2 or 3) 80 mg/kg bw/d (Study 2; only 14 foetuses from 2 litters available for evaluation): • Exencephaly; retarded ossification skull • Dilated brain ventricles; hypoplastic cerebellum • Short, kinky or twisted tail • Micrognathia; partial hemimelia; oligodactyly; coloboma of eyelids; kyphoscoliosis 40 mg/kg bw/d: • Exencephaly (up to 32%); retarded ossification parietal and interparietal • Dilated brain ventricles (up to 100%); hypoplastic cerebellum (up to 100%) • Short tail (up to 43%); kinky or twisted tail (up to 57%) • Micrognathia (up to 20%); abnormal pelvic limb posture with equinovarus (up to 42%); oligodactyly (up to 3%) 20 mg/kg bw/d: • Exencephaly (1%); encephalocele or meningocele (up to 7%); retarded ossification parietal (up to 20%)

		• Dilated brain ventricles (up to 45%); hypoplastic cerebellum (up to 64%) • Kinky or twisted tail (up to 15%) • Micrognathia (up to 3%); abnormal pelvic limb posture with equinovarus (up to 8%) 10 mg/kg bw/d: • Exencephaly (up to 3%); retarded ossification parietal (up to 42%) • Dilated brain ventricles (up to 6%), hypoplastic cerebellum (up to 10%) • Kinky or twisted tail (up to 1%) 5 mg/kg bw/d: • Retarded ossification parietal
PNDT study, gavage Teramoto *et al.* 1978 (Summary is based on the RAR and abstract)	Study from literature Strain: Wistar-Imamichi Doses: 0, 10, 20, 30, 40, 50 mg/kg bw/d Dosing GD 6-15	<u>Maternal toxicity</u> ≤ 50 mg/kg bw/d: No effects <u>Developmental toxicity</u> 50 mg/kg bw/d: • Reduced foetal wt • Meningocele • Dilated brain ventricles (all foetuses) • Short or kinky tail • Micrognathia; curved clavicles, fused/wavy ribs, fused sternebrae, malformed vertebrae, scoliosis 40 mg/kg bw/d: • Reduced foetal wt • Meningocele • Dilated brain ventricles (all foetuses) • Short or kinky tail • Curved clavicles, fused/wavy ribs, fused sternebrae, malformed vertebrae, scoliosis 30 mg/kg bw/d: • Reduced foetal wt • Dilated brain ventricles (all foetuses) • Short or kinky tail • Curved clavicles 20 mg/kg bw/d: • Dilated brain ventricles (39% of foetuses) 10 mg/kg bw/d: • Dilated brain ventricles (2% of foetuses)
PNDT study, gavage Chernoff *et al.* 1979 (Summary is based on the RAR)	Study from literature Strain: Sprague-Dawley Doses: 0, 5, 10, 20, 30, 40, 80 mg/kg bw/d Dosing GD 7-21	<u>Maternal toxicity</u> 80 mg/kg bw/d: mortality (25%), reduced bw gain ≤ 40 mg/kg bw/d: no effects <u>Developmental toxicity</u> 80 mg/kg bw/d: • Increased foetal mortality • Reduced foetal wt

	8-27 litters/group	• Encephalocele • Hydrocephalus • Cleft palate • Kyphosis • Micrognathia • Micromelia/hemimelia • Oedema 40 mg/kg bw/d: • Reduced foetal wt • Encephalocele • Hydrocephalus 30 mg/kg bw/d: • Reduced foetal wt (by 10%) • Hydrocephalus (10 out of 31 litters) 20 mg/kg bw/d: • Reduced foetal wt (by 7%) • Hydrocephalus (5 out of 19 litters) 10 mg/kg bw/d: reduced foetal wt (by 7%) 5 mg/kg bw/d: no effects
PNDT study, gavage Saillenfait *et al.* 1991 (Summary is based on the article)	Study from literature Purity ≥ 98% Strain: Sprague-Dawley Doses: 0, 15, 25, 35 mg/kg bw/d Dosing GD 6-20 20-23 females/group	<u>Maternal toxicity</u> ≤ 35 mg/kg bw/d: No effects <u>Developmental toxicity</u> 35 mg/kg bw/d: • Reduced foetal wt • Cranial meningocele (10 out of 20 litters) • Severe hind limb talipes (14 out of 20 litters) • Short and/or kinky tail (13 out of 20 litters) • Dilated brain ventricles (92 out of 100 foetuses, 20 out of 20 litters) 25 mg/kg bw/d: • Dilated brain ventricles (33 out of 85 foetuses, 12 out of 16 litters) • Short/kinky tail (2 foetuses) • Severe hind limb talipes (1 foetus) 15 mg/kg bw/d: no effects
PNDT study, gavage Anon. 2015a (Summary is based on the CLH report)	OECD 414 GLP Purity ≥ 98% Strain: Crl:CD(SD) Doses: 0, 2.5, 5, 15, 30 mg/kg bw/d Dosing GD 6-19 24 females/group Toxicokinetic investigations	<u>Maternal toxicity</u> ≤ 30 mg/kg bw/d: no effects <u>Developmental toxicity</u> 30 mg/kg bw/d: • Reduced foetal wt (by 13%) • Meningocele (22 out of 24 litters); reduced ossification of the skull (24 out of 24 litters vs 3 out of 24 litters in control) • Hydrocephaly (24 out of 24 litters) • Short tail (15 out of 24 litters), bent tail (24 out of 24 litters) • Malrotated limb (11 out of 24 litters)

		15 mg/kg bw/d: • Hydrocephaly (7 foetuses, 2 out of 23 litters) ≤ 5 mg/kg bw/d: no effects
Developmental toxicity, single dose on different days of gestation, gavage Ruddick and Khera 1975 (Summary is based on the RAR)	Study from literature Single dose of 240 mg/kg bw on one of days 6-21 of gestation	Maternal toxicity: none Malformations induced by treatment on: • GD 6-9: no malformations • GD 10: abnormal external genitalia, hydronephrosis or hypoplastic kidneys, absent or short tail • GD 11: lumbar spina bifida, fused ribs, kidney and tail anomalies • GD 12-15: hydrocephalus, exencephaly, brachygnathia, hydronephrosis, absent tail, forelimb micromelia Resorptions increased by treatment on GD 11, 14, 19
Developmental toxicity, investigations into effects on developing brain and limb buds, gavage Teramoto 1978b (Summary is based on the article)	Study from literature Strain: Wistar-Imamichi Single oral dose on GD 12 or 13 Doses: 0, 50, 100, 200 mg/kg bw 5-8 litters/group Treatments for histological examinations: 100 mg/kg bw on GD 12; 200 mg/kg bw on GD 13 3 treated females for each timepoint (3, 6, 12, 24, 48, 72 and 96 hours after treatment), 3 control females for each timepoint	200 mg/kg bw: • Cranial meningocele (100% of foetuses GD 12) • Dilatation of the brain ventricles, especially the 4th ventricle • Cleft palate (100% GD 12) • Micrognathia (100% GD 12 and 13) • Short tail (100% GD 12) • Oligodactyly (100% GD 13), syndactyly • Anal atresia (63% GD 12) • Hypoplastic genital tubercle (100% GD 13) • Reduced foetal wt 50 mg/kg bw: • Dilatation of the lateral, 3rd and 4th ventricles (96% GD 12, 100% GD 13), brain mantle reduced in thickness (hydromicrocephalus) • Short tail (89% GD 12) • Cranial meningocele (1% GD 12) • Cleft palate (1% GD12) Histological examinations – neural tube, 100 mg/kg bw on GD 12: • 12 h after treatment: Some pyknotic and necrotic cells in the wall of the neural tube. • 24 h after treatment: Severe cell necrosis and desquamation associated with destruction of the neural tissues throughout the neural tube. Ventricles filled with cellular debris. • 48 h after treatment: The neural tube still contained many necrotic cells. Rosette formation in the wall of the neural tube, but

		development of the brain already retarded compared to control. • 96 h after treatment: Most of the necrotic cells disappeared from the brain wall. Development of the brain remarkably retarded and the ventricles widely enlarged. Histological examinations – neural tube, 200 mg/kg bw on GD 13: • 72 h after treatment: Rosettes started to appear. Development of the brain so remarkably retarded that the ventricles tended to form a single cavity. The development of the cerebellar plate also retarded compared to control.
Developmental toxicity, postnatal pathogenesis of ETU-induced hydrocephalus, gavage Khera and Tryphonas 1977 (Summary is based on the article; only selected results relevant for classification of mancozeb are reported here)	Study from literature Strain: Wistar Single oral dose on GD 15 Experiment I Doses: 0, 30, 45 mg/kg bw 6-12 dams/group Cross-fostering test on additional 6 control dams and 6 dams given 30 mg/kg bw ETU Necropsy on all surviving progeny on PND 115 F1 females of 30 mg/kg bw/d paired and allowed to deliver a litter Experiment II Doses: 0, 15, 30 mg/kg bw Necropsy of all surviving progeny at week 64, histopathology Experiment III Doses: 0, 45 mg/kg bw	Experiment I: 45 mg/kg bw/d: all pups died within 4 weeks; none of the 23 pups which died in the first week were anomalous but 25 of the 26 pups dying in the next 3 weeks had hydrocephalus and microphthalmia 30 mg/kg bw/d: 155 pups born alive; 90 of pups died within 9 weeks; 63 of the 70 examined foetuses were hydrocephalic and micropthalmic; 16 out of the 65 foetuses surviving for 9 weeks had motor deficits (mild paraplegia, difficulty in turning, hopping gait) Experiment II: 30 mg/kg bw/d: all adverse effects from Experiment I were reproduced; moderate hydrocephalus in 4 rats and extreme hydrocephalus in 4 with no apparent obstruction of the ventricular system 15 mg/kg bw/d: no adverse developmental effects Experiment III (45 mg/kg bw): GD17: widespread cellular necrosis, spongy change in the subependymal plate, diminution of brain mass but with unaltered general conformation of the brain and spinal cord GD 20: first appearance of rosette formation PND 2: second wave of necrosis and spongy change; substantial increase in the size of the ventricular system, reduction in brain mass PND 7: patchy disorganization and uniform thinning of the cerebral cortex, spongy change, exhaustion of the subependymal plate; degenerative processes continued until PND 15; no blockage of the ventricular system

	13 dams/group Necropsy of 2 dams/group on GD 17, 20, 22; necropsy of 16-18 pups/group on PND 2, 7, 10, 15	
Developmental toxicity, dermal Stula and Krauss 1977 (Summary is based on the article)	Study from literature Strain: Sprague-Dawley Two dermal applications on two consecutive days: GD 10+11 or GD 12+13 Doses: GD 10+11: 0, 25 mg/kg bw/d (i.e. 0, 50 mg/kg bw in total) GD 12+13: 0, 25, 50 mg/kg bw/d (i.e. 0, 50, 100 mg/kg bw in total) 6-8 dams/group Vehicle: DMSO	50 mg/kg bw/d on GD 10+11: short tail (3/83), fused ribs (2/83) 50 mg/kg bw/d on GD 12+13: All 73 foetuses malformed. Defects included encephalocele, part/all of the tail missing, missing leg bones, hunchback curvature of the spine, short mandible, fusion of ribs, fusion of sternebrae 25 mg/kg bw/d on GD 10+11: no foetal abnormality No maternal toxicity
PNDT study, impact of coadministration of T3/T4 or NaI on developmental toxicity of ETU, gavage Emmerling 1978 (Summary is based on the CLH report and RAR)	Non-guideline Non-GLP Purity 97% Strain: Sprague-Dawley Dosing GD 7-20 Up to 16 females/group Groups: T3 (20 µg/kg bw/d) + T4 (100 µg/kg bw/d) NaI (333 µg/kg bw/d) ETU 20 mg/kg bw/d ETU 20 mg/kg bw/d + NaI ETU 20 mg/kg bw/d + T3 + T4 ETU 40 mg/kg bw/d	The teratogenic response to ETU was reduced for some malformations (micrognathia, cleft palate, syndactyly, ablepharia) when T3/T4 was co-administered, but not for malformations such as hydrocephaly, meningocele, short and kinky tail. Sodium iodide did not provide any protective effect Malformations at 20 mg/kg bw/d ETU without co-administration: meningocele (14% of foetuses), hydrocephalus (98%), kinky tail (88%), talipes (16%)

	ETU 40 mg/kg bw/d + NaI ETU 40 mg/kg bw/d + T3 + T4	
PNDT study, impact of thyroid status on ETU-induced teratogenicity, gavage Lu and Staples 1978 (Summary is based on the CLH report and RAR)	Study from literature Purity 97% ETU: 40 mg/kg bw/d Dosing GD 7-15 To euthyroid rats, thyroidectomised rats, and to rats given exogenous T4 6-12 females/group	84-100% of the foetuses in all groups given ETU were malformed regardless of the thyroid status of the dams 10% of the foetuses of thyroidectomized dams were malformed vs no malformations in the control Administration of ETU to euthyroid rats: malformations mostly of CNS, rib and tail, clubbed foot; delayed ossification of the skull Administration of ETU to thyroidectomized rats: additionally to the malformations in the ETU group also oedema, micrognathia, cleft palate and micromelia. These malformations were not seen in thyroidectomized rats not given ETU
Developmental toxicity, effect of inhibitors or inducers of ETU metabolism on teratogenicity, gavage Khera and Iverson 1981 (Summary is based on the CLH report and abstract)	Study from literature Single dose of 60 mg/kg bw on GD 13 5 females/group In combination with the P-450 inhibitor SKF-525A (40 mg/kg bw, i.p. 1 hour before ETU) and antithyroid drug methimazole (MMI; 200 mg/kg bw p.o., simultaneously with ETU) Control groups: only ETU; only SKF; only MMI; SKF+MMI Further groups were pre-treated with enzyme inducers phenobarbital or methylcholanthrene	SKF, MMI or SKF+MMI: not teratogenic MMI+SKF+ETU, SKF+ETU: increased incidence and severity of anomalies compared to the group given only ETU, similar to those previously reported at 120 mg/kg bw or higher doses of ETU Pre-treatment with phenobarbital or methylcholanthrene had no effect on the incidence of ETU-induced malformations
In vitro, effect of ETU on rat foetal neuronal and non-neuronal cells Khera 1987	Study from literature Rat foetal neuronal and non-neuronal cells grown in the presence of ETU	ETU at concentrations greater than 0.5 mM caused necrosis of neuronal cells and a reduction in the formation of neurites and fascicles. Non-neuronal cells were unaffected

(Summary is based on the CLH report)		
Whole embryo culture Stanisstreet *et al.* 1990 (Summary is based on the CLH report)	Study from literature Rat embryos at GD 9.5 incubated for 48 h with ETU or methimazole	ETU caused abnormalities at 0.5 mM and above, most commonly of the neural tube. Methimazole also caused abnormalities at 0.5 mM and above but the spectrum of changes was different from ETU The authors concluded that ETU has a direct effect on the rat embryo rather than through thyroid hormone changes
Whole embryo culture Daston *et al.* 1987 (Summary is based on the CLH report)	Study from literature Rat embryos at GD 10 incubated with ETU for 48 h	A dose-related inhibition of crown-rump length, protein and DNA content, somite number and increased abnormalities were seen from 0.04 mM ETU. Abnormalities included hydrocephalus, decreased mandibular size, decreased telencephalon size, abnormal dorsiflexion and subectodermal blisters. Osmolarity of the exocoelomic fluid was lowered and the authors suggested that ETU alters osmotic balance, which may contribute towards the formation of defects
Rabbit		
PNDT study, gavage Khera 1973 (Summary is based on the RAR)	Study from literature Purity 100% Strain: NZW Doses: 0, 10, 20, 40, 80 mg/kg bw/d Dosing GD 7-20	<u>Maternal toxicity</u> ≤ 80 mg/kg bw/d: no effects <u>Developmental toxicity</u> 80 mg/kg bw/d: increased incidence of resorptions and kidney microscopic alterations ≤ 40 mg/kg bw/d: no effects
PNDT study, gavage Anon. 2010c (Summary is based on the RAR)	OECD 414 GLP Purity 99.4% Strain: NZW Doses: 0, 5, 15, 50 mg/kg bw/d Dosing GD 7-28	<u>Maternal toxicity</u> 50 mg/kg bw/d: reduced bw gain and food consumption <u>Developmental toxicity</u> 50 mg/kg bw/d: reduced foetal wt 15 mg/kg bw/d: no adverse effects Dose selection was based on a range-finding study where excessive toxicity was observed at 150 mg/kg bw/d
Mouse		
PNDT study, gavage Teramoto 1978	Study from literature Strain: JCL-ICR Doses: 0, 200, 400, 800 mg/kg bw/d	<u>Maternal toxicity</u> ≤ 800 mg/kg bw/d: no effects <u>Developmental toxicity</u> ≤ 800 mg/kg bw/d: no effects

(Summary is based on the RAR)	Dosing GD 7-15	
PNDT study, gavage Chernoff 1979 (Summary is based on the RAR)	Study from literature Strain: CD-1 Doses: 0, 100, 200 mg/kg bw/d Dosing GD 7-16	Maternal toxicity 200 mg/kg bw/d: increased liver wt 100 mg/kg bw/d: no effects Developmental toxicity 200 mg/kg bw/d: supernumerary ribs 100 mg/kg bw/d: no effects
Hamster		
PNDT study, gavage Teramoto 1978 (Summary is based on the RAR and the abstract)	Study from literature Syrian golden hamster Doses: 0, 90, 270, 810 mg/kg bw/d Dosing GD 6-13	Maternal toxicity ≤ 810 mg/kg bw/d: no effects Developmental toxicity 810 mg/kg bw/d: reduced survival; cleft palate, kinky tail, oligodactyly, anal atresia; reduced foetal wt 270 mg/kg bw/d: malformations, reduced foetal wt 90 mg/kg bw/d: no effects

Derivation of a conversion factor from a dose of ETU to a corresponding dose of mancozeb causing the same developmental effects

Conversion factor derived from the studies Anon. (2015a) and Anon. (2015c): 3.5%

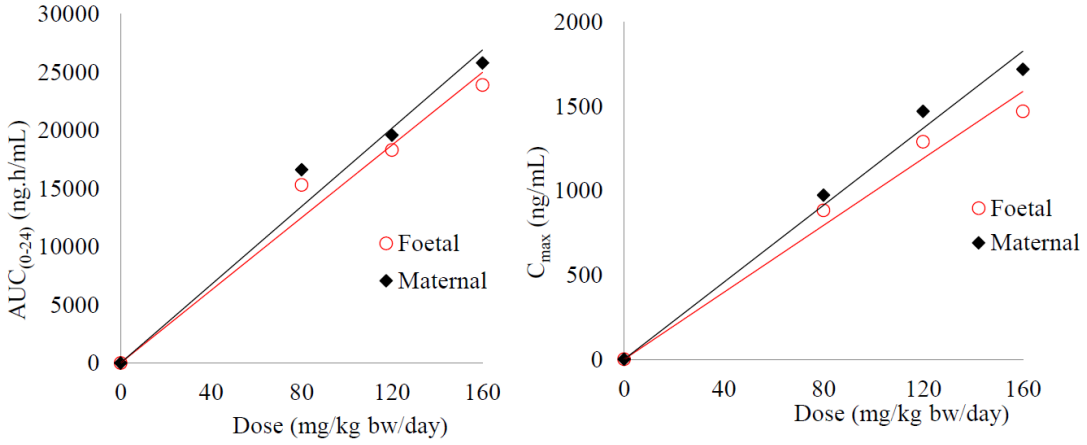
The PNDT gavage study with ETU in rats Anon. (2015a) and the preliminary PNDT gavage study with mancozeb Anon. (2015c) in rats of the same strain included toxicokinetic investigations subsequently used to derive a conversion factor between a dose of ETU and a dose of mancozeb producing the same peak plasma concentration (C_{max}) of ETU. The methodology is described in detail in Annex II to the CLH report and is briefly summarised below.

1. In the study with ETU (Anon., 2015a), employing doses up to 30 mg/kg bw/d, maternal and foetal plasma ETU concentrations were determined 24 h after administration of the last dose (last dose on GD 19, termination on GD 20). Maternal and foetal plasma concentrations of ETU were found to be similar and increased linearly with dose.
2. In the study with mancozeb (Anon., 2015c), employing doses up to 160 mg/kg bw/d, maternal and foetal plasma ETU concentrations were determined at various time points (0, 2, 4, 6, 12 and 24 h) after the last dose. Peak concentration (C_{max}) of ETU was reached at the 6 h timepoint. Both C_{max} and $AUC_{(0-24)}$ of ETU increased approximately linearly with dose and there was no marked difference in C_{max} and AUC between dams and foetuses.
3. C_{max} of ETU was chosen as the basis for comparison by the DS. As C_{max} was not measured in the study Anon. (2015a), it was estimated on the assumption of the same T_{max} and the same rate constant (k) for the decline in ETU concentrations as in the study with mancozeb (Anon., 2015c).

4. Finally, the C_{max} data from the two studies were compared using a linear regression model assuming a linear dose-response relationship and it was estimated that a dose of 429 mg/kg bw/d mancozeb would be needed to produce the same C_{max}(ETU) as a dose of 15 mg/kg bw/d ETU, a developmental LOAEL in the study Anon. (2015a).

While comparison of toxicokinetic parameters in two parallel studies, one with ETU and one with mancozeb, using animals of the same strain and source is scientifically the best approach to derivation of a conversion factor, RAC identifies several deficiencies and uncertainties in this particular case:

- Neither C_{max} or AUC were actually measured in the study with ETU (Anon., 2015a).
- The rise in plasma ETU after administration of ETU is likely to be faster than after administration of mancozeb as the latter has to be metabolized to ETU first. This is supported by the study by Ruddick *et al.* (1977) reporting a T_{max} of 1.3 h after administration of a single dose of ETU to pregnant rats on GD 15. Use of lower T_{max} would lead to a higher estimate of C_{max} (ETU) in the study Anon. (2015a) and consequently higher concentrations of mancozeb needed to reach the teratogenic level than assumed by the DS.
- The DS did not justify why C_{max} should be a better basis for comparison than AUC. An ETU concentration above a certain threshold for a certain period of time is probably needed for induction of malformations and it is not obvious whether C_{max} is the relevant parameter to capture this. Ideally, both AUC and C_{max} should have been measured and presented.
- A linear dose-response relationship can be reasonably assumed within the measured range (i.e., up to 160 mg/kg bw mancozeb). However, extrapolation above 160 mg/kg bw mancozeb is rather uncertain (cf. Figure A2-4 in the CLH report, copied below).



Conversion factor from the studies Anon. (1986f) and Anon. (1986g): 7%

The study by Anon. (1986g) investigated metabolism of mancozeb upon oral administration to male and non-pregnant female rats at two dose levels, 1.5 mg/kg bw or 100 mg/kg bw. The DS described derivation of the conversion factor as follows: "In this study the conversion was calculated based on the recovery of ETU from urinary and bile samples (18.2% of the administered dose) following an oral dose of mancozeb. The bioconversion of mancozeb to ETU on weight percentage basis was 6.8% (average 18.2% of the

administered 14C mancozeb dose recovered as ETU in urine and bile x 102 g ETU/mole/271 g mancozeb/mole = 6.8%)."

The robust study summary of this study received in August 2018 in response to the EFSA data call 4 and 5 provided additional details of this study and of the preceding study Anon. (1986f) investigating basic toxicokinetics. The information in the robust study summaries indicates that the mean conversion factor of approx. 7% on a weight basis relates to the administered, not absorbed dose.

Example calculation (by RAC) for female rats within 24h at a dose of 100 mg/kg bw:

- Ratio between faecal and urinary excretion of the ^{14}C label: 0.497 : 0.503
- Biliary excretion (measured in a separate group of animals): 2.0% of the recovered ^{14}C
- ETU concentration in urine: 42.7% of the ^{14}C activity in the urine
- ETU concentration in bile: 14.5% of ^{14}C activity in the bile
- Conversion of mancozeb to ETU: 0.497 x 0.427 + 0.02 x 0.145 = 0.215 = 21.5% on a mole/mole basis, corresponding to 8.1% on a weight basis

The value of approx. 7% is a mean from all four groups (low dose males, low dose females, high dose males, high dose females).

RAC notes that this method provides only a rough approximation of the parameter sought, i.e. of the conversion factor from a dose of ETU to a corresponding dose of mancozeb causing the same developmental effects; a comparison based on C_{max} and AUC would be preferable. However, as the C_{max}-based factor derived in the new studies Anon. (2015a,c) is associated with major uncertainties, the result from the studies Anon. (1986f,g) is a valuable contribution to the WoE assessment. Similarly to the value of 3.5% from the new studies, this value of 7% has been derived from measurements in the non-teratogenic range.

Conversion factor based on comparison of developmental effects in the study Anon. (1980) and 5 studies with ETU: 5–7%

As both previous factors are associated with uncertainties, RAC attempted to derive another independent estimate. This estimate is based on biological response.

The top dose of 512 mg/kg bw/d mancozeb in the study Anon. (1980) produced dilated brain ventricles in 54% of foetuses and 69% of litters. The 5 standard PNDT studies with ETU available (Khera, 1973; Teramoto *et al.*, 1978; Chernoff *et al.*, 1979; Saillenfait *et al.*, 1991; Anon., 2015a) indicate that a dose of ETU producing a comparable incidence of brain malformations was approx. 25–35 mg/kg bw/d ETU as shown in the table below. This corresponds to a conversion factor of mancozeb to ETU of ca. 5–7%.

Reference	**Dose of ETU producing a higher incidence of brain malformations than 512 mg/kg bw/d mancozeb**	**Dose of ETU producing a lower incidence of brain malformations than 512 mg/kg bw/d mancozeb**
Khera (1973), study 2	40 mg/kg bw/d: dilation of lateral ventricles (95% of foetuses)	20 mg/kg bw/d: dilation of lateral ventricles (23% of foetuses)
Teramoto *et al.* (1978)	30 mg/kg bw/d: dilation of the lateral or 4th ventricle (100% of foetuses)	20 mg/kg bw/d: dilation of the lateral or 4th ventricle (39% of foetuses)

Chernoff *et al.* (1979)	40 mg/kg bw/d: hydrocephalus (91% of litters)	30 mg/kg bw/d: hydrocephalus (32% of litters)
Saillenfait *et al.* (1991)	35 mg/kg bw/d: dilated brain ventricles (92% of foetuses)	25 mg/kg bw/d: dilated brain ventricles (39% of foetuses)
Anon. (2015a)	30 mg/kg bw/d: hydrocephaly (100% of litters)	15 mg/kg bw/d: hydrocephaly (9% of litters)

The main uncertainties associated with this estimate are the following:

- The animals in different studies were from different strains and sources; the sensitivity of animals to the effects ETU and mancozeb varies from study to study.
- The comparison is based on only one dose in only one study with mancozeb.

Acute oral toxicity of mancozeb in the rat

The key information from the rat acute oral toxicity studies with mancozeb presented in the RAR is summarised in the following table.

Overview of acute oral toxicity studies with mancozeb in the rat

Year	Dose (mg/kg bw)	Sex and no. of animals	Observations
1979	5000	10 m	No mortality No significant clinical signs No gross anomalies at necropsy
1984	5000	10 m	No mortality Clinical signs: passiveness, laboured respiration, abdominal respiration, lachrymation No gross anomaly at necropsy
1986	5000	5 m + 5 f	No mortality Piloerection shortly after dosing, recovery by day 2 Autopsy findings normal
1996	4000	5 m + 5 f	Mortality: 1 female
1997	2000	5 m + 5 f	No mortality No clinical signs of toxicity Vascular changes in the lung, liver, spleen and mesentery

These studies were conducted in nonpregnant animals. However, the available data do not indicate a markedly higher sensitivity of pregnant rats compared to nonpregnant rats upon oral exposure (see the summary table on mortality in the STOT RE section of the opinion).

10.9 Specific target organ toxicity-single exposure

Not addressed in this dossier.

10.10 Specific target organ toxicity-repeated exposure

The specific target organ toxicity of mancozeb upon repeated exposure has been investigated in regulatory 28 day, 90 day and 1 year studies in rats, mice and dogs, 28 and 90 day inhalation studies in rats and 28 and 90 day dermal studies in rats. There are also numerous human and animal studies from the open literature.

Table 20: Summary table of animal studies on STOT RE

Method, guideline, deviation(s) from the guideline (if any)	Species, strain, sex, number/group	Dose levels, duration of exposure	CLP Guideline value for classification (mg/kg bw/d or mg/l 6h/d rat study)	Results (Effects statistically significantly different unless stated otherwise)
Oral studies in rats (28 days)				
28 day oral (gavage) OECD 407 (1981) Deviations: control and test animals were not age matched, no statistics, not all groups reported. GLP Vehicle: distilled water. Mancozeb Purity: 89.83% Anonymous, 1994b	Rat, Sprague Dawley (SD) 6/sex/group (8/sex/group for 200 mg/kg bw/day)	0, 50, 200 or 500 mg/kg bw/day	Cat 1 = 30 Cat 2 = 300	<u>500 mg/kg bw/day</u> 1/6 females killed for humane reasons on day 21; 5/6 females ataxia, 2/6 females hind limb paralysis day 28 ↓ Body weight gain males, females overall loss week 1-5 Clinical chemistry: ↓ 11.3% glucose, 31.6% creatinine, 23.1% potassium in females Haematology: ↑ 34.7% lymphocytes in females Histopathology: no effects reported. <u>200 mg/kg bw/day</u> 1/8 females killed for humane reasons on day 13; 2/8 females ataxia. ↓ Body weight gain, males and females week 1-5 Clinical chemistry: ↑ 59.7% ALT males, 14.7% creatinine males, 38.6%/35.0% glucose males/females; ↓ 54.0% bilirubin males, 42.5%/43.6% phosphorus males/females, 37.4% AST in females, 18.1% potassium in females Haematology: ↑ 31.2% lymphocytes in males, 39.8% WBC in females; ↓ 40.7% segmented cells in males Histopathology: Liver degeneration: 8/8 males mild to severe; 4/6 females mild to moderate – effect considered incidental as not observed at the higher dose of 500 mg/kg bw/day. <u>50 mg/kg bw/day</u> No treatment related findings
Oral studies in rats (90 days)				

Sub chronic 12 week oral (dietary) Non guideline Non GLP Purity 80% Szepvolgyi *et al* (1989) (from literature review)	Rat, H-Wistar 12/males/group	1, 10, 50, 75, 113, 169, 253 and 379 mg/kg bw/day	Cat 1 = 10 Cat 2 = 100	<u>379 mg/kg bw/day</u> Mortality: 4/12 died week 1-6 Clinical observations: prostration, weakness and posterior distal paralysis, resolved in survivors by end of study. Body weight: ↓ (34.7% week 4) and body weight gain (83.4% weeks 1-12) Food consumption: ↓ 34.8% weeks 1-12 Clinical chemistry: cholinesterase ↑ 77.3%; AST ↓ 20.0%; serum protein ↓ 15.9%; Liver enzymes: APDM ↓ 37.7%; AHO ↓ 37.2%; Iodine: Thyroid ↓ 91%, protein bound ↓ 63.2% Relative organ weights: ↑ thyroid (~ 3-fold), liver (48.9%), kidneys (36.4%), testes (59.4%), adrenals (46.8%) Histopathology: Thyroid enlarged and proliferating epithelial cells (incidences not reported) <u>253 mg/kg bw/day</u> Body weight*:* ↓ (20.3% week 4) and body weight gain (52.9% weeks 1-12) Food consumption: ↓ 21.9% weeks 1-12 Clinical chemistry: Cholinesterase ↑ 50.1%; serum protein ↓ 10.1%; Liver enzymes: APDM ↓ 20.5%, liver triglycerides ↑ 51.1% Iodine: Thyroid ↓ 90%, protein ↓ 52% Relative organ weight: ↑ thyroid (~1.5-fold), liver (41.9%), kidneys (27.3%), testes (31.7%), adrenals (35.7%) Histopathology*:* Thyroid enlarged and proliferating epithelial cells (incidences not reported) <u>169 mg/kg bw/day</u> Body weight: ↓ (13.3% week 4) and body weight gain (24.2% weeks 1-12) Food consumption: ↓ 17.7% weeks 1-12 Clinical chemistry: cholinesterase ↑ 56.5%; Liver enzymes*:* triglycerides ↑ 47.6% Iodine: Thyroid ↓ 81%, protein bound ↓ 48.6% Relative organ weight: ↑ thyroid (77.3%), liver (22.5%) Histopathology: thyroid follicular cells columnar, some lumens proliferation (incidences not reported) <u>113 mg/kg bw/day</u>

				Liver enzymes: ↑ triglycerides 44.7% Iodine: Thyroid ↓ 80%, protein bound ↓ 48.6% Relative organ weight: ↑ thyroid (55.4%), liver (14.5%) Histopathology: thyroid follicular cells columnar, some lumens proliferation (incidences not reported) <u>75 mg/kg bw/day</u> Iodine: ↓ 83% in thyroid Relative organ weight*:* ↑ thyroid (50.2%), liver (14.5%) <u>50 mg/kg/bw/day</u> Iodine: ↓ 83% in thyroid <u>10 mg/kg/bw/day</u> Iodine: ↓ 21% in thyroid
Subchronic 90 day oral (dietary) OECD 408 GLP Mancozeb technical Purity 88.2% (Batch BL.I.850.930) Anonymous, 1989	Rat, Crl (SD) BR 10/sex/group 10/sex/group control & high dose to investigate recovery	0, 28; 113 and 454 ppm Equivalent to: Males 0, 1.7, 7.0 and 29.2 mg/kg bw/day; Females 0, 2.1, 8.4 and 33.4 mg/kg bw/day 90 days with 4-week recovery period for 454 ppm group.	Cat 1 = 10 Cat 2 = 100	<u>454 ppm (29.2 and 33.4 mg/kg bw/day in males and females respectively)</u> ↓ Body weight gain: 14.9% females weeks 0-13 ↓ Haematology: 40.2% neutrophils females ↓ T4: 18.6 and 15.2% in males and females respectively ↑ T3: 22.2% in females Pathology*:* no treatment related findings All effects recovered after 28 days without treatment <u>113 ppm (7.0 and 8.4 mg/kg bw/day in males and females respectively)</u> ↓ T4: 15.2% in females (not statistically significant) <u>28 ppm (1.7 and 2.1 mg/kg bw/day in males and females respectively)</u> No effects
Three-month oral (dietary) OECD 408 GLP Mancozeb Purity 84% Anonymous, 1986b	Rat, CRL-CD (SD) 14/sex/group	0 (control), 30, 60, 125, 250 and 1000 ppm Equivalent to: Males: 0, 1.8, 3.5, 7.4, 15.0 and 57.3 mg/kg bw/day Females: 0, 2.2, 4.4, 9.2, 17.8 and 74.6 mg/kg bw/day	Cat 1 = 10 Cat 2 = 100	<u>1000 ppm (57.3 and 74.6 mg/kg bw/day in males and females respectively)</u> ↓ Body weight: 3-8% males weeks 3 to 13, 3-14% females weeks 2 to 13 (females statistically significant weeks 7-10) Organ weights: thyroid (absolute ↑ 32.0% males; relative ↑ 49.2% and 33.1% in males and females respectively; liver (relative ↑ 10.7 and 23.8% in males and females); testes (↑ absolute 10.6% and relative 4.8%); spleen (↑ relative 21.9% females) ↓ *T4*: 33.8 and 42.9% in males and females respectively ↑ *TSH*: 3.6-fold and 2.7 fold in males and

				females respectively ↓ Hepatic mixed function oxidase activity: 31-35% males and 34-40% females Histopathology: Thyroid follicular cell hyperplasia: 9/10 males, 9/10 females (0/10 controls both sexes); adrenal hypertrophy zona glomerulosa 6/10 males, 3/10 females (1/10 control both sexes); liver hypertrophy of the centrilobular hepatocytes 2/10 males 250 ppm (15.0 and 17.8 mg/kg bw/day in males and females respectively) ↓ T4: 28.3% in females; ↑TSH: 56.7 and 93.9% in males and females respectively (not statistically significant) 125 ppm (7.4 and 9.2 mg/kg bw/day in males and females respectively) No treatment related effects 60 ppm (3.5 and 4.4 mg/kg bw/d in males and females respectively) No treatment related effects 30 ppm (1.78 and 2.2 mg/kg bw/day in males and females respectively) No treatment related effects
Sub chronic 90 day toxicity oral (gavage) OECD 408 (1981) Minor deviations THs were not investigated GLP Vehicle distilled water Sanachem Mancozeb technical Purity 89.1% Anonymous, 1997c	Rats Sprague Dawley 12/sex/group	0, 15, 25, 40 or 50 mg/kg bw/day Dosed 5 days/week, 90 days	Cat 1 = 10 Cat 2 = 100	50 mg/kg bw/day No treatment-related effects 40 mg/kg bw/day No treatment-related effects 25 mg/kg bw/day No treatment-related effects 15 mg/kg bw/day No treatment-related effects

Sub chronic toxicity oral (90 days) (Gavage) OECD 408 (1981) Minor deviations THs were not investigated GLP Vehicle peanut oil Mancozeb Purity 85% Anonymous, 1999c	Rat Wistar 12/sex/group 12/sex/group control and high dose recovery groups	0, 64, 160, 400 mg/kg bw Daily for 90 days Recovery after 28 days	Cat 1 = 10 Cat 2 = 100	<u>400 mg/kg bw/day</u> Mortality: 1/24 males (day 14), 1/24 females (day 86, values include main study and recovery animals) Haematology: neutrophils: ↑ (23.2% females); ↓ lymphocytes (6.1% females) Organ weights: adrenals ↑ (60% absolute and 33.3% relative in females); liver ↑ (18-21% relative weight recovery group both sexes and absolute weight recovery males) Histopathology*:* hepatocellular degeneration/necrosis 11/11 males; 2/12 recovery; inflammatory changes 9/12 females; 0/12 recovery <u>160 mg/kg bw/day</u> Organ weights: ↑ adrenals (80% absolute and 33.3% relative in females) females <u>64 mg/kg bw/day</u> No treatment related effects.

Method, guideline, deviation(s) from the guideline (if any)	**Species, strain, sex, number/group**	**Dose levels, duration of exposure**	**CLP Guideline value for classification (mg/kg bw/d or mg/l 6h/d rat study)**	**Results (Effects statistically significantly different unless stated otherwise)**
Oral studies in mice (28 days)				
Four week range finding dietary study in mice. Non guideline Deviations, weight and pathology of liver and thyroid only, limited clinical pathology parameters Dose mg/kg bw not reported. Non GLP Mancozeb: purity 83% Anonymous, 1985b	Mouse, Charles River COBS-CD-1 5/sex/group	0, 1, 10, 100, 1000 or 10000 ppm (mancozeb) Equivalent to 0, 0.2, 2, 20, 200 and 2000 mg/kg bw/day	Cat 1 = 30 Cat 2 = 300	No treatment-related effects on mortality. No treatment-related overt clinical signs of toxicity. <u>10000 ppm (2000 mg/kg bw/day)</u> ↓ Body weight: 9.2% males, 11.7% females day 28 ↑Organ weights: ↑ absolute thyroid and liver weight (66.7% and 20.2% respectively females); ↑ relative thyroid weight: (25.8% males, 57.9% females); ↑ relative liver weight (9.6% males, 19.7% females) Thyroid hyperplasia: 4/5 males, 5/5 females <u>1000 ppm (200 mg/kg bw/day)</u> ↓ Body weight: 18.6% males day 14 9.5% females day 21 ↑ Organ weights*:* absolute thyroid and liver weight (33.3% and 15.1% respectively females) Thyroid hyperplasia: 1/5 females <u>100 ppm (20 mg/kg bw/day DAR)</u> No treatment-related findings.

Oral studies in mice (90 days)				
Sub chronic toxicity oral. (dietary) OECD 408 Few minor deviations GLP Purity 83.1% Anonymous, 1985c	Mouse Charles River COBS-CD-1 15/sex/group	0 (control), 10, 100, 1000 or 10000 ppm Equivalent to: 0, 1.8, 18, 167 nd 1663 mg/kg bw/day in males; 0, 2.3, 22, 234 and 2160 mg/kg bw/day in females 3 months	Cat 1 = 10 Cat 2 = 100	No treatment-related effects on mortality. No treatment-related overt clinical signs of toxicity. <u>10000 ppm (1663 and 2160 mg/kg bw/day in males and females respectively)</u> ↓ Body weight: 8.9% males, 6.8% females (day 28 values). Difference from control less marked at end of study. Food consumption: ↓ 12.9% males, 19.9% females (day 28 values) Liver enzymes: ↓ aniline hydroxylase activity (55% males, 48% females); ↓ aminopyrine-demethylase activity (34% males) Organ weights: ↑ absolute thyroid (76.3% and 62.2% in males and females); ↑ relative liver (12.3% and 10.5% in males and females); ↑ relative kidney (22.9% and 10.5% in males and females) Histopathology: Thyroid follicular cell hyperplasia, vacuolation of epithelium, congestion and decreased colloid density in 11-15/15 males and females (control 0-5/30); liver possible hepatocytic nuclear pleomorphism 10/15 females (4/30 controls) <u>1000 ppm (167 and 234 mg/kg bw/day in males and females respectively)</u> Body weight: ↓ 5% males only (day 28 value) Liver enzymes: ↓ aminopyrine-demethylase activity (38% males) <u>100 ppm (18 and 22 mg/kg bw/day in males and females respectively)</u> No treatment-related findings <u>10 ppm (1.8 and 2.3 mg/kg bw/day in males and females respectively)</u> No treatment-related findings

Method, guideline, deviation(s) from the guideline (if any)	**Species, strain, sex, number/group**	**Dose levels, duration of exposure**	**CLP Guideline value for classification (mg/kg bw/d or mg/l 6h/d rat study)**	**Results (Effects statistically significantly different unless stated otherwise)**
Oral studies in dogs (90 days)				
Three month dietary toxicity study in dogs OECD 409 GLP Purity 83.35%) Anonymous, 1986c	Dog: Beagle 6/sex/group	0, 10, 100, 1000 or 5000 ppm adjusted to give 100% a.i. Equivalent to: Males: 0, 0.29, 2.98, 28.62 & 101.53 mg/kg bw/day Females: 0, 0.32, 3.35, 28.91 &108.67 mg/kg bw/day 3 months	Cat 1 = 10 Cat 2 = 100	5000 ppm (101.53 and 108.67 mg/kg bw/day in males and females respectively) Mortality: Two males and one female dog killed weeks 8 - 10 due to poor health Clinical observations: dehydration all animals, thinness 2/6 males 6/6 females, few no faeces 6/6 males 5/6 females Body weight: ↓ 21.3% and 23.3% males and females week 13; lost weight over course of study. Food consumption*:* ↓ ~ 40% both sexes (overall). Haematology: ↓ RBC (25%), Hb (21.6%), Hct (22.4%) females week 5; Hb (15.9%) and RBC (17.7%) males week 5 T3: ↓ 58.3% and 40.0% in males and females week 5 T4: ↓ 7% and 81% in males and females week 5 ALT: ↓ ~ 40% week 5 both sexes Histopathology: thyroid follicular cell hyperplasia 6/6 males and 6/6 females; thymic cortical lymphoid depletion 6/6 males and 5/5 females; Reproductive organs hypoplastic changes: aspermatogenesis testis 3/6; hypogenesis of the epididymis (2/6), prostate (6/6), ovaries (4/6) and uteri (4/6) 1000 ppm (28.62 and 28.91 mg/kg bw/day in males and females respectively) Clinical observations*:* Occasional dehydration. Body weight: ↓ 9.6% males and 15.1% females week 13; loss or lack of gain during course of study Food consumption: ↓10.2% males, 18.7% females (overall value) Haematology: ↓ RBC (15.9%), Hb (15.0%), Hct (18.2%) females week 5 100 ppm (2.98 and 3.35 mg/kg bw/d in males and females respectively) No treatment-related changes 10 ppm (0.29 and 0.32 mg/kg bw/day in males and females respectively) No treatment-related changes

13 weeks oral toxicity study in dogs. (gavage in gelatine capsules) OECD 409 GLP Mancozeb Purity 88.2% Anonymous, 1987c	Dog: Beagle 4/sex/group, plus 2/sex/group (high dose and control) for recovery (6 weeks)	0, 5.7, 34.0 and 340/204 mg/kg bw/day Equivalent to 5, 30 and 300/180 mg ai/kg bw/day 13 weeks followed by 6 week recovery for the 340/204 mg/kg bw/day group. High dose reduced from day 17.	Cat 1 = 10 Cat 2 = 100	<u>340/204 mg/kg bw/day</u> *Clinical signs*: emesis, yellow faeces, pale mucous membranes, emaciation, subdued behaviour and isolated occurrence of convulsion or collapse *Body weight and body gain*: ↓ 16% weight week 13, 60% gain week 1-13 full recovery during the reversibility period *Food consumption*: ↓ 21% males, 24% females (weeks 1-13); full recovery during the reversibility period *Haematology:* ↓ PCV, Hb and Hct (~ 25% in males and 30% in females after 12 weeks) *Clinical chemistry*: ↓ glucose (13.3% males, 14.5% females after 12 weeks); ↑ ALP activity (31.3% after 6 weeks, 58.6% after 12 weeks females only); ↑ cholesterol (81.8% males after 12 weeks); ↑ bilirubin (200% males, 50% females after 12 weeks) *T4*: ↓ 30.0% males and 35.0% female after 6 weeks and 55.6% females after 12 weeks *T3*: ↓ 33.3% females after 12 weeks *Organ weights:* ↑ thyroid (~ 120% absolute and 170% relative); ↑ relative liver weights (39.0% males, 47.1% females); ↑ relative adrenal weights (42.2% females only) *Histopathology*: ↑ thyroid follicular hyperplasia (4/4 males and 3/4 females), colloid pallor and accumulation of colloid in the thyroid; mesenteric lymph node: ↑ hypoplasia 2/4 males 1/ 4 females; ↑ testes immaturity 2/4 All effects recovered after 6 week recovery period <u>34 mg/kg bw/day</u> *Clinical chemistry:* ↑ ALP activity (38.6% females after 12 weeks) *T4*: ↓ 40% females after 6 weeks; *Histopathology:* ↑ thyroid follicular hyperplasia (2/4 males and 2/4 females), colloid pallor in thyroid (3/4 males 2/4 females); mesenteric lymph node: ↑ hypoplasia 3/4 males 2/4 females; ↑ testes immaturity 2/4 <u>5.7 mg/kg bw/day</u> *Histopathology*: ↑ Thyroid follicular hyperplasia (3/4 males and 2/ 4 females), colloid pallor in thyroid 3/4 males 3/4 females

Oral studies in dogs (1 year)				
52 week oral toxicity study in the dog (dietary) OECD 452 GLP Mancozeb Purity 84.5% Anonymous, 1990c	Dog: Beagle 4/sex/group	0 (control), 50, 200, 800 or 1600 ppm Equivalent to: Males: 0, 1.8, 7.6, 28.4 & 53.0 mg/kg bw/day Females: 1.9, 7.8, 29.2 & 59.7 mg/kg bw/day in females 52 consecutive weeks	Cat 1 = 2.5 Cat 2 = 25	<u>1600 ppm (53.0 and 59.7 mg/kg bw/day in males and females respectively)</u> Mortality*:* 2/4 killed *in extremis* weeks 10 and 11. One acute urogenital tract lesion; other chronic regenerative anaemia. Body weight and body gain: ↓ 11.9% weight week 52, 30.6% gain week 1-53 females, not evident in 2 surviving males Food consumption ↓ (not quantified) Haematology: ↓ Hb (17.8% and 11.5% at 13 and 52 weeks in females), RBC (16.7%), PCV (15.3% at 13 weeks in females); ↑ MCV (7.3-9.3% weeks 13, 26 and 52 in males) Clinical chemistry: ↑ Serum cholesterol (41.1% and 54.4% weeks 13 and 26 in females). *T4*: ↓ 20-25% males, 20-39.5% females, not statistically significant; Organ weights: ↑ thyroid weights (absolute 47.0% males, 72.1% females; relative 50.0% males and 57.5% females); ↑ liver weights (relative 12.8% males, 27.9% females, not statically significant) Histopathology: ↑ Thyroid follicular distension (2/2 males, 4/4 females) <u>800 ppm (28.4 and 29.2 mg/kg bw/day in males and females respectively)</u> Body weight and body gain*:* ↓ ~ 17% both sexes week 52, 80.7% males, 35.4% females gain week 1-52 Food consumption: ↓ both sexes Haematology: ↓ Hb (11.0% and 12.1% at 13 and 52 weeks in females), PCV (9.7% at 13 weeks in females) <u>200 ppm(7.6 and 7.8 mg/kg bw/day in males and females respectively)</u> No treatment-related effects. <u>50 ppm (1.8 and 1.9 mg/kg bw/day in males and females respectively)</u> No treatment-related effects.

Oral toxicity study to dogs for 52 weeks. (capsule) EPA 83-1, consistent with OECD 452 GLP Mancozeb technical Purity 88.6 % Anonymous, 1991c	Dog: Beagle 4/sex/group	0, 2.3, 22.6 and 113 mg /kg bw/day Daily for 52 weeks except for 113 mg/kg bw/day group which were sacrificed in week 26	Cat 1 = 2.5 Cat 2 = 25	<u>113 mg /kg bw/day</u> One male died on week 13 with marked anaemia. Rest of group killed week 26 on humane grounds. Clinical signs: Underactivity, thin and pale, emesis, yellow/green faeces Body weight gain: ↓ 47.1% females days 0-90 Food consumption: ↓ 3% males, 18% females weeks 1-13 Haematology: ↓ 35-39% PCV, Hb, RBC in females after 24 weeks; ↑ MCV 4.8% males after 24 weeks Clinical chemistry: ↑ cholesterol (81.3% males after 24 weeks); ↑ ALP (156.3% males); ↑ inorganic phosphorus (18.5% males); ↑ total protein (7.1% males); ↓ ALT (51.4% females); ↓ AST (32.1% females) T4: ↓ 38.9% males, 41.4% females after 24 weeks (female value not statistically significant). <u>22.6 mg/kg bw/day:</u> Body weight gain: ↓ 45.2% females days 0-364 Food consumption*:* ↓ 4% males, 13% females weeks 1-52 Haematology: ↑ MCV (4.5% males after 50 weeks) Clinical chemistry: ↑ cholesterol (37.0% females after 24 weeks); ↓ AST (25.0% females after 24 weeks); ↓ urea (25.7% males); ↓ creatinine (41.7% males); T4: ↓ 26.3% males after 50 weeks. Organ weights*:* ↑ relative liver weight (44.6% in females); ↑ relative thyroid weight (35.2% in males (not statistically significant, increased in 2/ 4 males)). <u>2.3 mg /kg bw/day</u> No treatment-related findings.

52 week oral (capsule) toxicity study in dogs. EPA 83-1, consistent with OECD 452 but only 2 groups GLP Mancozeb Purity 88.6 % Anonymous, 1991d	Dog, Beagle 4/sex/group	Orally by capsules 0 and 40 mg/kg bw/day Daily for 52 weeks	Cat 1 = 2.5 Cat 2 = 25	40 mg/kg bw/day *Clinical observations:* 2/4 females thin, 1/4 females hypothermic and of pale appearance from week 21 *Body weight:* ↓ 26.7% weight week 52, 82.8% gain weeks 1-13 females *Food consumption*: ↓ 20-26% females *Clinical chemistry*: ↓ ALAT (46.2% and 33.3% in females after 24 and 52 weeks); ↓ ASAT (44.4% in females after 24 weeks); ↑ inorganic phosphorous (25.0% and 20% in females after 24 and 50 weeks); ↑ ALP (73.0-121.6% both sexes after 24 and 50 weeks); ↑ Cholesterol (14.0-37.0% both sexes after 24 and 50 weeks,(only stat. sig. in females after 24 weeks). *T4*: ↓ 36.4%, 42.1% in males and females respectively after 50 weeks; *T3*: ↓ 14.3% females after 50 weeks *Organ weights*: ↑ Thyroid weight (22.2% and 17.1% absolute values in males and females) *Histopathol*ogy: Liver: iron pigment deposition in Kuffer cells (4/4 females), periacinar lipofuscinosis 4/4 males (incidence in controls 1/4 for both findings)

Method, guideline, deviation(s) from the guideline (if any)	**Species, strain, sex, number/group**	**Dose levels, duration of exposure**	**CLP Guideline value for classification (mg/kg bw/d or mg/l 6h/d rat study)**	**Results (Effects statistically significantly different unless stated otherwise)**
Inhalation studies in rats (28 days)				
Subacute inhalation toxicity study in rats (nose-only) Mancozeb Purity: 83.35% Anonymous, 1986d	Rat/ Crl:CD (SD)BR 38/sex/group 5/sex/group killed after 4 weeks	6 hours a day and 5 days per week Concentration 0, 0.022, 0.086, 0.308 mg/L Respirable concentration 0, 0.008, 0.04, 0.127 mg/L Mass median diameter 3.7-4.4 µm GSD 2.1-2.3 4 weeks	Cat 1 = 0.06 Cat 2 = 0.6	0.127 mg/L respirable (0.308 mg/L total aerosol) Body weights: ↓ 5.5% males weight week 4, 10.9% males gain week 0-4 0.04 mg/L respirable (0.086 mg/L total aerosol) No treatment related findings 0.008 mg/L respirable (0.022 mg/L total aerosol) No treatment related findings
Inhalation studies in rats (90 days)				
Subchronic inhalation toxicity study in rats (Nose-only) EPA guidelines 82-4 GLP purity 83.35% Anonymous, 1986d	Rat/ Crl:CD (SD)BR 38/sex/group 10/sex/group killed after 13 weeks	6 hours/day, 5 days/week Concentration 0, 18, 79, 326 mg/m^3 Respirable concentration 0, 8, 36, 144 mg/m^3 Mass median diameter 3.8-4.2 µm, GSD 2.1 13 weeks	Cat 1 = 0.02 Cat 2 = 0.2	144 mg/m^{3}respirable (326 mg/m^3 total aerosol) Body weights: ↓ 6.8% males weight week 13, 10.4% males gain week 0-13 T4: ↓ 31.2% females Triglycerides: ↓ 36.2% males after 13 weeks Histopathology: Thyroid follicular epithelium hyperplasia (mild) 3/10 females 36 mg/m^{3}respirable (79 mg/m^3 total aerosol) No treatment related findings 8 mg/m^{3}respirable (18 mg/m^3 total aerosol) No treatment related findings

Method, guideline, deviation(s) from the guideline (if any)	**Species, strain, sex, number/group**	**Dose levels, duration of exposure**	**CLP Guideline value for classification (mg/kg bw/d or mg/l 6h/d rat study)**	**Results (Effects statistically significantly different unless stated otherwise)**
Dermal studies in rats (28 days)				
4 week repeat dermal toxicity study in rats OECD 410 GLP Vehicle: distilled water Mancozeb Purity 82.4% Anonymous, 1988d	Rat, Crl: CDBR, SD 10/sex/group	0 (control), 10, 100 or 1 000 mg/kg bw/day 6 hours/day 20 or 21 applications	Cat 1 = 60 Cat 2 = 600	10, 100 or 1000 mg/kg bw/day No effects
Dermal 28 day study in rat OECD 410 GLP Mancozeb, purity of test substance not reported Vehicle: peanut oil Anonymous, (1999d)	Rat: Sprague-Dawley Crl: CD(SD)BR 5/sex/group, including recovery groups for control and 750 mg/kg bw/day	0, 120, 300, 750 mg/kg bw/day 6 hours/day, 5 days/week for 4 consecutive weeks	Cat 1 = 60 Cat 2 = 600	750 mg/kg bw/day Clinical signs: lethargy in both sexes. Haematology: ↑ lymphocytes 13.5% males; ↓ 26.7% males Organ weight*:* relative brain weight ↑ 19.3% females. Macroscopic pathology: liver congestion/hard in consistency: 4/5 females (0/5 controls and 3/5 recovery controls). Microscopic pathology: Liver foci of round cell infiltration: 3/5 females (1/5 main study controls); skin acanthosis and hyperkeratosis 3/5 males (0/5 main study controls) 300 mg/kg bw/day Organ weight: relative brain weight ↑ 12.4% females. Macroscopic pathology: liver congestion/hard in consistency: 4/5 females (0/5 controls and 3/5 recovery controls). 120 mg/kg bw/day No effects

Dermal studies in rats (90 days)				
90 day dermal toxicity in rats. OECD (411) GLP Vehicle distilled water Mancozeb Purity 89.1% Anonymous, 1997d	Rat: SD 12/sex/group	0 and 1000 mg/kg bw/day at least six hours per day, 5 days /week 90 days	Cat 1 = 20 Cat 2 = 200	1000 mg/kg bw/day No treatment-related effects

Table 21 Summary table of human data on STOT RE

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations
Thyroid function in male workers manufacturing mancozeb, an agricultural fungicide, and in men not exposed to mancozeb. Anonymous (1985a)	Mancozeb	Thyroid function in men currently or previously exposed to EBDCs for many years at a manufacturing site was compared to 153 men not exposed to EBDC, its products or ETU, who worked at the same plant. Blood was analysed for thyroid hormones, complete blood count and chemistry profile. A 24 hour urine sample was analysed for iodide, creatinine, ETU and EBDC.	Exposure to EBDC manufacture was not associated with an increased prevalence of thyroid abnormalities
Worker exposure data Smith (1984)	ETU	Clinical examinations and thyroid function tests were carried out over a period of 3 years in the UK on 8 workers involved in the manufacture of ETU and 5 workers involved in the mixing of ETU with rubber.	Mixers but not process workers had significantly lower levels of T4 in their blood compared to controls. With the exception of one mixer with elevated TSH levels, who was evaluated as hypothyroid on further testing, no effects were found on TSH or thyroid binding globulin
Worker exposure data Anonymous (1990b)	Mancozeb	In workers in an EBDC fungicide factory in the Netherlands dermal exposure to mancozeb varied from 290.19 mg to 3305.87 mg outside clothing (including hands and head) and from 7.92 mg to 118.7 mg inside clothing. Respiratory exposure was minor when compared to dermal exposure, resulting in 290.30 to 4818.27 µg. Thyroid hormone levels were determined.	No biologically significant differences were observed for the concentrations of T3, T4 and TSH in blood samples collected before and after each shift. No effects on thyroid hormones were detected.
Worker exposure data Steenland *et al* (1997)	EBDC pesticides	Sprayers using EBDCs (49 men), landowners (lightly exposed, 14 men) and a non-exposed control group (31 men) compared for blood T4 and TSH levels. ETU was determined in urine	Low levels of ETU were detected in the urine of EBDC workers. Although an apparent increase in age-adjusted TSH occurred, T4 and TSH levels were within

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations
			normal levels in all groups.
Cross-sectional worker exposure study Baranska *et al* (2008)	Pesticides	Immune function was determined by measurement of antibody titres against two vaccinations of hepatitis B	Exposure to EBDCs and other pesticides did not affect the ability of the immune system to respond to vaccination, but pesticide exposed workers who were genetically characterized by the 2.2 IL-1α polymorphism showed a lower antibody response than unexposed controls with the same genotype
Cross-sectional study of female spouses of pesticide workers (Agricultural Health Study) Goldner *et al* (2010)	Organochlorine pesticides	Self-reported history of physician diagnosed thyroid disease and exposure to organochlorines was assessed among 16,529 female spouses of pesticide applicators. The risk of thyroid disease was also assessed in relation to ever use of herbicides, insecticides, fungicides including maneb or mancozeb, and fumigants.	Ever use of maneb/mancozeb was associated with hypothyroidism and hyperthyroidism but further studies are needed to confirm the findings.
Cross-sectional study of pesticide workers (Agricultural Health Study) Goldner *et al* (2013)	Organochlorine pesticides	Self-reported history of physician diagnosed thyroid disease and exposure to pesticides was investigated in 22,246 male pesticide applicators. The association between ever use of 50 pesticides and the occurrence of any thyroid disease (hypothyroidism, hyperthyroidism, or other disease vs controls with no thyroid disease) was evaluated.	There was no evidence that exposure to maneb/mancozeb increased the risk of either hypothyroidism or hyperthyroidism as observed previously among the spouses of applicators.
Cross-sectional case-control study Panganiban *et al* (2004)	EBDCs	The study was a cross-sectional investigation of the incidence of thyroid gland disorders among banana plantation workers exposed to EBDCs and the relation between the incidence of thyroid gland disorders and blood and urinary ETU. The study group included 57 directly exposed workers, 31 indirectly exposed workers randomly selected from four banana plantations and 43 comparison workers from an organic farm. All subjects underwent complete medical examinations and laboratory tests.	There were no statistically significant differences in thyroid function tests of exposed and control groups and no statistically significant differences in the prevalence of abnormal thyroid ultrasound results, or solitary nodules in exposed workers compared to controls.
Worker exposure study Colosio *et al* (2007);	Mancozeb	This study investigated immunotoxic effects induced by exposure to the fungicide mancozeb in Italian vineyard workers. The study included 48 vineyard workers intermittently exposed to mancozeb and 45 healthy controls. The subjects were investigated three times: before the seasonal application of pesticides and at 30 and 45 days after the beginning of the application period.	No relevant adverse immune effects were found in the pesticide exposed workers compared with the non-exposed controls

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations
Worker exposure study Corsini *et al* (2005)	Mancozeb	The study, conducted in Italy, included 26 subjects: 13 vineyard workers (12 male, 1 female) engaged in mancozeb application by tractor, and 13 control subjects (12 male, 1 female) never exposed to mancozeb or to other plant protection products. The potential immunotoxic effects of mancozeb were investigated using several blood cellular and serum parameters.	Comparisons within exposed workers suggest that low-level exposure to mancozeb may have slight immunomodulatory effects. However, the results are difficult to interpret because of the small sample size. The investigators concluded that the prognostic significance of the slight changes observed in the study is unclear.
Worker exposure study (EUROPIT) van Amelsvoort *et al* (2008)	Mancozeb	This study was conducted in the Netherlands to investigate the short-term and long-term immune effects of occupational exposure to EBDCs, in particular mancozeb. 41 re-entry workers and 40 non-exposed controls were investigated before the seasonal application of pesticides and 30 days after the beginning of the application period. Immune parameters were determined in blood and all participants filled in a questionnaire regarding exposure and outcome parameters, and all subjects underwent a comprehensive medical examination	No relevant adverse immune effects were found in the pesticide exposed workers compared with the non-exposed controls.
Cross-sectional worker exposure study Steerenberg *et al* 2008	EBDCs	The study involved five field studies in: the Netherlands (flower bulb growers, mainly re-entry workers), Italy (vineyard workers), Finland (potato farmers), and Bulgaria (workers from a zineb factory and greenhouse workers) to evaluate a possible association between occupational exposure to pesticides and haematological parameters and components of the immune defence.	EBDCs do not influence the immunologic system in a clinically significant fashion, and do not pose a significant health risk to the exposed subjects.
Cross-sectional study of pesticide workers (Agricultural Health Study) Beard *et al* (2011)	Mancozeb	The authors identified 110 suicides among 81,998 cohort members. Effects on state of mind, as suicide, were determined	No association was established between pesticide and subsequent incidence of suicide in pesticide applicators and their spouses in the US Agricultural Health Study
Case-control study Dhillon *et al* (2008)	Pesticides	Associations between self-reported exposure to pesticide products, organic pesticides, and other occupational and environmental exposures were determined. 100 cases of Parkinson's Disease (PD) and 84 controls were evaluated for associations between self-reported exposure to pesticide products, organic pesticides, and other occupational and environmental exposures and PD.	No relationship between exposure to mancozeb and risk of PD was found
Cross-sectional study of pesticide workers (Agricultural Health Study) Kamel *et al* (2007)	Mancozeb	Data from pesticide applicators and spouses participating in the Agricultural Health Study (USA) were evaluated in relation of self-reported PD and pesticide exposure.	No evidence of an association between incident or prevalent PD and mancozeb exposure

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations
Case control study Tanner *et al* (2009); Tanner *et al* (2011)	Mancozeb	519 PD cases from 8 movement disorder clinics across the US and 511 controls were investigated. In follow-up study lifetime use of pesticides and PD investigated.	No evidence of association of PD with mancozeb exposure.
Cross-sectional study of pesticide workers (Agricultural Health Study) Mills *et al* (2009)	Mancozeb	The study examined myocardial infarction (MI) mortality and nonfatal MI incidence among male pesticide applicators	The study does not provide convincing evidence that maneb/mancozeb, or any individual pesticide or pesticide class, is associated MI mortality or nonfatal MI incidence. The investigators concluded that there was little evidence of increased risk of MI mortality or nonfatal MI associated with the occupational use of pesticides in a population with low risk for MI

Table 22: Summary table of other studies relevant for STOT RE

Method, guideline, deviation(s) from the guideline (if any)	Species, strain, sex, number/group	Dose level, duration of exposure	CLP Guideline value for classification (mg/kg bw/d or mg/l 6h/d rat study)	Results (Effects statistically significantly different unless stated otherwise)
Subchronic oral neurotoxicity (dietary) Non guideline GLP Deviations: no clinical chemistry, organ weights or standard histopathology (neuropathology only) Mancozeb Purity 79.3% Anonymous, 1991e	Rat, Crl:CD BR, 10/sex/group; plus 2 groups of 16 females for 14 days only	0, 20, 125, 750 and 5000 ppm for 90 days Equivalent to: Males: 0, 1.35, 8.21, 49.7 and 339 mg/kg bw/day Females: 0, 1.67, 10.5 and 63.3 mg/kg bw/day 5000 ppm and 5000 ppm for 14 days Due to mortality the initial group of females at 5000 ppm were fed control diet from day 14.	Cat 1 = 10 Cat 2 = 100	5000 ppm (339 and 413 mg/kg bw/day in males and females, respectively): Mortality*:* 1/10 males and 4/10 females weeks 2-4. 0/16 and 0/16 in females fed diet for 2 weeks. Clinical signs: abnormal gait and/or limited or no use of hindlimbs, muscle wasting, from week 2/3. Body weight: ↓ 44.7% in males day 91; initial females weight loss to day 14; additional females reduced body weight gain (not significant). Food consumption*:* ↓41% in males; ↓ 74% in females week 2; additional females ~ ↓ 33%. Histopathology: intrasheath ellipsoids, myelin phagocytosis, Schwann cell proliferation; demyelinated nerves, myelin sheath thickening, myelin bubbles, neurofibrillary degeneration of nerve tissues in males and females; 10/16 females had posterior thigh muscle atrophy 750 ppm (49.7 and 63.3 mg/kg bw/day in males and females, respectively): Body weight: ↓9% (not significant) in females Histopathology*:* myelin phagocytosis, Schwann cell proliferation; demyelinated nerves, myelin sheath thickening, myelin bubbles in males; demyelination and myelin ovoids/debris in females. 125 ppm (8.21 and 10.5 mg/kg bw/day in males and females, respectively): No treatment-related effects. 20 ppm (1.35 and 1.67 mg/kg bw/day in males and females, respectively): No treatment-related effects.

Oral Investigation into serum T4 and liver microsomal enzyme activity (gavage) Non guideline investigative study Non-GLP Mancozeb Purity not reported Vehicle: corn oil Flippin *et al* 2009 (from literature)	Rat: Long Evans female rats 8-16/group	0, 3.9, 7.8, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 for 4 days. Serum T4 and liver microsomal enzyme measured 24h after last dose	N/A	Predictive modelling of a mixture of thyroid hormone disrupting chemicals that affect production and clearance of thyroxine. Mancozeb produced a dose-dependent decrease in circulating levels of T4 with an ED_{50} of 259 mg/kg bw/day No induction of EROD, PROD or T4-UDPGT was seen.
Non guideline oral investigative study Oral (drinking water) Non-GLP Mancozeb Purity 80% Vehicle: water Kechrid *et al* 2011 (from literature)	Rat: Wistar 8/group	0, 2 or 3.5 g/L for 4 weeks	N/A	<u>3.5 g/L:</u> ↓ Body weight gain, ↓ blood glucose, ↑ ALT, AST and ALP ↑ cholesterol, serum triglycerides, serum creatinine and serum urea concentrations <u>2g/L</u> No effects
Non guideline investigative study Non GLP Mancozeb Purity 75% Vehicle: olive oil Ksheerasagar *et al* 2010 (from literature)	Mice, swiss albino, male 10/group	200, 400, 600, 800 mg/kg bw/day Administered for 30 days	N/A	<u>800 mg/kg bw/day</u> ↓ kidney, spleen & liver weights ↑ thyroid & thymus weights ↓ testes, prostate & cowper's gland weight ↓ number & diameter of spermatogenic and leydig cells ↓ diameter of spermatogonia & primary spermatocytes ↓ number of small follicles ↑number of medium, large and total follicles in the thyroid Hypertrophy of & hyperplasia of thyroid follicular cells with loss of colloid ↓ protein (in testes, liver & kidney) & glycogen (liver & kidneys)

				↑ lipids in testes <u>600 mg/kg bw/day</u> ↓ kidney, spleen & liver weights ↑ thyroid & thymus weights ↓ testes, prostate & cowper's gland weight ↓ number & diameter of spermatogenic and leydig cells ↓ number & diameter of spermatogenic and leydig cells ↓ number of small follicles ↑number of medium, large and total follicles in the thyroid Hypertrophy of & hyperplasia of thyroid follicular cells with loss of colloid ↓ glycogen (liver & kidney) & lipids (kidney) ↑ lipids (liver) <u>400 mg/kg bw/day</u> ↓ testes weight Hypertrophy of & hyperplasia of thyroid follicular cells with loss of colloid <u>200 mg/kg day</u> No effects
Non guideline investigative study Oral Non guideline Non GLP Mancozeb Purity not reported Sakr 2007 (from literature)	Rats, albino	313.6 mg/kg bw/day 3 times a week for 6 weeks	N/A	<u>313.6 mg/kg bw/day</u> Histopathological changes in the liver ↑ ALT & AST, malondialdehyde, ↓ superoxide dismutase Treatment with mancozeb & ginger extract (120 mg/kg bw/day) improved effects.
Non guideline investigative study Oral Non GLP Mancozeb Purity not reported Sakr *et al* 2009	Rats, albino	313.6 mg/kg bw/day 3 times a week for 6 weeks	N/A	<u>313 mg/kg bw/day</u> ↓ serum testosterone & LH ↓ diameters of seminiferous tubules Inhibition of spermatogenesis, degeneration of spermatogenic cells, destruction of leydig cells & congestion of blood vessels Treatment of rats with mancozeb and with ginger led to an amelioration of the observed testicular damage.

(from literature)				
Non guideline investigative study Oral (gavage) Non GLP Mancozeb Purity not reported Vehicle: olive oil) Joshi *et al* 2005 (from literature)	Rats, Wistar, male	500 mg/kg bw/day	N/A	500 mg/kg bw/day No changes in body weight ↓ Testis, epididymis, ventral prostate & seminal vesicle weights ↓ sperm density & motility 80% negative fertility ↓ sialic acid & glycogen content ↑ testicular protein & cholesterol ↓ reduction in testosterone & ALP ↑ acid phosphatase

10.10.1 Oral

Rats

28-day studies

Groups of 6 Sprague Dawley (SD) rats/sex were administered mancozeb by gavage for 28 days at 0, 50, 200 or 500 mg/kg bw/day. The top dose of 500 mg/kg bw/day resulted in hind limb ataxia/paralysis in females, leading to the early termination of one female. Abnormal clinical and haematological parameters were observed in this dose group, as were reductions in body weights and body weight gain. Females appeared to be more susceptible to mancozeb than males. Ataxia in females was also noted in the 200 mg/kg bw/day dose group, resulting in the premature termination of another female. Reduced body weight and body weight gain, and increases in liver and kidney weights were also observed at 200 mg/kg bw/day. Abnormalities in clinical chemistry parameters also occurred at this dose. No treatment related effects were observed at 50 mg/kg bw/day (Anonymous, 1994b).

90-day studies

Five key studies investigating the oral administration of mancozeb over 90 days were available.

In the study by Szepvolgyi *et al* (1989), groups of 12 male Wistar rats were given mancozeb in the diet for 90 days at 0, 1, 10, 50, 75, 113, 169, 253 or 379 mg/kg bw/day. The top dose of 379 mg/kg bw/day resulted in significant toxicity (prostration, weakness and paralysis) and the premature death of 4 males within 6 weeks. Survivors at this dose had enlarged thyroids and showed signs of thyroid toxicity, both biochemical (reduced iodine) and histopathological (proliferating epithelial cells). Liver effects included reductions in liver enzymes and increases in relative organ weight, without accompanying histopathological signs. Similar effects on the thyroid and liver were seen at the 253, 169, 113 and 75 mg/kg bw/day doses. At lower doses decreases in thyroid iodine and increases in liver and thyroid weight were unaccompanied by histopathological change in either organ.

In the study by Anonymous (1989), groups of 10 SD rats/sex were given mancozeb in the diet for 90 days at 0, 28, 113 or 454 ppm. Mancozeb had a minimal effect amongst males and females receiving 454 ppm, characterised by a reduced body weight gain during the treatment period. Other changes from controls included decreases in neutrophils, increases in T3 and suppression of T4. These were unaccompanied by organ weight change and histopathology and so were considered to be equivocal.

In the study by Anonymous (1986b), groups of 14 SD rats/sex were given mancozeb in the diet for 90 days at 0, 30, 60, 125, 250 or 1000 ppm. No treatment related mortality or clinical signs were seen at any dose. No effects on body weight or food consumption were recorded in animals fed concentrations up to and including 250 ppm. At 1000 ppm, male rats showed a statistically significant decrease in body weight from week 3 through week 13, whereas in females the 3 to 14% body weight decrease was significant only between weeks 7 and 10. At 1000 ppm, food consumption was decreased in males during weeks 3 to 13, but not in females. Thyroid follicular cell hyperplasia was seen in 90% of the males and females receiving 1000 ppm. A small, well-defined focus of hyperplastic follicular epithelial cells was seen in one male. There was an increased incidence and severity of hypertrophied vacuolated cells in the pituitary of male rats. The kidneys of males and females administered 125-1000 ppm had minimal to moderate amounts of a yellow-brown pigment in the lumen of the cortical tubules. These pigment deposits were not accompanied by any histopathological effects and were attributed to ethylene-bisisothiocyanate sulfide (EBIS), a yellow-coloured metabolite of mancozeb; hence, they were not considered to be adverse. There was also an increased incidence of hypertrophy of the cells of the adrenal zona glomerulosa, and of hypertrophy of the centrilobular hepatocytes in males fed 1000 ppm mancozeb. There was hepatocytic centrilobular hypertrophy in the 1000 ppm male rats. The only clinical sign which was considered to be possibly related to treatment was diarrhoea which was noted sporadically in a few animals from Day 57 onwards. However, this did not appear to be dose-dependent and together with the other observed clinical signs was not reflected in the macroscopic pathology, histopathology, haematology or clinical biochemistry. These signs were therefore considered not to be treatment-related. When fed for 3 month to rats, mancozeb induced thyroid, pituitary and liver alterations, both macroscopic and microscopic at the top dose of 1000 ppm (57 mg/kg bw/day). Effects on thyroid hormones were also present at 250 ppm (15 mg/kg bw/day).

In the study by Anonymous (1999c), groups of 12 Wistar rats/sex were given mancozeb by gavage for 90 days at 0, 64, 160 or 400 mg/kg bw/day. At the top dose, one male and one female were found dead. The treatment-related effects at this dose consisted of changes in haematological parameters, increased liver and adrenal weights and hepatocellular degeneration. Increased adrenal weight was also noted at 160 mg/kg bw/day.

In the study by Anonymous (1997c), groups of 12 SD rats/sex were given mancozeb by gavage 5 days/week for 90 days at 0, 15, 25, 40 or 50 mg/kg bw/day. No treatment-related effects were noted at any dose.

In a subchronic neurotoxicity study (Anonymous, 1991e), groups of 10 SD rats/sex were given mancozeb in the diet for 90 days at 0, 20, 125, 750 or 5000 ppm. One male and 4 females died between the second and fourth week of administration in the 5000 ppm group. These deaths resulted in a cessation of test compound to females of the high dose only and administration of control feed from the 15th day through the remainder of the study. Clinical signs of hindlimb effects were observed in all rats fed 5000 ppm diets. Onset of clinical signs in the second and third week of administration consisted of generalised weakness, abnormal gait or mobility, and limited or no use of the rear legs. Loss of muscle mass was also reported in the males. However, by day 60 some males in the high-dose group appeared clinically normal and females showed improvement after one week on control diets. Females of the satellite group fed mancozeb for two weeks also manifested limited rear limb mobility. No other group of rats had clinically observable signs of toxicity or mortality attributable to the intake of the test compound. Body weight, food consumption and food efficiency were substantially decreased (40-45%) in males by day 90 in the high-dose group. Females receiving 5000 ppm also showed initial decreases in food consumption and weight loss that were reversed after cessation of test compound and their placement on control feed. Body weights were also 9% lower than controls among females at 750 ppm. Both male and female rats of the 5000 ppm groups presented with myelin damage and Schwann cells proliferation in sections of nerve tissue and myelin damage in teased nerve fibres. A lower incidence of these lesions was observed in rats fed the 750 ppm diets. Muscular atrophy was also noted microscopically, but only at the 5000 ppm level. Overall, in this dietary 90-day neurotoxicity study in rats, severe neurotoxic effects (hindlimb, loss of muscle mass, muscular atrophy and myelin damage with Schwann cells proliferation of nerve tissue) were seen at the top dose of 5000 ppm (328/420 mg/kg bw/d in M/F) in the presence of generalised toxicity (large reductions in body weight and food consumption and deaths). Less severe

neurotoxicity (myelin damage with Schwann cells proliferation of nerve tissue) was also seen at 750 ppm (49/63 mg/kg bw/d in M/F), in association with small reductions in body weight.

Hind limb paralysis was also noted in maternal animals at 150 mg/kg bw/day in an investigative developmental neurotoxicity study (Axelstad *et al*, 2011 – summarised in the developmental toxicity section).

Other studies (from the literature review)

Weanling Long Evans female rats (8-16 per group) were dosed at 0, 3.9, 7.8, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 mg mancozeb/kg bw for 4 days (Flippin *et al*., 2009). 24 hours after the last dose, serum T_4 was measured by radioimmunoassay and liver microsomal enzyme activity was assayed. Mancozeb produced a dose-dependent decrease in circulating levels of T_4 with an ED_{50} of 259 mg/kg bw/d. There were no statistically significant decreases in T_4 levels from 15.6 mg/kg bw/d. No induction of EROD (ethoxyresorufin O-deethylase), PROD (pentoxyresorufin O-deethylase) or T_4-UDPGT (uridine diphosphate glucuronyl transferase) was seen.

Mancozeb (in olive oil) was administered to 6 male albino Wistar rats orally by gavage at 500 mg/kg bw/d for 30 days (Joshi et al., 2005). Sex organ weight analysis, reproductive, biochemical and enzymatic parameters and testosterone levels were evaluated to determine the toxicity of mancozeb on the male rat. There was no significant difference in body weight at the end of the experimental period. Testis, epididymis, ventral prostate and seminal vesicle weights were decreased. Significant testicular toxicity and a marked reduction in testosterone were observed. Overall, oral gavage administration of 500 mg mancozeb/kg bw/d to male Wistar rats for 30 days did not affect body weight development but was associated with testicular toxicity. However, the reliability of this study is questionable. Despite the use of a high dose of mancozeb, no systemic toxicity, as seen in other studies, was reported. The testicular findings are also questionable as they have not been reported in numerous other studies.

Three groups of eight rats were administrated with drinking solutions of 0 (control), 2 g/L and 3.5 g/L mancozeb (Kechrid *et al.*, 2011). The animals were maintained for 4 weeks; body weight gain was recorded regularly. At the end of the study the animals were fasted overnight, killed and serum glucose, serum cholesterol, serum triglycerides, serum creatinine, serum urea concentrations and serum glutamic oxaloacetic transaminase (GOT), serum glutamic pyruvic transaminase (GPT) and serum alkaline phosphatase activities were determined. A treatment level of 3.5 g/L resulted in decreased body weight gain, deceased blood glucose and increased activities of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP). In addition serum cholesterol, serum triglycerides, serum creatinine and serum urea concentrations were significantly elevated. The authors concluded that a 3.5 g/L dose of mancozeb has a toxic effect on body weight and blood biochemistry parameters. However there were no toxic effects at a dose of 2 g/L dose.

Mancozeb dosed orally at 313.6 mg/kg bw/d, 3 times/week for 6 weeks to 20 albino rats induced histopathological changes in the liver and elevation of liver function enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). There was an increase in the lipid peroxidation marker, malondialdehyde (MDA) and a decrease in the level of the antioxidant enzyme superoxide dismutase (SOD). Treating animals with mancozeb + ginger extract (120 mg/kg bw/d) led to improvement in the histopathological lesions, decreases in ALT, AST and MDA and increases in SOD compared to mancozeb alone (Sakr, 2007).

Groups of 20 albino rats were orally dosed with 1/10 LD_{50} (313.6 mg/kg bw) of mancozeb (in water), 3 times per week for 6 weeks (Sakr *et al*., 2009). A significant decrease in serum testosterone and a decrease in luteinizing hormone (LH) were observed. There was also significant testicular toxicity. Treatment of rats with mancozeb and with ginger led to an amelioration of the observed testicular damage. Overall, oral administration of mancozeb at 313.6 mg/kg bw/d for 6 weeks to rats caused toxic effects in the testes. The

reliability of this finding is questionable as it is inconsistent with the observations of numerous other repeated dose studies and multigeneration studies in rats.

Mice

28-day studies

In a 28 day range finding study by Anonymous (1985b), groups of 5 CD-1 mice/sex were given mancozeb in the diet at 0, 10, 100, 1000 or 10,000 ppm. Administration of 10,000 ppm resulted in decreased body weight and increased thyroid weight of females. Increased liver weights and thyroid hyperplasia were observed in both sexes at 10,000 (2000 mg/kg bw/day) and in females at 1,000 ppm (200 mg/kg bw/day). There were no associated histopathology effects.

90-day studies

In the main 90 day study (Anonymous, 1985c) groups of 15 CD-1 mice/sex were given mancozeb in the diet at 0, 10, 100, 1000 or 10,000 ppm. No treatment related mortality or signs of toxicity were observed at any dose level. Decreased body weight and food consumption were noted in the 10000 ppm group for both sexes, and body weight decrease in the 1000 ppm males only. No treatment related haematological and clinical chemistry effects were seen in any group. Hepatic mixed function oxidase activity (as measured by aniline hydroxylase levels) was decreased in both sexes at 10000 ppm, and aminopyrine-demethylase activity was decreased in males at 1000 and 10000 ppm. Thyroid weights (absolute and relative) were increased in both sexes at the 10000 ppm level; also, at this dose, increased liver and kidney weights were present in both sexes. Histopathology revealed an increased incidence of follicular cell hyperplasia and hypertrophy in both sexes at 10000 and 1000 ppm. In addition, increased vacuolation interstitial congestion and decreased colloid density were observed in both sexes in the high dose group. Histopathologic changes in the liver were limited to a possible increase in hepatocytic nuclear pleomorphism in the females at 10000 ppm. Also noted among the 10000 ppm females only were increased deposits of brownish pigment in the adrenal reticularis. Overall, when orally administered (via the diet) to mice for 3 months, mancozeb produced decreases in body weight and induced thyroid and liver effects at concentrations of 1000 (167/234 mg/kg bw/day in M/F) and 10000 ppm (1663/2160 mg/kg bw/day in M/F).

Other studies (from the literature review)

Mancozeb (in olive oil) was orally administered at doses of 200, 400, 600 or 800 mg/kg bw/d to groups of 10 male Swiss albino mice for 30 days (Ksheerasagar *et al*., 2010). All treated mice were depressed and adopted abnormal posture with the head held in between the forelegs. They were trying to huddle at the corner of the cage and showed more running activity immediately after the administration of mancozeb. In mice treated with 600 or 800 mg/kg bw/d a significant decrease in the kidney, spleen and liver weights and an increase in thyroid and thymus weights were observed. There was no significant change in body weight. There was a significant decrease in the weight of the testes at ≥ 400 mg/kg bw/d and in the weight of the prostate and Cowper's glands at ≥ 600 mg/kg bw/dy. There was no significant change in the weight of the epididymides, vasa deferentia, seminal vesicles and coagulating glands. Testicular toxicity was observed at 600 and 800 mg/kg bw/d. In the thyroid, treatment with 600 or 800 mg/kg bw/d caused a decrease in the number of small follicles and an increase in the number of medium, large follicles and total number of follicles. Histological observation of the thyroid revealed hypertrophy and hyperplasia of follicular cells with loss of colloid at 400, 600 and 800 mg/kg bw/d. Liver and kidney toxicity were also observed at 600 and 800 mg/kg bw/d. Overall, oral administration of mancozeb to mice for 30 days caused toxic effects on the testis and thyroid from approximately 400 mg/kg bw/d. No such effects were observed at 200 mg/kg bw/d at which clinical signs of toxicity were reported. Although these behavioural findings observed from the lowest dose of 200 mg/kg bw/d indicate systemic toxicity, changes in body weights were not reported. This questions the reliability of these findings in the mouse.

Dogs

90-day studies

Two 90 day studies in dogs are available.

In a 90 dietary study in Beagle dogs (Anonymous 1986c), groups of 6 Beagle dogs/sex were given mancozeb at 0, 10, 100, 1000 or 5000 ppm. Three top dose dogs (one female and two males) were sacrificed during Weeks 8-10 due to a severe deterioration in their physical condition. Signs associated with reduced food consumption and low body weights were dehydration, thinness, and pale mucous membranes. A slight to moderate and moderate to marked decrease in food consumption was noted for the 1000 and 5000 ppm dogs, respectively. This was possibly due to a lack of palatability. There was a marked decrease in body weight for the top dose dogs and a lack of body weight gain, or slight loss of body weight in the 1000 ppm dogs. Evaluation of the clinical pathology data revealed a slight reduction in red cell mass in the top dose dogs as indicated by decreased erythrocyte counts, haematocrit values, and haemoglobin concentrations at Weeks 5 and 13. A reduction in red blood cell mass was also seen in the 1000 ppm females at Week 5 but not at Week 13. Increased values of total bilirubin and total cholesterol and decreased values of T3, T4, alanine aminotransferase (ALT) and calcium were also noted for the top dose dogs. In addition, total cholesterol was increased in the 1000 ppm females at Week 13 and total bilirubin was increased in the 1000 ppm males at Week 5. The clinical pathology findings, in general, are consistent with the syndrome of hypothyroidism in the canine. However, some of the clinical pathology changes seen in this study may be related to malnourishment resulting from decreased food intake. Treatment-related findings at gross necropsy were noted in the 1000 and 5000 ppm dogs and included enlarged and/or dark thyroids and decreased thymus size. Analysis of the organ weights revealed significantly greater mean values of the thyroid/parathyroid weights for the top dose male and female dogs when compared to the respective control values. Treatment-related findings at gross necropsy were noted in the 1000 and 5000 ppm dogs and included enlarged and/or dark thyroids and decreased thymus size. Analysis of the organ weights revealed significantly greater mean values of the thyroid/parathyroid weights for the top dose male and female dogs when compared to the respective control values. Analysis of the urine samples indicated the presence of dose-related levels of EBDC (assayed as CS_2) and ETU. ETU was detected in the blood in the 1000 and 5000 ppm groups. No EBDC was detected in the thyroid at 5000 ppm, while ETU residues increased in the thyroids of the 1000 and 5000 ppm groups. Overall, when orally administered (via the diet) to dogs for 3 months, mancozeb produced decreased feed consumption and body weight gain, haematology and clinical-chemistry findings and thyroid histopathology from a dose of 1000 ppm (30 mg/kg bw/day) and clinical signs of toxicity and mortality at the top dose of 5000 ppm (101 mg/kg bw/day).

In a 90 day (Anonymous, 1987c) capsule dosing study, groups of 4 Beagle dogs/sex were given mancozeb at 0, 5.7, 34 or 340/204 mg/kg bw/day. Administration of mancozeb at doses of 340/204 mg/kg bw/day for 90 days resulted in marked decreases in food consumption and body weight that were accompanied by clinical chemistry changes (decreases in red cell parameters, lower plasma glucose, increases in ALP, cholesterol, bilirubin and decreases in T3 and T4). Increases in thyroid, liver and adrenal weight were observed with associated thyroid histopathology (increased thyroid follicular hyperplasia, colloid pallor and accumulation of colloid in the thyroid). There were also increases in hypoplasia of the mesenteric lymph node. Similar histopathological findings (without organ weight change) were noted at both 34 and 5.7 mg/kg bw/day.

1-year studies

In a 1 year dietary study by Anonymous (1990c), groups of 4 Beagle dogs/sex were given mancozeb at 0, 50, 200, 800 or 1600 ppm. Two males given 1600 ppm were killed in extremis in weeks 10 and 11. One animal was found to have an acute urogenital tract lesion and the other a chronic regenerative anaemia. The urogenital tract lesion was probably an incidental event but the probability that the anaemia was an idiosyncratic treatment-related reaction cannot be excluded. All other animals survived and there were no clinical signs or palpable masses related to treatment. There were no apparent neurological or ophthalmic changes related to treatment. Animals in the 800 ppm group gained less weight than controls. There were no other treatment-related effects on body weight gain. Food consumption of males and females given 800 ppm was generally less than controls. Throughout the study females given 800 and 1600 ppm had lower haemoglobin, red blood cell count and packed cell volume values than controls. This was not apparent in males surviving to termination Other than elevated cholesterol levels in females given 1600 ppm there were no changes in clinical chemistry parameters associated with treatment. There was a reduction in T4 levels in animals given 1600 ppm, but no

trends were apparent in T3 levels. The cellular and chemical constituents of urine were unaffected by treatment. Treatment-related thyroid follicular distension (increased amount of colloid) was seen at the terminal kill in all the top dose animals, while increased incidence of Kupffer cell pigment was seen in the liver of the top dose (females only). In conclusion, in this 52-week dietary study in dogs, mortality occurred at the top dose of 1600 ppm. The only consistent treatment-related effects during the study were a reduction in T4 levels (not statistically significant) with increased thyroid weight and thyroid follicular distension in animals given 1600 ppm and transient reductions in haemoglobin, red blood cell count and packed cell volume and elevation in cholesterol in females given 800 (28 mg/kg bw/day) or 1600 ppm (53 mg/kg bw/day). There were no apparent effects of treatment of up to 200 ppm in mancozeb in the diet.

In another 1-year study (Anonymous, 1991c), groups of 4 Beagle dogs/sex were given capsules containing mancozeb at 0, 2.3, 22.6 or 113 mg/kg bw/day. The top dose of 113 mg/kg bw/day was not tolerated for more than 26 weeks with animals showing changes consistent with severe hypothyroidism, which was probably primarily responsible for all other observed changes such as inappetence, body weight loss and anaemia; this dose clearly exceeded the maximum tolerated dose. Similar but minimal changes were seen in the 22.6 mg/kg bw/d dose group.

In a subsequent 1-year study (Anonymous, 1991d), groups of 4 Beagle dogs/sex were given capsules containing mancozeb at 0, or 40 mg/kg bw/day. Clinical signs of toxicity, effects on body weights and food consumption, changes in clinical-chemistry parameters, decreased thyroid hormones, increased thyroid weight and liver histopathology were seen at the only dose tested.

10.10.2 Other routes

Dermal

In a 28 day dermal study (Anonymous, 1988d), groups of 10 SD rats/sex were treated with mancozeb in water at 0, 10, 100 or 1000 mg/kg bw/day. There was no evidence of any treatment related effects.

When mancozeb was administered for 28 days to rats using peanut oil, rather than water as the vehicle (Anonymous, 1999c) the treatment-related effects consisted of sparse variations from normal in haematological parameters, organ weights and histopathological examination in females only at dose levels of 300 and 750 mg/kg bw/day. At 750 mg/kg bw/day 3/5 females were also noted to have liver foci of round cell infiltration.

Dermal application of mancozeb for 90 days (Anonymous, 1997d) to SD rats at 1000 mg/kg bw/day resulted in no treatment-related effects.

Inhalation

In a 28-day study (Anonymous 1986d), groups of 5 SD rats/sex were exposed by inhalation nose-only (6 hr/day, 5 day/week) to mancozeb aerosol at respirable concentrations of 0, 8, 40 or 127 mg/m^3. The only effect noted in this study was a decrease in body weights at the top concentration.

A 90 day inhalation study in the rat (Anonymous., 1986e) is also available. In this study, groups of 10 SD rats/sex were exposed by inhalation nose-only (6 hr/day, 5 day/week) to mancozeb aerosol at respirable concentrations of 0, 8, 36 or 144 mg/m^3. Treatment-related effects (decreased body weights, effects on the respiratory tract, decreased T4 levels and thyroid follicular hyperplasia) were only seen at the top concentration.

10.10.3 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

The repeated dose toxicity of mancozeb by the oral route has been extensively investigated in studies ranging from 4-days to 90 days in rats and mice. Studies in dogs (90 days and 1 year) are also available. Inhalation studies in rats (28 and 90 days) and three dermal studies (28 and 90 day studies) in rats have also been conducted.

The effects of mancozeb after repeated oral administration are consistent with those of its ETU metabolite, which is responsible for the toxicity of the parent compound. ETU interferes with the production of thyroid hormone by inhibition of the thyroid peroxidase enzyme responsible for iodination and coupling of tyrosine residues into the thyroid hormone precursor, thyroglobulin. This mechanism leads to hypertrophy and hyperplasia of the follicular cells of the thyroid gland, and ultimately tumours of this organ after long term exposure to very high doses.

Thyroid toxicity has been seen in all three species investigated, rats, mice and dogs, representing the most sensitive effect of mancozeb repeated oral dose toxicity. In rats exposed to mancozeb for 3 months, thyroid toxicity tended to appear at lower dose levels (from 15 mg/kg bw/d) than in mice (from 180 mg/kg bw/d). Thyroid toxicity was accompanied at higher dose levels by effects on the liver and adrenal, changes in haematological and clinical-chemistry parameters and reduced food consumption and body weights. In rats, neurotoxicity (neurohistopathology findings) also started to occur from a dose of 49 mg/kg bw/d for 90 days; these neurotoxic effects became more severe (hindlimb, and muscular atrophy) at the high dose of 328 mg/kg bw/d for 90 days. In dogs exposed to mancozeb for 3 months or 1 year, thyroid toxicity started to appear from around 22.6 mg/kg bw/d (for 52 weeks). Thyroid toxicity was accompanied by anaemia, effects on the liver and reductions in food consumption and body weight.

Repeated dermal administration of mancozeb to rats for up to 13 weeks had no effects up to the limit dose of 1000 mg/kg bw/d. Repeated inhalation exposure of rats to mancozeb aerosol for 13 weeks resulted in decreased body weights, effects on the respiratory tract, reduced serum T4 levels and thyroid hyperplasia at the top respirable concentration of 144 mg/m^3.

Animal data from the literature are either unreliable or confirm that the thyroid is the main target organ of repeated oral dose toxicity of mancozeb.

Epidemiology and medical studies indicated that, in reliable studies, mancozeb does not affect the thyroid or have adverse endocrine-related effects in humans. The possible appearance of neurological effects in populations exposed to pesticides has been investigated in several studies. Effects on state of mind, as suicide, were determined by Beard *et al* (2011). No association was established between pesticide and subsequent incidence of suicide in pesticide applicators and their spouses in the US Agricultural Health Study. This finding was consistent for use of any pesticide, including mancozeb.

Possible associations with pesticide use and Parkinson's disease (PD) have been studied. Dhillon *et al* (2008), Kamel *et al* (2007) and Tanner *et al* (2009 & 2011) investigated associations with individual pesticides, including EBDCs. None of these studies found that exposure to mancozeb was associated with increased incidences of PD.

Also, there is no convincing evidence that mancozeb has adverse effects on the immune system in exposed workers.

10.10.4 Comparison with the CLP criteria

Classification into STOT-RE is warranted when significant or severe toxicity is observed after repeated exposure to doses at or below the reference values assigned in the guidance on the application of the CLP criteria. These values are extrapolated from the value for oral administration to the rat in 90 day studies and are given in the table below:

Route	Duration	Category 1 (mg/kg bw/day)	Category 2 (mg/kg bw/day)
Oral	28 day	≤ 30	> 30 ≤ 300
Oral	90 day	≤ 10	> 10 ≤ 100
Oral	52 weeks	≤ 2.5	> 2.5 ≤ 25

The values for other routes of administration, also based on 90 day studies in the rat are <20 ≤ 200 mg/kg bw/day for dermal exposure and < 0.02 ≤ 0.2 mg/l 6h/day for inhalation exposure. STOT-RE is assigned on the basis of either significant or severe toxicity. 'Significant' is taken to mean changes which clearly indicate toxicologically relevant functional disturbances or morphological changes. 'Severe' refers to profound effects of a considerably more adverse nature which significantly impact on health.

No adverse effects were noted when mancozeb was administered dermally to rats for 28 days or 90 days up to the limit dose (Anonymous, 1988a; Anonymous, 1999c; Anonymous 1997d). Effects on body weights, respiratory tract and thyroid were seen when mancozeb was given to rats via the inhalation route for 90 days (6 hr/day) at the top concentration of 0.3 mg/L total aerosol (i.e. at a concentration above the CLP guidance value of 0.2 mg/L 6h/day for classification) (Anonymous, 1986e). Therefore, no classification of mancozeb for STOT-RE by the dermal or inhalation route is required.

However in repeated dose oral studies in rats, mice and dogs significant effects were observed at doses around or below the CLP guideline values for classification into STOT-RE 2; target organs appeared to be the liver, thyroid, nervous system and adrenals. Mice appeared to be less sensitive (Anonymous, 1985b; Anonymous, 1985c) than rats and dogs to the toxicity of mancozeb, with significant effects occurring at dose levels above the CLP guidance values for classification. Therefore, only the findings in rats and dogs are considered further in this section. It is also noted that Wistar rats (Szepvolgyi et al., 1989; Anonymous, 1999c) appeared to be less sensitive than Sprague-Dawley (SD) rats and that some rat studies were relatively clean because used low dose levels (Anonymous, 1989; Anonymous,, 1997c). It is further noted that in dogs, more significant effects tended to appear after 90 days of treatment (Anonymous, 1986c; Anonymous, 1987c) rather than 1 year (Anonymous, 1990c; Anonymous, 1991c and 1991d). No significant effects were seen in studies from the literature review at dose levels below the guidance values for classification.

When adverse effects are observed at or around the guidance values, it should be considered whether the effects are significant enough to warrant classification. According to the guidance on the application of the CLP criteria the following effects might be considered as significant and thus warrant classification for STOT-RE.

(a) *Morbidity or death resulting from repeated or long term exposure.*

(b) *Significant functional changes in the central or peripheral nervous system or other organ systems.*

(c) *Any consistent or adverse change in clinical biochemistry, haematology or urinalysis parameters.*

(d) *Significant organ damage noted at necropsy.*

(e) *Multifocal or diffuse necrosis, fibrosis or granular formation in organs with regenerative capacity.*

(f) *Morphological changes that are reversible but provide clear evidence marked organ dysfunction.*

(g) *Evidence of cell death in organs incapable of regeneration.*

Conversely the following effects may be observed, but are not considered to be supporting evidence for classification for STOT-RE.

(a) *Clinical observations or small changes in body weight gain, food consumption or water intake.*

(b) Small changes in clinical biochemistry, haematology or urinalysis parameters.

(c) *Changes in organ weights with no evidence of organ dysfunction.*

(d) *Adaptive responses that are not considered toxicologically relevant.*

(e) *Substance induced species specific mechanisms of toxicity.*

The findings of the oral studies in rats and dogs that are specific to each target organ are compared with these criteria below.

Liver

Liver hypertrophy of the centrilobular hepatocytes was observed in 2/10 males when 57 mg/kg bw/day of mancozeb (a dose below the guidance value of 100 mg/kg bw/d for classification with STOT-RE 2) were administered to SD rats for 90 days (Anonymous, 1986b). This effect is not severe enough to warrant classification. Clinical-chemistry findings, indicative of liver toxicity, were seen at 34 mg/kg bw/day (a dose below the guidance value of 100 mg/kg bw/d for classification with STOT-RE 2) in a 90-day study in dogs (Anonymous, 1986c). However, in the absence of liver histopathology, these effects are also considered insufficient for classification.

Therefore, classification of mancozeb with STOT-RE 2 for effects on the liver is not justified.

Thyroid

Thyroid follicular cell hyperplasia was observed in 9/10 males and 9/10 females at 57/74 (M/F) mg/kg bw/day (i.e. below the guidance value of 100 mg/kg bw/d for classification with STOT-RE 2) in a 90-day study in SD rats (Anonymous, 1986b). These effects were accompanied by changes in thyroid hormones. Thyroid follicular cell hyperplasia was also noted in a 90 day dog study (Anonymous, 1986c) in 6/6 males and 6/6 females at 101/108 (M/F) mg/kg bw/day, i.e. just above the guidance value of 100 mg/kg bw/day for classification. Changes in thyroid hormones were also seen at this dose. In another 90 day dog study (Anonymous, 1987c), thyroid follicular hyperplasia was observed in 2/4 males and 2/4 females at 34 mg/kg bw/day. This effect also occurred at the lower dose of 5.7 mg/kg bw/day where thyroid follicular hyperplasia was observed in 3/4 males and 2/4 females.

Therefore, significant thyroid toxicity occurred in rats and dogs at dose levels below the guidance values for classification with STOT-RE 2. Although there is evidence that rats are more sensitive than humans to perturbation of thyroid homeostasis, this evidence is less clear for the dog. In addition, it is noted that in a 6-month study in monkeys with ETU (Leber et al., 1978 – see RAR, section B6 for further details), thyroid toxicity was observed from the relatively low dose of 2.5 mg/kg bw/day.

Overall, therefore, it is concluded that mancozeb could induce thyroid toxicity in humans at dose levels relevant for classification. Hence, classification of mancozeb with STOT-RE 2 for effects on the thyroid is justified. This conclusion may appear in conflict with the conclusion that mancozeb-induced thyroid tumours in rats (and not mice) are unlikely to occur in humans and therefore that classification of mancozeb for carcinogenicity is not appropriate (see section 10.7). The dossier submitter considers that there is no inconsistency as although it is plausible that mancozeb would induce thyroid toxicity in humans at dose levels relevant for classification, it is highly unlikely that mancozeb would cause thyroid hyperplasia and tumours in humans since there is no clear evidence that hypothyroidism (goitre) in humans progresses to neoplasia and because whilst thyroid hypertrophy has been observed in humans, thyroid hyperplasia is rare.

Nervous system

In a 28 day oral study in SD rats (Anonymous, 1994b), 2/8 females displayed hind limb ataxia after administration of mancozeb at doses of 200 mg/kg bw/day (below the guidance value of 300 mg/kg bw/day for classification with STOT-RE 2). Hind limb paralysis was noted in 2/6 females at the same dose.

In addition, in a 90 day oral neurotoxicity study in SD rats (Anonymous, 1991e), myelin phagocytosis, Schwann cell proliferation, demyelinated nerves, myelin sheath thickening and myelin bubbles were observed in males and demyelination and myelin ovoids and debris were noted in females at 750 ppm (49/63 mg/kg bw/day; M/F) (below the guidance value of 100 mg/kg bw/day for classification with STOT-RE 2). Detailed incidences (information on severity not available in the study report) of these neurohistopathological findings seen at 750 ppm in this 90 day oral neurotoxicity study are shown in the table below. None of the findings were seen in control animals apart from myelin bubbles in the lumbar, dorsal root ganglion sections (indicated with * in the table) which were also observed in one control female.

Pathological findings	Males 750 ppm (Group VII) Incidence (no. animals affected)	Females 750 ppm (Group VIII) Incidence (no. animals affected)
Number animals examined	10	10
Cervical, dorsal root ganglion sections: Myelin bubbles Myelin phagocytosis Schwann cell proliferation	 1 1 1	 1
Lumbar, dorsal root sections: Myelin bubbles Myelin phagocytosis Schwann cell proliferation	 1 1 1	
Lumbar, dorsal root ganglion sections: Myelin bubbles* Myelin phagocytosis Schwann cell proliferation	 2 1 1	
Lumbar, ventral root sections: Myelin bubbles Myelin phagocytosis Schwann cell proliferation	 3 1 1	 1
Tibial nerve, lower: Myelin bubbles Myelin phagocytosis Schwann cell proliferation	 2 2	

Therefore, based on these findings, mancozeb should be classified for STOT-RE category 2 for effects on the nervous system.

Adrenals

Adrenal hypertrophy of the zona glomerulosa was observed in 6/10 males and 3/10 females when mancozeb was administered at doses of 57 and 74 mg/kg bw/day (below the guidance value of 100 mg/kg bw/day for classification with STOT-RE 2) to SD male and female rats respectively in a 90-day study (Anonymous, 1986b). However, these effects were not replicated in numerous other studies; therefore, classification of mancozeb with STOT-RE 2 for effects on the adrenals is not appropriate.

Other effects

In addition, deaths were seen at 200 mg/kg bw/day (below the guidance value of 300 mg/kg bw/day) in a 28-day study in SD rats (Anonymous, 1994b) and at 101/108 (M/F) mg/kg bw/day (just above the guidance value of 100 mg/kg bw/day) in a 90-day study in dogs (Anonymous, 1986c). In dogs, mortality was also accompanied by severe clinical signs of toxicity and anaemia.

Overall

Significant effects were seen with mancozeb at oral dose levels below the guidance values for classification with STOT-RE 2 in the thyroid (rats and dogs) and nervous system (rats). In addition, deaths in rats and dogs and severe clinical signs of toxicity in dogs were seen at oral doses below or marginally above the STOT-RE 2 guidance values. Therefore, classification of mancozeb with STOT-RE 2 (H373) for the oral route is justified. No adverse effects were noted when mancozeb was administered dermally to rats for 28 days or 90 days up to the limit dose (Anonymous, 1988a; Anonymous, 1999c; Anonymous 1997d). Effects on body weights, respiratory tract and thyroid were seen when mancozeb was given to rats via the inhalation route for 90 days (6 hr/day) at the top concentration of 0.3 mg/L total aerosol (i.e. at a concentration above the CLP guidance value of 0.2 mg/L 6h/day for classification) (Anonymous, 1986e). Therefore, no classification of mancozeb for STOT-RE by the dermal or inhalation route is required.

10.10.5 Conclusion on classification and labelling for STOT RE

STOT-RE 2; H373: May cause damage to organs (thyroid, nervous system) through prolonged exposure (oral route)

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS proposed classification with STOT RE 2 (thyroid, nervous system) limited to the oral route. This proposal was based on the observation of reduced T4 levels and thyroid hyperplasia in the dog and the rat and on evidence of neurotoxicity (hind limb ataxia, demyelination) found in some rat studies. Mortality in rats and dogs and clinical signs of severe toxicity in dogs were considered to provide further support for classification. The proposal to limit the classification to the oral route was based on the absence of effects below the Guidance Values (GVs) in rat dermal and inhalation studies. The DS's justification is described in more detail below.

Thyroid

Thyroid follicular cell hyperplasia accompanied by changes in thyroid hormones occurred below the GVs for classification in Category 2 in 90-day oral studies in the rat (Anon., 1986b) and the dog (Anon., 1986c; Anon., 1987c). These effects were attributed to inhibition of thyroid peroxidase (TPO) by ETU, a metabolite of mancozeb.

Although there is evidence that rats are more sensitive than humans to perturbation of thyroid homeostasis, this evidence was considered less clear for the dog. In addition, the DS noted that in a 6-month study in monkeys with ETU (Leber *et al.*, 1978) thyroid toxicity was observed from a relatively low dose. Therefore, the DS concluded that mancozeb could induce thyroid toxicity in humans at dose levels relevant for classification and proposed classification in Category 2 for thyroid effects.

Nervous system

In a 28-day oral study in rats (Anon., 1994b), hind limb ataxia or paralysis was observed in several females below the GV for STOT RE 2. In addition, a 90-day oral neurotoxicity study in rats (Anon., 1991e) reported myelin damage and Schwann cell proliferation at a dose below the GV for STOT RE 2. Based on these findings, the DS proposed classification in Category 2 for effects on the nervous system.

Liver

Liver hypertrophy in the rat (Anon., 1986b) and increased ALP in the dog (Anon., 1987c), both occurring below the GVs for STOT RE 2, were considered insufficient for classification; the former because of low severity of the effect and the latter due to lack of associated histopathological findings.

Adrenals

Adrenal hypertrophy of the zona glomerulosa was observed in one 90-day rat study (Anon., 1986b) below the GV for STOT RE 2. As this effect was not replicated in numerous other studies, no classification was proposed by the DS for effects on adrenals.

Other effects

Mortality was seen at 200 mg/kg bw/d (i.e., below the GV of 300 mg/kg bw/d) in a 28-day oral study in the rat (Anon., 1994b), and just above the guidance value of 100 mg/kg bw/d in a 90-day oral study in the dog (Anon., 1986c). In the dog, mortality was also accompanied by severe clinical signs of toxicity and anaemia. The DS considered these findings to provide further support for classification with STOT RE 2.

Specifying the exposure route

No adverse effects were noted when mancozeb was administered dermally to rats for 28 or 90 days up to the limit dose (Anon., 1988d; Anon., 1999d; Anon., 1997d). In a 90-day rat inhalation study (Anon., 1986d), thyroid effects were only observed at doses above the GV for classification. Therefore, the DS proposed to limit the STOT RE classification to the oral exposure route.

Comments received during public consultation

5 MSCAs, 1 industry association and 2 individuals commented on the dossier submitter's STOT RE classification proposal.

Two MSCAs supported STOT RE 2 (thyroid, nervous system). One of them explicitly expressed their support for stating the oral route.

The third MSCA supported STOT RE 2 for both thyroid and nervous system but proposed to check whether the thyroid findings in the 90-day dog study by Anon. (1987c) could trigger classification in Category 1. The DS responded that the follicular cell hyperplasia seen at 5.7 mg/kg bw/d in this study was not accompanied by changes in thyroid weight or in thyroid hormone levels. They added that in several other dog studies (including those of longer duration) thyroid effects started to appear only from higher dose levels (around

23-28 mg/kg bw/d). Therefore, the DS did not consider classification with STOT RE 1 warranted.

The fourth MSCA commented that the mancozeb-induced thyroid follicular cell hyperplasia, resulting from decreased T4 levels and a subsequent increase in TSH, might be considered as an adaptive response and a potential preneoplastic lesion, which should instead be discussed under the carcinogenicity endpoint. The DS replied that the proposed STOT RE classification was not intended to cover thyroid carcinogenicity.

The fifth MSCA supported classification with STOT RE 2 based on neurotoxicity and mortality. However, this MSCA did not support stating the thyroid as a target organ as they did not consider the thyroid effects occurring at doses below the GVs sufficient to trigger classification on their own. Additionally, this MSCA proposed to include the eye as a target organ based on increased incidence and severity of bilateral retinopathy seen from 6.7 mg/kg bw/d in females in the rat carcinogenicity study of Anon. (1990). The DS responded that the incidence of bilateral retinopathy at 6.7 mg/kg bw/d was only marginally increased in females without a concomitant increase in severity, was not increased in males at this dose, and was not reproduced in a second rat carcinogenicity study (Anon., 1992a). For these reasons, the DS did not consider classification for effects on the eye justified.

The industry association presented a case against a STOT RE classification. They argued that the effects on the thyroid observed in the animal studies did not lead to other long-term toxicological effects and were reversible where this was tested. The thyroid findings in the dog were considered mild and possibly confounded by malnourishment. The rat thyroid findings were not considered relevant to humans due to the differences in thyroid physiology between these two species. In addition, the commenter pointed out the kinetic differences in the metabolism of ETU between rodents and humans, with humans possessing a particularly high ETU metabolising capacity. As to neurotoxicity, the commenter emphasized that only a few animals per group were affected below the GVs in the rat studies and that neurotoxicity was only observed in some studies while others were negative in this regard. The histopathological findings in the neurotoxicity study of Anon. (1991e) were considered by the industry association of limited toxicological relevance as they were not accompanied by significant functional effects. It was also pointed out that ETU produced no evidence of neurotoxicity (summarised in the Mancozeb RAR, 2017) or developmental neurotoxicity in an extended one generation reproduction toxicity study in rats, with inclusion of the cohort for developmental neurotoxicity (Anon., 2013). Finally, the commenter mentioned negative epidemiology data for both the thyroid and nervous system.

The two individuals who confidentially commented during the public consultation argued against the proposed classification for thyroid effects using reasoning similar to those presented by the industry association. On the other hand, they did not express any clear preference regarding the neurotoxic effects and considered the case borderline between no classification and Category 2. In response to this, the DS repeated the arguments for classification presented in the CLH report, expressed their disagreement with the use of the very limited human data as an argument against classification, and regarding the lack of neurotoxicity with ETU they replied that the neurotoxicity of mancozeb might not be caused by this metabolite.

Additional key elements

6-month study with ETU in monkeys by Leber et al. (1978)

This study was conducted on request of US EPA to investigate human relevance of the rodent thyroid findings with ETU. As the study is not described in detail either in the CLH report or in the RAR, a summary of the most significant findings based on the full study report is provided below.

The study consisted of two experiments. Due to the high incidence of tuberculosis in the first experiment (phase I), the study was repeated with a new group of animals (phase II). Only the results of the second experiment (phase II) are reported here.

Summary of the study with ETU in rhesus monkeys (Leber *et al.*, 1978) – only phase II reported here

Type of study; Reference	**Method**	**Observations**
6-month dietary, rhesus monkey Leber *et al.* 1978	Non-guideline Non-GLP Doses: 0, 50, 150, 450 ppm; probably corresponding to approx. 0, 1.9, 6.3, 19 mg/kg bw/d [a] Positive control: propyl thiouracil (PTU) 125 ppm, raised to 250 ppm approx. in the middle of the study 5/sex/dose Examinations: haematology, clinical chemistry, serum hormones (T3, T4, TSH, FSH, GH, PRL), ^{125}I thyroid uptake, urinary excretion of ETU, organ weights, histopathology	450 ppm (≈ 19 mg/kg bw/d): • ↑ thyroid weight; ↑ pituitary weight (not stat. sign.) • Thyroid follicular hyperplasia (moderate to severe 10/10 vs none in controls) • Pituitary pars distalis cytoplasmic vacuolization/swelling (10/10 vs none in controls) • ↓ T4 (week 24 by approx. 50/70% m/f) • ↓ T3 (from week 20 by approx. 55/65% m/f) • ↑ TSH (from week 18 approx. 6-/8-fold m/f) • ↑ ^{125}I uptake 150 ppm (≈ 6.3 mg/kg bw/d): • ↑ thyroid weight • Thyroid follicular hyperplasia (mild to marked 5/10 vs none in controls) • Pituitary pars distalis cytoplasmic vacuolization/swelling (5/10 vs none in controls) • ↓ T4 (week 24 by approx. 60/50% m/f) • ↑ TSH (m from week 18 approx. 4-fold) • ↑ ^{125}I uptake 50 ppm (≈ 1.9 mg/kg bw/d): • ↑ thyroid weight (m) • Thyroid follicular hyperplasia (mild 3/10 vs none in controls)

		• ↑ ^{125}I uptake Positive control (125/250 ppm PTU): • Pituitary pars distalis cytoplasmic vacuolization/swelling (3/10 vs none in negative controls) • ↑ ^{125}I uptake

[a] Neither equivalent doses in mg/kg bw/d nor data on food consumption are provided in the study report. The equivalent doses have been calculated by RAC using mean body weights (week 0 to 27) on the assumption that the whole daily ration (200 g) was consumed by the animal. For comparison, the CLH report (p. 154) states that the equivalent doses were 0, 2.5, 7.5 and 22.5 mg/kg bw/d.

A summary of the thyroid-related findings at the end of the study (week 26) in a tabular form is provided below.

Thyroid-related findings at the end of the 6-month monkey study with ETU by Leber *et al.* (1978)

	ETU				**PTU (positive control)**
Dose (ppm)	**0**	**50**	**150**	**450**	**125/250**
Dose (mg/kg bw/d)	**0**	**≈ 1.9**	**≈ 6.3**	**≈ 19**	
Males					
No. examined	5	5	5	5	5
TSH (ng/mL)	0.42	0.93	2.19*	3.28*	0.48
T3 (ng %)	231	199	185	119*	198
T4 (µg %) (wk 24)[b]	5.80	5.62	2.26*	2.82*	4.28
Thyroid weight abs. (g)	1.11	1.69	2.68*	3.45*	1.21
Thyroid follicular hyperplasia, mild to moderate	0	2	2	3	0
Thyroid follicular hyperplasia, marked to severe	0	0	1	2	0
Females					
No. examined	5	5	5	5	5
TSH (ng/mL)	0.41	0.61	0.82	3.95*	0.53
T3 (ng %)	195	237	209	92.4*	220
T4 (µg %) (wk 24)[b]	6.48	7.00	3.40*	1.78*	5.04
Thyroid weight abs.	0.71	1.57*	1.95*	3.69*	0.83

Thyroid follicular hyperplasia, mild to moderate	0	1	2	1	0
Thyroid follicular hyperplasia, marked to severe	0	0	0	4	0

* statistically significantly different from control, $p < 0.05$

[b] T4 levels at week 24 are presented instead of those at week 26 because control T4 levels were markedly higher (by approx. 40%) on week 26 compared to the preceding weeks

Epidemiology study by Medda et al. (2017)

This recent epidemiology study investigated a possible association between exposure to mancozeb and thyroid effects in Italian grapevine workers. Additionally, the study attempted to verify whether iodine uptake may modulate the risk of thyroid disruption by the mancozeb metabolite ETU (results not shown here).

The study population consisted of 177 male workers and 74 male controls. The workers were recruited randomly from vineyards where mancozeb was systematically used and all had ≥ 1 year history of direct exposure to mancozeb. The controls were recruited from healthcare personnel. None of the recruited subjects were known to be affected by thyroid diseases or were taking drugs interfering with thyroid function at the recruitment.

The workers had slightly (by 6%) but statistically significantly lower T4 levels compared to controls. The values of the parameters investigated and their statistical evaluation (multivariate comparisons by separate regression models, linear or logistic as appropriate) are provided in the table below. Potential confounders considered included age at enrolment, wine consumption, tobacco smoking and history of thyroid disorders in first degree relatives.

Results of the epidemiology study by Medda *et al.* (2017)			
	Workers	**Controls**	**β Coeff., p-value**
Urinary iodine concentration (µg/L) Geometric mean	114.8	97.3	0.18, 0.11
Urinary ETU concentration (µg/L) Geometric mean	12.2	8.2	0.35, **0.03**
Thyroid volume (mL) Geometric mean	14.3	12.0	n/a
Frequency of subjects with thyroid nodules	15.3%	20.6%	-0.73, 0.06
Frequency of subjects with a solitary thyroid nodule	11.3%	10.8%	-0.32, 0.50
Frequency of subjects with thyroid hypoechoic pattern	10.2%	9.6%	n/a
T3 (ng/mL) mean	1.9	1.9	-0.01, 0.77
T4 (ng/mL) mean	88.8	94.2	-5.41, **0.03**
FT3 (pg/mL) mean	3.8	3.6	0.20, **0.008**

FT4 (pg/mL) mean	12.8	12.0	0.92, **0.001**
TSH (µUI/mL) mean	1.6	1.6	0.01, 0.84
Tg (ng/mL) mean	14.6	11.7	0.05, 0.73
Frequency of subjects positive for TgAb and/or TPOAb	10.2%	13.5%	-0.25, 0.57

Assessment and comparison with the classification criteria

Repeated dose toxicity of mancozeb was investigated in rats, mice and dogs. Most of the studies were oral studies but for the rat several dermal and inhalation studies have also been provided. Further, rabbit developmental studies have been included for completeness.

The effects potentially relevant for classification are as follows:

- Effects on the thyroid
- Neurotoxicity
- Effects on the liver
- Effects on the adrenals
- Effects on the eyes
- Mortality

Thyroid

Reduced T4 levels, increased TSH, increased thyroid weight and follicular cell hyperplasia were observed in the rat at doses below the GVs for classification in Category 2. The most comprehensive investigation into the thyroid effects was conducted in a 90-day dietary study in the rat by Anon. (1986b). The values of thyroid-related parameters at the three highest dose levels and in the control are summarised in the table below. At the top dose of 57/75 mg/kg bw/d (m/f), T4 levels were reduced by ≈ 40%, TSH levels increased approx. 3-fold, thyroid weight increased by approx. 30% and follicular cell hyperplasia was present at a high incidence. At the next lower dose of 15/18 mg/kg bw/d T4 was lower in females by 28%.

Thyroid-related parameters in the rat study Anon. (1986b)					
Dose (ppm)		**0**	**125**	**250**	**1000**
Dose (mg/kg bw/d) m/f		0	7.4/9.2	15/18	57/75
T4 (µg/dl)	m	5.3	5.7	5.3	3.5*
	f	3.8	3.2	2.7*	2.2*
TSH (ng/ml)	m	1.2	1.6	1.9	4.3*
	f	0.5	0.4	1.0	1.3*
Thyroid weight (mg)	m	25	23	27	33*
	f	19	18	18	24
Follicular cell hyperplasia (n=10)	m	0	0	0	9*
	f	0	0	0	9*

* statistically significantly different from control, $p < 0.05$

Although many other repeated dose studies in the rat are available, few of them investigated thyroid hormone levels (Anon., 1989 – 90-day oral; Anon., 1986d – 90-day inhalation; Anon., 1988d – 28-day dermal) and their results do not contradict those of Anon. (1986b). Therefore, the study of Anon. (1986b) is considered as the key rat study regarding classification for thyroid effects. Further, a non-guideline 4-day gavage study in female weanlings by Flippin *et al.* (2009) is available, indicating a LOEL for T4 reduction of approx. 30 mg/kg bw/d and an ED_{50} for T4 reduction at 259 mg/kg bw/d.

The observed pattern of effects indicates perturbation of the hypothalamic-pituitary-thyroid (HPT) axis. Reduced thyroid hormone (TH) levels, when detected by the hypothalamus and the anterior pituitary, result in increased TSH production and thyroid stimulation in order to return the thyroid hormone levels to normal. If the TSH elevation is persistent and the thyroid is not able to keep up with the demand, the follicular cells undergo hypertrophy and cell division, leading to hyperplasia.

Although not all possible mechanisms have been investigated, it is plausible that the main initiating event in the adverse outcome pathway is inhibition of thyroid peroxidase (TPO), a key enzyme in the production of thyroid hormones, by ETU, a metabolite of mancozeb. Inhibition of pig and rat TPO by ETU was demonstrated *in vitro* (Doerge and Takazawa, 1990; Freyberger and Ahr, 2006; Paul *et al.*, 2014). No induction of liver T4-UDP glucuronosyltransferase by mancozeb in the rat was observed by Flippin *et al.* (2009).

Out of the individual thyroid-related findings, only a reduction in thyroid hormone levels is considered by RAC to be an adverse effect for the purpose of STOT RE classification. Thyroid follicular cell hyperplasia or hypertrophy are adaptive, potentially reversible effects with no residual adverse consequences on cessation of exposure except for possible development of neoplasia, which is addressed under the carcinogenicity hazard class.

Thyroid effects were also observed in the mouse and in the dog. The investigations in the mouse were limited (THs were not measured) and thyroid hypertrophy and hyperplasia were observed only above the GVs for classification. The thyroid-related findings in the dog studies are summarised in the following table.

Thyroid-related and selected other findings in the dog repeat dose toxicity studies

Type of study; Reference	**Method**	**Observations**	**GV for STOT RE 2**
90-day dietary Anon. 1986c	OECD 409 GLP Doses: 0, 10, 100, 1000, 5000 ppm; corresponding to 0, 0.29/0.32, 3.0/3.4, 29, 102/109 mg/kg bw/d (m/f) Beagle 6/sex/dose	102/109 mg/kg bw/d: • Mortality 3/12 (killed *in extremis* due to poor condition) • Markedly reduced food consumption (by ≈ 40%), body weight loss • ↓ T4 (by ≈ 90/80% m/f week 5) • ↓ T3 (by ≈ 60/40% m/f week 5) • ↑ thyroid weight (absolute ≈ 2-fold)	100 mg/kg bw/d

		• Thyroid follicular cell hyperplasia (in all animals) ≤ 29 mg/kg bw/d: no thyroid-related findings	
90-day capsule Anon. 1987c	OECD 409 GLP Doses: 0, 5.7, 34, 340/204 mg/kg bw/d; high dose reduced from day 17 Beagle 4/sex/dose + 2/sex/dose (high dose and control) for recovery (6 weeks)	340/204 mg/kg bw/d: • Reduced food consumption and body weight gain; clinical signs of toxicity • ↓ T4 (f by ≈ 50% week 12) • ↓ T3 (f by ≈ 30% week 12) • ↑ thyroid weight (absolute ≈ 2-fold) • Thyroid follicular cell hyperplasia (m 4/4, f 3/4) 34 mg/kg bw/d: • ↓ T4 (f by 40% week 6) • Thyroid follicular cell hyperplasia (m 2/4, f 2/4) 5.7 mg/kg bw/d: • Thyroid follicular cell hyperplasia (m 3/4, f 2/4)	100 mg/kg bw/d
1-year dietary Anon. 1990c	OECD 452 GLP Doses: 0, 50, 200, 800, 1600 ppm; corresponding to 0, 1.8/1.9, 28/29, 53/60 mg/kg bw/d Beagle 4/sex/dose	53/60 mg/kg bw/d: • 2/4 males killed *in extremis* (one was found to have acute urogenital tract lesion and the other a chronic regenerative anaemia) • ↓ T4 (by ≈ 20-30%, not stat. sign.) • ↑ thyroid weight (absolute 1.5/1.7-fold m/f) • Thyroid follicular distension (m 2/4, f 4/4) ≤ 28/29 mg/kg bw/d: no thyroid-related findings	25 mg/kg bw/d
1-year capsule Anon. 1991c	EPA 83-1, consistent with OECD 452 GLP Doses: 0, 2.3, 23, 113 mg/kg bw/d Beagle 4/sex/dose	113 mg/kg bw/d: • The physical condition deteriorated, particularly in females, and the group was terminated in week 26 • Clinical signs: underactivity, pallor • ↓ T4 (by ≈ 40% week 24) 23 mg/kg bw/d: • ↓ T4 (m by 26% week 50)	25 mg/kg bw/d

		• ↑ thyroid weight (m absolute 1.4-fold, not stat. sign.) 2.3 mg/kg bw/d: no effects	
1-year capsule Anon. 1991d	EPA 83-1, consistent with OECD 452 but only 2 groups GLP Doses: 0, 40 mg/kg bw/d Beagle 4/sex/dose	40 mg/kg bw/d: • ↓ T4 (by ≈ 40% week 50; no significant effect on week 24) • ↓ T3 (f by 14% week 50) • ↑ thyroid weight (absolute 1.2-fold)	25 mg/kg bw/d

Slight reductions in T4 levels accompanied by thyroid hyperplasia and/or hypertrophy were observed in two dog studies (Anon., 1987c; Anon., 1991c) at doses below the GVs for classification in Category 2. In addition, marked reductions in T4 and T3 were observed in the 90-day dietary study (Anon., 1986c) slightly above 100 mg/kg bw/d. However, as this dose was associated with pronounced general toxicity, part of the reduction in thyroid hormone levels might have been an adaptive response to chronic stress. Still, the thyroid-related effects in the dog are considered to support classification in Category 2.

RAC agrees with the DS that the increased incidence of follicular cell hyperplasia at 5.7 mg/kg bw/d in the 90-day capsule study in dogs (Anon., 1987c) does not warrant classification in Category 1 as this effect was not seen in other studies at higher dose levels (Anon., 1986c; Anon., 1990c).

RAC notes the differences in physiology of the HPT axis between rodents and humans. The rat thyroid is much more active and the turnover of thyroid hormones (especially T4) is higher in rats than in humans. This is thought to be related to a lower TH reserve in the rats compared to humans, who, unlike rats, also possess a high-affinity thyroxine-binding globulin in addition to albumin and transthyretin. Consequently, if TH synthesis is disrupted, rats deplete their hormone stores much more rapidly than humans do. The activity of the dog thyroid gland is intermediate between that of humans and rodents.

On the other hand, this does not mean that impairment of thyroid hormone synthesis cannot have adverse consequences in humans. In fact, two drugs for treatment of hyperthyroidism in humans, methimazole and propylthiouracil, exert their effects by inhibiting thyroid hormone synthesis. Then the question is whether the effect would occur below the guidance values for classification in humans.

The HTP axis physiology of monkey is comparable to that of humans (monkeys also have a thyroxine-binding globulin). In a 6-month study in rhesus monkeys (Leber *et al.*, 1978; see also the background document, section 'additional key elements'), ETU caused an increase in TSH levels after 3 months, followed by a decrease in T4 levels after an additional 2 months. The top dose of approx. 20 mg/kg bw/d caused a more pronounced effect than the mid dose of approx. 6 mg/kg bw/d, but the T4 reduction at 6 mg/kg bw/d was already severe (T4 was reduced from week 24 by approx. 50% in both sexes). The time-course of the effects in monkeys indicates that the presence of TBG stabilizes the thyroid hormone levels and makes them react only to prolonged disruption of synthesis, and with some delay compared to rats. In the rat, T4 reduction by ETU is already observed after 1 month (data on shorter exposure durations were not available for ETU; the study with mancozeb

by Flippin *et al.*, 2009, in rat weanlings reported an effect on T4 levels already after 4 days of exposure). The rat LOAELs vary somewhat between studies with the lowest LOAEL being approx. 2 mg/kg bw/d (Anon., 2013) and a corresponding T4 reduction by approx. 30%. This indicates that the threshold for T4 reduction by ETU is comparable between the monkey and the rat and the only substantial difference is in the exposure duration necessary to induce the effect.

The marked increases in TSH in the high dose group of the monkey study are not consistent with 'euthyroid sick syndrome'.

RAC also notes that a reduction in T4 levels was reported (Smith, 1984) in a small group of workers exposed to ETU levels that were relatively high, but still unlikely to exceed the effect level in rats (for details please refer to the background document, section 'Supplemental information').

An additional factor to consider is the interspecies difference in the rate of ETU metabolism. *In vitro* studies with liver S9 or primary hepatocytes (Saghir *et al.*, 2005; Zhu, 2015) indicate that ETU might be more readily eliminated in humans than in rats while metabolism in the dog appeared similar to humans. (For more information on these two studies see the background document, section 'Supplemental information'.) However, translation of the differences observed *in vitro* into quantitative relationships *in vivo* is not straightforward. In addition, the differences in ETU metabolism are already accounted for in the study in monkeys (Leber *et al.*, 1978) that showed an effect level comparable to that in rats. Finally, effects below GVs were also seen in the dog, which showed similar rate of ETU metabolism in primary hepatocytes to those from humans (Zhu, 2015). Therefore, RAC is of the opinion that the available information on interspecies differences in ETU metabolism has no impact on the classification of mancozeb for STOT RE.

RAC concluded that classification of mancozeb in Category 2 for effects on the thyroid is justified. This classification is based on reduced T4 levels in the dog and rat studies with mancozeb. Qualitative and quantitative human relevance of these findings is inferred from the proposed mode of action (TPO inhibition leading to disruption of TH synthesis) and from a comparison of LOAELs for T4 reduction by ETU in the rat and the monkey.

Neurotoxicity

The table below summarises findings related to neurotoxicity in the rat oral and inhalation studies including several studies which were negative with respect to neurotoxicity (however, negative studies with relatively low top doses have been omitted from the table). No specific signs of neurotoxicity were seen in the other three species tested (mouse, dog, rabbit) nor the in the rat dermal studies.

RAC notes that functional tests were probably not conducted in any of the available studies with the exception of the developmental neurotoxicity study by Anon. (2008c), which was negative. However, the top dose in this study was rather low (30 mg/kg bw/d).

Observations related to neurotoxicity in rat oral and inhalation repeated dose studies	
Type of study; Reference	**Observations related to neurotoxicity**
28-day gavage Anon. 1994b	500 mg/kg bw/d: 1/6 females hind limb paralysis and killed for humane reasons on day 21; 2/6 females **hind limb paralysis** on day 28 200 mg/kg bw/d: 1/8 females ataxia and killed for humane reasons on day 13; 2/8 females **ataxia** 50 mg/kg bw/d: no effects
12-week dietary (males only) Szépvölgyi *et al.* 1989	379 mg/kg bw/d: mortality 4/12 week 1-6; clinical signs: prostration, weakness and **posterior distal paralysis** before death of the 4 animals; signs were transient in survivors and absent at 12 weeks 253 mg/kg bw/d: no neurotoxicity-related findings
90-day gavage Anon. 1999c	400 mg/kg bw/d: mortality 2/24; no neurotoxicity-related findings
90-day neurotoxicity, dietary Anon. 1991e	339/413 mg/kg bw/d (m/f): • Mortality: 1/10 males and 4/10 females weeks 2-4; treatment of females discontinued from day 15; large reductions in food consumption and bw loss in initial females during the first 2 weeks • Clinical signs: abnormal gait and/or **limited or no use of hind limbs** (all animals, from week 2-3, some improvement by day 60), general weakness • Histopathology: **demyelination and Schwann cell proliferation** in males and females; posterior thigh muscle atrophy (10/16 females) 50/63 mg/kg bw/d (m/f): • No clinical signs of toxicity • Histopathology: **demyelination, Schwann cell proliferation** (incidences provided in a separate table below) 8.2/10.5 mg/kg bw/d (m/f): no effects
90-day dietary Anon. 1986b	57/75 mg/kg bw/d: no neurotoxicity-related findings
Prenatal developmental toxicity, gavage, dosing GD 6-15 Anon. 1980	512 mg/kg bw/d: 1/22 died, 2/22 killed after abortion; clinical signs: lethargy, ataxia, scruffy coat, diarrhoea or soft faeces, hunched, dehydrated
Prenatal developmental toxicity, gavage, dosing GD 6-15 Anon. 1988c	360 mg/kg bw/d: 1/25 killed *in extremis*, preceded by clinical sings (marked body weight loss, **hind limb paralysis**); further 4/25 **slight, transient hind limb paralysis** at the end of the dosing period

2-week gavage (females only) Anon. 2015b	300 mg/kg bw/d: no clinical signs of toxicity
Prenatal developmental toxicity, gavage, dosing GD 6-19 Anon. 2015d	160 mg/kg bw/d: no clinical signs of toxicity
Developmental neurotoxicity, gavage, dosing GD 7 – PND 16 Axelstad *et al.* 2011	Range-finding study: 500, 350 and 200 mg/kg bw/d: severe weight loss and **hind limb paralysis** in all groups by GD 12 (severity was dose-dependent), most dams sacrificed on GD 14 Main study: 150 mg/kg bw/d: severe weight loss, **mild hind limb paralysis**
Prenatal developmental toxicity, inhalation, GD 6-15 Lu and Kennedy 1986	0.89 mg/L: mortality 30/37; **hind limb weakness** 11 animals (mild to moderate) 0.11 mg/L: mortality 3/37; **hind limb weakness** 24/37 (mild; onset after 7 exposures, persisted for 3 days after the last exposure) 0.055 mg/L: **hind limb weakness** 6/27 (mild)
90-day inhalation Anon. 1986d	0.33 mg/L: no clinical signs of toxicity

The following table shows incidences of the histopathological findings at the mid-dose in the 90-day dietary neurotoxicity study Anon. (1991e).

Incidences of histopathological findings in the 90-day neurotoxicity study Anon. (1991e) at 750 ppm (50/63 mg/kg bw/d)		
	Males	Females
Number of animals examined	10	10
Cervical, dorsal root ganglion sections:		
Myelin bubbles	1	1
Myelin phagocytosis	1	
Schwann cell proliferation	1	
Lumbar, dorsal root sections:		
Myelin bubbles	1	
Myelin phagocytosis	1	
Schwann cell proliferation	1	
Lumbar, dorsal root ganglion sections:		
Myelin bubbles*	2	
Myelin phagocytosis	1	
Schwann cell proliferation	1	
Lumbar, ventral root sections:		

Myelin bubbles	3	1
Myelin phagocytosis	1	
Schwann cell proliferation	1	
Tibial nerve, lower:		
Myelin bubbles	2	
Myelin phagocytosis	2	
Schwann cell proliferation		

* observed also in 1 control female

Clinical signs of neurotoxicity (hind limb weakness or paralysis, ataxia) were observed in several rat studies, in the oral studies mostly above 200 mg/kg bw/d, starting within the first few weeks of treatment. Demyelination and Schwann cell proliferation were identified as the histopathological correlate in the 90-day neurotoxicity study (Anon., 1991e) and appeared at a low incidence without clinical signs also at 50 mg/kg bw/d, which was below the GV for Category 2.

RAC considers the histopathological findings at the mid-dose in the 90-day neurotoxicity study sufficient for classification in Category 2, noting that similar damage may have occurred also in other rat studies without being detected due to lack of specific investigations (e.g., nerve fibre teasing). The clinical signs of neurotoxicity, although appearing mostly above the GVs, are considered to provide additional support for classification. Moreover, manganese, contained in mancozeb at a relatively high percentage, is an established neurotoxicant, and mancozeb itself has been shown to affect various neuronal cell populations *in vitro* (Domico *et al.*, 2006), which further strengthens the case for classification.

ETU is most likely not the metabolite causing the neurotoxic effects, since ETU produced no evidence of neurotoxicity or developmental neurotoxicity in an extended one-generation reproduction toxicity study in rats, which included the cohort for developmental neurotoxicity (Anon., 2013).

Although neurotoxicity was only observed in the rat and not in the other species (the mouse and the dog), there is no information disproving human relevance of the rat findings. RAC notes that the mode of action of mancozeb-induced neurotoxicity is largely unknown.

Therefore, RAC agrees with the DS that classification of mancozeb with STOT RE 2 for effects on the nervous system is justified.

The consideration of other organs by RAC did not lead to proposed STOT RE classification and is discussed in the Background Document.

In agreement with the DS, findings reported in other organs (liver, adrenals, eyes) and the mortality (spontaneous or sacrifice in extremis) observed in the dog, rat and rabbit studies are not considered sufficiently adverse by RAC to warrant classification (see background document under 'supplemental information').

Route of exposure

According to the CLP regulation, the exposure route should be stated if it is conclusively proven that no other routes of exposure cause the hazard. As thyroid effects in the dog contributed to classification and no dog studies via the dermal and inhalation routes are

available, effects warranting classification via those routes cannot be excluded in this species. In addition, neurotoxicity below the GVs was seen not only in oral studies but also after inhalation exposure in the rat (Lu and Kennedy, 1986). Hence, the conditions for specifying the exposure route are not fulfilled.

Overall conclusion on classification

RAC agrees with the DS that classification of mancozeb with **STOT RE 2; H373 (thyroid; nervous system)** is justified. This classification is based on reduced T4 levels in the dog and the rat and on neurotoxic findings in the rat. Mortality in the rat, the dog and the rabbit provides additional support for classification but in this case is not sufficient to trigger classification on its own. Contrary to the dossier submitter's proposal, RAC does not consider it appropriate to specify the exposure route.

Supplemental information - In depth analyses by RAC

Liver

Modest increases in liver weight were observed below the GVs for classification in a few studies (Szépvölgyi *et al.*, 1989; Anon., 1986b; Anon., 1985b; Anon., 1991c; Anon., 1988b); in one rat study (Anon., 1986b) this was associated with a slightly increased incidence of centrilobular hypertrophy. One dog study (Anon., 1987c) reported increased ALP activity (1.4-fold) without any concomitant liver findings. Overall, these findings are not considered sufficiently adverse to warrant classification.

Adrenals

Adrenal hypertrophy of the zona glomerulosa was observed at 57/75 mg/kg bw/d (m/f) in a rat 90-day study (Anon., 1986b; incidences 6/10 in males, 3/10 in females vs. 1/10 in the controls of both sexes). RAC agrees with the DS that as this effect was not replicated below the GVs for classification in other studies (e.g., Anon., 1999c; Anon., 1997c), classification of mancozeb with STOT RE 2 for effects on the adrenals is not appropriate.

Eyes

Increased incidence and severity of bilateral retinopathy was reported at 31/40 mg/kg bw/d (m/f) in both sexes in a 2-year combined chronic toxicity and carcinogenicity study (Anon., 1990a). A marginal increase was also observed in females at 6.7 mg/kg bw/d. Incidences are provided in the table below.

Incidence of bilateral retinopathy in the 2-year rat study Anon. (1990a)

	Males					**Females**				
Dose (mg/kg bw/d)	**0**	**0.77**	**2.3**	**4.8**	**31**	**0**	**1.1**	**3.1**	**6.7**	**40**
No. examined	60	62	61	58	61	62	60	62	61	61
Bilateral retinopathy	4	2	1	3	19*	21	28	24	31*	49*

* statistically significant difference from the control group, $p < 0.05$ (Fisher's test)

The indicative GV for classification in Category 2 for a 2-year study is 12.5 mg/kg bw/d. As the increase in incidence at 6.7 mg/kg bw/d was only marginal, without a concomitant increase in severity (according to the DS's response to a comment raised in the public consultation), limited to one sex, and the effect was not reproduced in another 2-year rat study (Anon., 1992a) up to doses of 17/21 mg/kg bw/d (m/f), RAC agrees with the DS that classification with STOT RE for effects on the eye is not justified.

Mortality

The following table summarises findings related to mortality in the studies available, including negative findings (with the exception of oral rat studies with a top dose below 100 mg/kg bw/d and dermal studies; there was no mortality or severe toxicity in these).

Observations related to mortality in oral repeated dose studies	
Type of study; Reference	**Observations related to mortality**
Rat	
28-day gavage Anon. 1994b	500 mg/kg bw/d: • 1/6 females hind limb paralysis and **killed** for humane reasons on day 21 • 2/6 females hind limb paralysis on day 28 200 mg/kg bw/d: • 1/8 females ataxia and **killed** for humane reasons on day 13 • 2/8 females ataxia 50 mg/kg bw/d: no effects
12-week dietary (males only) Szépvölgyi 1989	379 mg/kg bw/d: 4/12 **died** week 1-6; clinical signs: prostration, weakness, paralysis 253 mg/kg bw/d: no mortality
90-day gavage Anon. 1999c	400 mg/kg bw/d: **mortality** 1/24 males (day 14), 1/24 females (day 86) 160 mg/kg bw/d: no marked toxicity
90-day neurotoxicity, dietary Anon. 1991e	339/413 mg/kg bw/d (m/f): • **Mortality**: 1/10 males and 4/10 females weeks 2-4; treatment of females discontinued from day 15 • Clinical signs: abnormal gait and/or limited or no use of hind limbs, general weakness • Large reductions in food consumption and bw loss during the first 2 weeks 50/63 mg/kg bw/d (m/f): no mortality or severe toxicity
Prenatal developmental toxicity, gavage, dosing GD 6-15 Anon. 1980	512 mg/kg bw/d: 1/22 **died** (GD 18) and two other dams were terminated after signs of abortion; markedly reduced food consumption, bw loss and clinical signs of toxicity in most of the animals 128 mg/kg bw/d: no mortality or severe toxicity

Prenatal developmental toxicity, gavage, dosing GD 6-15 Anon. 1988c	360 mg/kg bw/d: 1/25 treatment-related death (**killed** *in extremis*); preceded by clinical sings (marked body weight loss, hind limb paralysis) 60 mg/kg bw/d: no effects
2-week gavage (females only) Anon. 2015b	300 mg/kg bw/d: no mortality or severe toxicity
Prenatal developmental toxicity, gavage, dosing GD 6-19 Anon. 2015d	160 mg/kg bw/d: no mortality or severe toxicity
Developmental neurotoxicity, gavage, dosing GD 7 – PND 16 Axelstad *et al.* 2011	Range-finding study: 500, 350 and 200 mg/kg bw/d: severe weight loss and hind limb paralysis, most dams **sacrificed** on GD 14 Main study: 150 mg/kg bw/d: severe weight loss, mild hind limb paralysis
Prenatal developmental toxicity, inhalation, GD 6-15 Lu and Kennedy 1986	0.89 mg/l (only 4-5 exposures): **mortality** 30/37 (average time for onset of mortality: GD 10); physical obstruction of the airways might have contributed to the mortality 0.11 mg/L: **mortality** 3/37 (GD 15-16) 0.055 mg/L: no mortality
90-day inhalation Anon. 1986d	0.33 mg/L: no mortality or severe toxicity
Mouse	
28-day dietary Anon. 1985b	2000 mg/kg bw/d: no mortality or severe toxicity
90-day dietary Anon. 1985c	1660/2160 mg/kg bw/d (m/f): no mortality or severe toxicity
Dog	
90-day dietary Anon. 1986c	102/109 mg/kg bw/d (m/f): • 2/6 males and 1/6 females **sacrificed** during weeks 8-10 due to poor condition • Markedly reduced food consumption (by ≈ 41%), body weight loss instead of gain (terminal bw lower by ≈ 22% compared to controls) • Clinical signs: few or no faeces, dehydration, thinness, pale mucous membranes 27/28 mg/kg bw/d (m/f): reduced weight gain or lack thereof, reduced food consumption

90-day capsule Anon. 1987c	340/204 mg/kg bw/d (dose reduced from day 17): • No mortality • Clinical signs: emesis, yellow faeces, pale mucous membranes, emaciation, subdued behaviour, isolated occurrence of convulsion or collapse • Reduced food consumption (by ≈ 23%) and body weight gain (terminal bw lower by ≈ 17% compared to controls) 34 mg/kg bw/d: no severe toxicity
1-year dietary Anon. 1990c	53/60 mg/kg bw/d (m/f): 2/4 males **killed** *in extremis* weeks 10 and 11. One was found to have acute urogenital tract lesion and the other a chronic regenerative anaemia. No treatment-related clinical signs. 28/29 mg/kg bw/d (m/f): no severe toxicity
1-year capsule Anon. 1991c	113 mg/kg bw/d: • 1/4 males **died** on week 13 with marked anaemia. The rest of the group **killed** week 26 on humane grounds. • Clinical signs: underactivity, thin and pale, emesis, yellow/green faeces 23 mg/kg bw/d: no severe toxicity
1-year capsule Anon. 1991d	40 mg/kg bw/d (single dose level): 2/4 females thin, 1/4 females hypothermic and of pale appearance from week 21
Rabbit	
Prenatal developmental toxicity, gavage, dosing GD 7-19 Anon. 1987b	80 mg/kg bw/d: • 2/20 **sacrificed** in a moribund condition due to weight loss • Other effects: abortions, alopecia, anorexia, ataxia, scant faeces 30 mg/kg bw/d: no effects
Prenatal developmental toxicity, gavage, dosing GD 6-18 Anon. 1991b	100 mg/kg bw/d: no mortality; reduced body weight gain

<u>Rat</u>

Mortality starting from approximately 300 mg/kg bw/d was seen in some oral rat studies, often in association with clinical signs of neurotoxicity (hind limb paralysis), and also in one inhalation study from approx. 0.1 mg/L (Lu and Kennedy, 1986). The spontaneous deaths or sacrifice *in extremis* (often due to neurotoxicity) usually occurred within the first few weeks of administration and was limited to a few animals per group while the rest of the animals, although showing signs of toxicity, survived until the end of the study without further deterioration of the general condition. This temporal pattern does not reflect Haber's rule, which assumes inverse proportionality between exposure duration and the threshold for the toxic effect. Thus, the default GVs for the 90-day study are more appropriate in this case and as a result, most of the mortalities are deemed to occur above the GVs for classification. Mortality in rats is considered to provide additional support to

classification in Category 2 triggered by other effects (thyroid toxicity, neurotoxicity) but is not sufficient to trigger classification on its own.

Mouse

No mortality or severe toxicity was seen in a 90-day oral study up to a dose level of approx. 2000 mg/kg bw/d.

Dog

Markedly reduced food consumption and body weight loss was apparent in a 90-day dietary study (Anon., 1986c) at 102/109 mg/kg bw/d (m/f). As this was a dietary study, some impact of reduced palatability is possible. 3 out of 12 animals were killed due to their deteriorated condition during weeks 8 to 10. Other effects at this dose included reduced erythrocyte count, reduced haemoglobin (by approx. 20%) and reduced thyroid hormone levels (T4 by approx. 70%). On the other hand, no mortality was seen at a 2-fold higher dose (approx. 200 mg/kg bw/d) in a 90-day study performed by another laboratory (Anon., 1987c), where mancozeb was administered via gavage in capsules.

Two out of four males were killed *in extremis* in weeks 10 and 11 at 53 mg/kg bw/d in a 1-year dietary study (Anon., 1990c). One animal was found to have an acute urogenital tract lesion and the other dog presented a chronic regenerative anaemia. According to the DS, the urogenital tract lesion was probably an incidental event but the possibility that the anaemia was an idiosyncratic treatment-related reaction could not be excluded. All other animals survived and there were no clinical signs.

In another 1-year oral study (Anon., 1991c), 1 out of 4 top dose (113 mg/kg bw/d) males died in week 13 with marked anaemia. Animals of this dose group showed underactivity, pallor, inappetence and weight loss. This group was terminated in week 26 for ethical reasons.

Mortality was observed in the dog in several repeated dose studies, but mostly above the GVs for classification. Similarly to the rat, mortality in the dogs is considered to provide additional support for classification in Category 2 triggered by other effects but is not sufficient to trigger classification on its own.

Rabbit

2 out of 20 dams were sacrificed moribund at 80 mg/kg bw/d in a prenatal developmental toxicity study (Anon., 1987b). On the other hand, no mortality or clinical signs of toxicity were observed in another study at a comparable dose of 100 mg/kg bw/d (Anon., 1991b). Due to this inconsistency, mortality observed in the rabbit is not considered sufficient to trigger classification.

Conclusion

The mortality (spontaneous or sacrifice *in extremis*) observed in the dog, rat and rabbit studies is not considered sufficient to trigger classification on its own but provides additional support for classification in Category 2 triggered by effects on the thyroid and nervous system.

In vitro comparative metabolic studies with ETU

Two *in vitro* studies were conducted to investigate the interspecies differences the rate of ETU metabolism between various animal species and humans. Liver-based systems were used as the liver was claimed to be the main site of metabolism of ETU.

Saghir *et al.* (2005) used liver S-9 fraction from rats (non-induced females and Aroclor 1254-induced males), mice (non-induced females and Aroclor 1254-induced males), and humans (three human pools from an unspecified number of female donors, age 17-41 years). The incubation time was 1 hour. The loss of ETU from human incubations was 11-13% compared to 2% for non-induced rats and 5% for non-induced mice.

Zhu (2015) used hepatocytes from female mice, rats, rabbits, dogs, and humans. Female hepatocytes were used because one of the aims was to investigate interspecies differences in ETU-induced teratogenicity. The human hepatocytes were pooled from 10 donors. ETU loss after 3 hours from incubations with hepatocytes from humans, dogs and rabbits was around 20%, whereas approx. 10% loss was observed in those form rats and no ETU loss in those from mice.

Summary of the epidemiology data on thyroid effects

The following table summarises the studies presented in the CLH report. Additionally, one recent study (Medda *et al.*, 2017) has also been included.

Human data on thyroid function		
Reference; **Substance and endpoint investigated**	**Description of the study**	**Results**
Manufacture		
Smith 1984 ETU; thyroid function	Exposed subjects: 8 workers involved in the manufacture of ETU and 5 in mixing of ETU with rubber Controls: Matched controls from other production workers and managerial staff Method: Blood was analysed for T4, TSH and TBG (thyroid-binding globulin). The measurements were repeated in the same subjects over 3 years Exposure data: Inhalation exposure of mixers up to 160 µg/m^3; dermal exposure not quantified Limitations: Low number of exposed subjects	Mixers but not process workers had statistically significantly lower T4 levels

Anon. 1985a EDBCs; thyroid function	Exposed subjects: 153 men currently or previously exposed to EBDC at a manufacturing site Controls: 153 matched non-exposed controls working in the same plant Method: Blood analysed for thyroid hormones and other parameters; a 24-hour urine sample analysed for iodide, ETU and EBDC Exposure data: Dermal exposure to mancozeb 6.3–140 mg, to ETU 0.11–1.5 mg; respiratory exposure to mancozeb 0.02–1.5 mg, to ETU 0.0000–0.0023 mg	No significant differences in thyroid function tests
Anon. 1990b EDBCs; exposure measurements	Exposed subjects: Workers involved in the manufacturing of EBDC fungicides (Netherlands) Method: Pre- and post-shift measurements of blood T3, T4 and TSH levels in the same workers Exposure data: Exposure inside the clothing 7.9 to 120 mg, potential dermal exposure 290 to 3300 mg. Respiratory exposure was minor compared to dermal exposure (respiratory exposure accounted for 0.1-0.4% of the total exposure) Limitations: Comparison of pre- and post-shift TH and TSH levels is not biologically meaningful due to the large reservoir of THs in humans	No biologically significant difference between pre- and post-shift T3, T4 or TSH levels
Agricultural use		
Steenland *et al.* 1997 EDBCs; thyroid function	Exposed subjects: 49 heavily exposed EDBC pesticide applicators (backpack sprayers without protective equipment), 14 lightly exposed landowners (present during application) Controls: 31 non-exposed subjects Method: Determination of blood T4 and TSH and urinary ETU Exposure data: Urinary ETU levels below the detection limit of 10 ppb in all non-exposed subjects; applicators 58 ppb; landowners 12 ppb	T4 levels not affected Increase in TSH of borderline stat. significance only after correction for age

Panganiban *et al.* 2004 EBDCs; thyroid function, examination for nodules	Exposed subjects: 57 directly exposed banana plantation workers, 31 indirectly exposed workers Controls: 43 workers from an organic farm Method: Determination of blood T4, TSH and ETU and urinary ETU; thyroid gland ultrasound Exposure data: Blood ETU levels were significantly higher in the exposed groups compared to the control group	No stat. sign. differences in thyroid function tests or the prevalence of solitary nodules
Goldner *et al.* 2010 (Agricultural Health Study) Organochlorine and other pesticides, including mancozeb/maneb; thyroid disease	Exposed subjects: 16,529 female spouses of pesticide applicators Method: Examination of the association between use of 50 specific pesticides and self-reported hypothyroidism, hyperthyroidism, and 'other' thyroid disease Limitations: self-reported thyroid disease, inability to determine whether exposure preceded disease onset	Ever use of maneb/mancozeb associated with an increased risk of both hypothyroidism and hyperthyroidism
Goldner *et al.* 2013 (Agricultural Health Study) Organochlorine and other pesticides, including mancozeb/maneb; thyroid disease	Exposed subjects: 22,246 male pesticide applicators Method: Examination of association between use of 50 specific pesticides and self-reported hypothyroidism, hyperthyroidism, and 'other' thyroid disease Limitations: self-reported thyroid disease, inability to determine whether exposure preceded disease onset	No evidence that exposure to maneb/mancozeb increased the risk of either hypothyroidism or hyperthyroidism
Medda *et al.* 2017 Mancozeb; thyroid function	Exposed subjects: 177 male grapevine workers Controls: 74 males, healthcare personnel Method: Determination of serum T4, T3, free T4, free T3, thyroglobulin, anti-thyroglobulin antibodies, anti-TPO antibodies; urinary iodine and ETU; thyroid gland ultrasound Exposure data: Serum ETU levels were significantly higher in the exposed group compared to the control group Limitations: Potential confounding by exposure to other pesticides not taken into account	Workers had slightly (by 6%) but statistically significantly lower serum T4 levels than controls

Three of the epidemiological studies indicated a possible association between exposure and thyroid effects: Smith (1984), Goldner *et al.* (2010), and Medda *et al.* (2017).

The study by Smith (1984), despite the low number of subjects, is considered by RAC to be a good-quality study involving a group exposed to relatively high levels of ETU. Only inhalation exposure was quantified, so it is difficult to estimate the total systemic exposure.

The study by Goldner *et al.* (2010) reported an association between ever use mancozeb/maneb with both hypothyroidism and hyperthyroidism in female spouses of pesticide applicators. It is noted that the study has some limitations, such as self-reported thyroid disease, and that no effect was reported in male pesticide applicators (Goldner *et al.*, 2013). Therefore the study by Goldner *et al.* (2010) is not considered to provide convincing evidence of an association between mancozeb exposure and thyroid disease.

The study by Medda *et al.* (2017) found a slight but statistically significant reduction in T4 levels in male grapevine workers compared to controls. However, not all potential confounders were taken into account; healthcare personnel serving as the control population might have differed from farmers in some parameters not captured by the study authors, e.g., in the overall level of exposure to pesticides.

It should also be noted that the exposure levels of pesticide applicators are relatively low; the Draft (Renewal) Assessment Report (RAR, 2017) indicates an upper boundary of systemic exposure to mancozeb in pesticide applicators around 0.3 mg/kg bw/d (Vol. 3 B.6), which is far below the LOAEL for thyroid disruption in the rat. Systemic exposure of mixers in the study by Smith (1984) was probably closer to the effect level in animals as there is no need for conversion from EBDCs to ETU, and dermal absorption of ETU is higher than that of mancozeb (17% vs 1%; RAR, 2017). Thus, an association of thyroid effects with exposure is more plausible for the Smith (1984) study than for the studies involving pesticide applicators.

10.11 Aspiration hazard

Not addressed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Note that in all cases, DT50 values refer to Single First Order (SFO) kinetics; the terms ‘half-lives’ and SFO DT50 values are used interchangeably as they relate to the same value. Where DT50 values are quoted for mancozeb, these relate to primary degradation, not dissipation or ultimate degradation. Degradation rates for ultimate degradation have not been submitted or calculated.

Where studies were conducted at temperatures other than 12°C, half-lives have been normalised to 12°C as necessary using equation 25 in Guidance on the BPR: Volume IV, Part B Risk Assessment (active substances) Version 1.0 April 2015, i.e. $DT50(X°C) = DT50(t) \cdot e^{(0.08 \cdot (T-X))}$.

Table 23: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Hydrolysis			
US EPA Subdivision N, 161-1, similar to OECD 111, predates GLP	25ºC pH 5: half-life 2.8 hours (7.9 hours at 12°C) pH 7: half-life 5.5 hours (15.5 hours at 12°C) pH 9: half-life 12.9 hours (36.5 hours at 12°C) main degradates: ETU – pH dependent, up to 100% EU – pH dependent, up to 11.5%	Variable mass balance attributed to inhomogeneity of dosing solution (test substance of low solubility). Biphasic degradation at pH 5 & 7, half-life expressed as DT90/3.32.	Lawrence *et al* 1988; kinetic calculations in Hardy 2015
US EPA Subdivision N, 161-1, similar to OECD 111, GLP compliant	25ºC pH 5: half-life 34.8 hours (98.4 hours at 12°C) pH 7: half-life 51.2 hours (144.9 hours at 12°C) pH 9: half-life 22.9 hours (64.8 hours at 12°C) main degradates: ETU – pH dependent, up to 94.5% EU – pH dependent, up to 25.6% 2 unidentified degradants >10%	Variable mass balance attributed to inhomogeneity of dosing solution (test substance of low solubility).	Wang 1995; kinetic calculations in Hardy 2015

OECD 111, GLP compliant	20°C pH 4: half-life 0.39 hours (0.7 hours at 12°C) pH 7: half-life 0.53 hours (1.0 hours at 12°C) pH 9: half-life 0.34 hours (0.6 hours at 12°C) 35°C pH 4: half-life 0.15 hours pH 7: half-life 0.52 hours pH 9: half-life 0.30 hours main degradates at 20°C: EBIS – pH dependent, up to 41.3% ETU – pH dependent, up to 90.7% EU – pH dependent, up to 31.2% Seven unknown degradants >10%	Study reliable	Völkel 2001b; kinetic calculations in Hardy 2015
OECD 111, GLP compliant	25°C pH 4: half-life 0.7 hours (2.0 hours at 12°C) pH 7: half-life 3.0 hours (8.5 hours at 12°C) pH 9: half-life 5.5 hours (15.6 hours at 12°C) 35°C pH 4: half-life 0.4 hours pH 7: half-life 1.5 hours pH 9: half-life 2.4 hours 50°C pH 4: half-life 0.3 hours pH 7: half-life 0.5 hours pH 9: half-life 0.4 hours main degradates at 25°C: ETU – pH dependent, up to 94.8% EU – pH dependent, up to 55.1% CPII – pH dependent, up to 11.1% CPIII – pH dependent, up to 24.5%	Study reliable for mancozeb, some concerns regarding degradant identification, particularly CPII and CPIII.	Cardinali 2007 (corrections submitted 2016)
Photolysis			
No stated guideline, pre-dates GLP	Mancozeb hydrolysed rapidly in dark control at 25°C, little difference between levels in exposed and dark control. Degradate EBIS degradation is enhanced by light exposure.	Reliability questionable due to use of mercury vapour lamp. However, practical influence of light exposure on mancozeb likely to be limited given rapid hydrolysis.	Yeh 1985

Ready biodegradability			
OECD 301B, GLP compliant	Test item 5-6% biodegradation in 36 days. Aniline reference 94% biodegradation (by % of theoretical CO_2).	Study performed with a formulated product. Study outcome classifies test substance as 'not readily biodegradable'.	Ectors 1995
Biodegradation			
OECD 309, GLP compliant	Carsington pelagic fresh water system, 20°C. Mancozeb DT90 < 3 days (< 5.7 days at 12°C). Degradants: M1 (max 11.15% AR at 60 days); M4 (ethanolamine; max 15.4% AR at 14 days); M8 (glycolic acid; 15.46% AR at 28 days); EU (41.22% AR at 60 days); M13 (ethylene glycol; 24.69% AR at 49 days); ETU (35.00% AR at 3 days).	More than 90% disappearance of mancozeb between dosing and second sampling time at 3 days after treatment. Mineralisation up to 16.8% at 60 days after treatment.	Dobson 2015
German BBA, US EPA 162-3 & 162-4, GLP compliant	Two water/sediment simulation study, four systems, 20°C. Mancozeb whole system DT50 0.05 – 0.28 days (0.09 – 0.53 days at 12°C). No partitioning of mancozeb to sediment detected. Degradants: EBIS – up to 30.9% AR total system ETU – up to 51.6% AR total system EU – up to 43.5% AR total system Hydantoin – up to 11.7% AR total system Unknown 2 - up to 15.2% AR total system	Two separate studies but very similar. Rapid primary degradation of mancozeb with significant degradant formation. Up to 57.8% AR mineralisation at 106 DAT, up to 36.7% AR unextracted at 106 days.	Müller-Kallert 1994 Völkel 1995 Kinetic calculations in Hardy 2015

All DT50 values in the table above are calculated at stated study temperature; DT50 values in parentheses have been normalised to 12°C using (DT50 at T)*$e^{(0.08*(T-12))}$ as recommended by ECHA. In hydrolysis studies where incubations at multiple temperatures were run, only the DT50 from the lowest temperature incubations have been normalised to 12°C.

11.1.1 Ready biodegradability

Ectors 1995; an OECD 301B, GLP-compliant study was conducted on a commercial formulation of mancozeb containing 80% w/w mancozeb; no details of the constituents of the remaining 20% w/w in the formulation were given. Mancozeb is of low solubility and the formulation was considered to be dispersible in water. For the study, the formulation was added directly to the activated sewage sludge/nutrient solution with additional water and stirred with magnetic stirrers to keep the test substance dispersed. No other solvents were used. The organic carbon content of the test item was 0.13 mg C/mg active substance and $ThCO_2$ was calculated as 0.47 mg CO_2/mg active substance. The test systems were 5L brown glass bottles containing activated sewage sludge as the inoculum in high quality water and nutrient solution; these bottles were connected to CO_2

absorber bottles containing 0.0125 M $Ba(OH)_2$. At the start of the incubation period, the mancozeb test item was added to the sewage sludge/nutrient solution and constantly stirred. The study author stated that the study was conducted at 'room temperature' and records of temperature indicated that the temperature varied between 20 - 25°C over the study duration. CO_2 liberated by biodegradation of the test item reacts with $Ba(OH)_2$ in the absorber bottles to form insoluble BaCO3 which precipitates. CO_2 production was thus determined by titration of remaining $Ba(OH)_2$ with 0.05 M HCl and using phenolphthalein as an indicator. The mancozeb test item was tested alongside aniline as a reference substance. Due to the physical nature of the test item, % DOC removal could not be measured. There was 96% DOC removal from the aniline reference and 94% biodegradation (% of theoretical CO_2). The biodegradation of the mancozeb test item as a % of $ThCO_2$ was 5 and 6% in the two test vessels. An intense smell of H_2S came free during the titration of the absorber solutions of the mancozeb test system (mancozeb contains S). It was concluded that the mancozeb test item was not readily biodegradable. The study is considered to be reliable.

11.1.2 BOD_5/COD

For the purpose of classification, data generated by the ready biodegradability study supersede direct BOD_5 and COD measurements.

11.1.3 Hydrolysis

Lawrence *et al*, 1988; the hydrolysis of ^{14}C-mancozeb was investigated at 25.4 °C in sterile aqueous buffer solutions of pH 5, 7 and 9; the study was conducted to the US EPA Subdivision N, 161-1 guideline, which is similar to OECD 111, and predated GLP. At 25 °C mancozeb underwent rapid degradation at all three pH. Degradation was pH related, ranging from 2.8 hours at pH 5 to 1.9 hours at pH 9; normalisation to 12°C results in DT50 7.9 hours at pH 5 – 36.5 hours at pH 9. The main hydrolysis product was ETU with up to 100% observed. A further major degradant was EU at a maximum of 11.5%. Validity of the study is limited due to variability of recovery but gives a reasonable understanding of hydrolytic behaviour.

Wang, 1995; the hydrolysis of ^{14}C-mancozeb was investigated at 25 °C in sterile aqueous buffer solutions of pH 5, 7 and 9; the study was conducted to the US EPA Subdivision N, 161-1 guideline, which is similar to OECD 111, and was GLP compliant. At 25 °C mancozeb underwent relatively rapid degradation at all three pH. The pH dependent degradation observed in Lawrence *et al*, 1988 was not demonstrated, but degradation still varied dependent on pH. Results normalised to 12°C were as follows: pH 5 – 4.1 days; pH 7 – 6.0 days; pH 9 – 2.7 days. The main hydrolysis product was ETU with up to 94.5% observed. A further major degradant was EU at a maximum of 25.6%. Two unidentified degradants also exceeded 10%. Validity of the study is limited due to variability of recovery.

Völkel 2001b; the hydrolysis of ^{14}C-mancozeb was investigated at 20 and 35 °C in sterile aqueous buffer solutions of pH 5, 7 and 9; the study was conducted to the OECD 111 guideline, and was GLP compliant. At 20 °C mancozeb underwent rapid degradation at all three pH. DT50s varied dependent on pH. Results at 20°C normalised to 12°C were as follows: pH 4 – 0.7 hours; pH 7 – 1.0 hour; pH 9 – 0.6 hours. The main hydrolysis products were EBIS up to 41.3%, ETU with up to 90.7% and EU with up to 31.2% observed. Seven unidentified degradants also exceeded 10%. The study is considered reliable.

Cardinali 2007 (update 2016): the hydrolysis of ^{14}C-mancozeb was investigated at 25, 35 and 50 °C in sterile aqueous buffer solutions of pH 5, 7 and 9; the study was conducted to the OECD 111 guideline, and was GLP compliant. At 20 °C mancozeb underwent rapid degradation at all three pH. DT50s varied dependent on pH, similar to seen in the study of Lawrence *et al* 1988. Results at 20°C normalised to 12°C ranged from 2.0 hours at pH 4 to 15.6 hours at pH 9. The main hydrolysis products were ETU with up to 94.8% and EU with up to 55.1% observed. Two additional degradants were also seen at 11.1% and 24.5%, however the reliability of the degradant identification is uncertain as these degradants were not seen in any other mancozeb study, let alone in hydrolysis studies. The study is considered reliable with respect to data on mancozeb, but of suspect reliability with respect to degradant identification.

11.1.4 Other convincing scientific evidence

No information.

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

No information.

11.1.4.2 Inherent and enhanced ready biodegradability tests

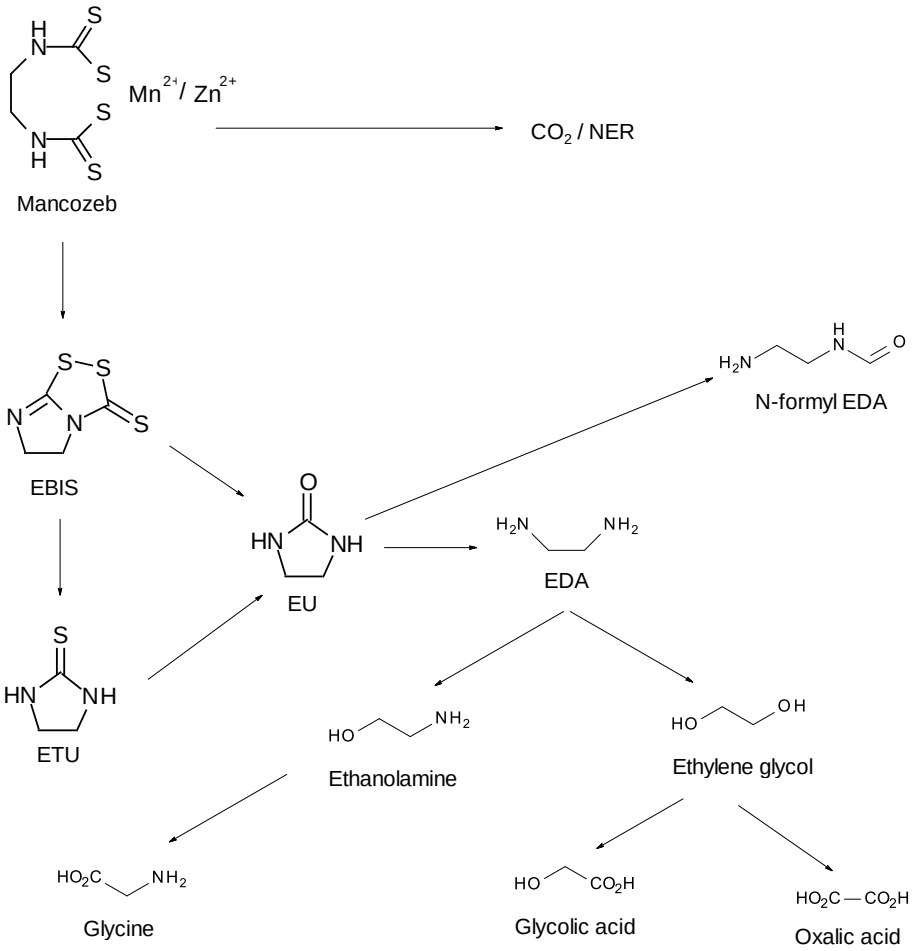
No information.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

Aerobic mineralisation in surface water

Dobson 2015; conducted on a fresh water pelagic water system at 20°C in the dark using two dose rates of ^{14}C-mancozeb as recommended in OECD 309. The water had a pH of 8.81, was non-turbid and colourless and had a dissolved organic carbon content of 4.2 mg/l. Study duration was 60 days. Sampling regime designed to consider degradant behaviour rather than to accurately quantify mancozeb decline as rapid decline of mancozeb was expected. Mancozeb was not detected by 3DAT and thus had declined by >90% of applied at 3 DAT (only previous sample event was at 0 DAT). Thus it is only possible to state that the DT90 of mancozeb at 20°C was <3 days, i.e. DT90 <5.7 days at 12°C. 16.8% mineralisation was seen at study termination in low dose incubation, 8.1% mineralisation in high dose incubation. A large number of degradants were generated. Unidentified M1 (max 11.15% AR at 60 days); ethanolamine (max 15.4% AR at 14 days); glycolic acid (15.46% AR at 28 days); EU (41.22% AR at 60 days); ethylene glycol (24.69% AR at 49 days); ETU (35.00% AR at 3 days). Study is reliable. A proposed metabolic pathway of mancozeb in surface water from the Dobson 2015 study is shown below.

Figure 6.1: Proposed metabolic pathway of mancozeb in surface water



Water/sediment study

Müller-Kallert 1994; conducted to US EPA 162-3 & 162-4 guidelines, similar to OECD 308, and GLP compliant. Conducted on two fresh water water/sediment systems incubated at 20°C in the dark, study duration 106 days. Properties of the water/sediment systems are given below.

Sediment	***River***	***Pond***
pH (KCl)	7.6	7.3
Redox potential (mV)	-174	-156
OC (g C/100g dry sediment)	1.06	1.59
Biomass (mg microb. C/100g dry sed.)	43.1	90.1
CEC (mVal N/100g dry sed)	4.6	17.8
USDA nomenclature	sand	loam
Clay (% < 2µm)	12.1	43.1
Silt (% 2-50 µm)	4.9	31.3
Sand (% >50 µm)	83.0	25.6
Water phase		
Oxygen concentration (mg/l)	10.2	7.7
pH	7.72	7.31
Total organic carbon (mg C/l)	6.5	12.1
redox potential (mV)	+230	+210

^{14}C-mancozeb was dosed. Mancozeb declined rapidly in the whole systems. Analysis for mancozeb was performed after complexing extract with EDTA. Degradation of mancozeb can be expressed either as the degradation of the main complexed fraction (i.e. sodium salt of ethylenebisdithiocarbamate, nabam) or as the sum of the complexed fractions. DT50 of the main complexed fraction whole system normalised to 12°C = 0.17 – 0.23 days. DT50 of sum of complexed fractions in whole system normalised to 12°C = 3.17 – 3.41 days. No partitioning of mancozeb to either sediment. Mineralisation 57.7 – 57.8% AR at 106 days. Unextracted residues 35.4 – 36.7% AR at 106 days. Significant formation of degradants: EBIS – up to 30.9% AR total system; ETU – up to 47.6% AR total system; EU – up to 29.5% AR total system. Study is reliable.

Völkel 1995; virtually a repeat of Müller-Kallert 1994 with similar water/sediment systems and study methodology. Properties of the water/sediment systems are given below.

Sediment	***River***	***Pond***
pH (KCl)	6.85	6.61
Redox potential (mV)	-105	-112
OC (g C/100g dry sediment)	1.35	5.03
Biomass (mg microb. C/100g dry sed.)	109.7	330.3
CEC (mVal N/100g dry sed)	2.77	7.05
USDA nomenclature	sand	loam
Clay (% < 2µm)	7.0	5.0
Silt (% 2-50 µm)	11.6	35.8
Sand (% >50 µm)	81.4	59.2
Water phase		
Oxygen concentration (mg/l)	2.5 - 7.2	4.5 - 7.8
pH	8.02	7.37
Total organic carbon (mg C/l)	2.6	1.1
redox potential (mV)	+142	+129

Study duration was 105 days. Mancozeb declined rapidly in the whole systems; DT50 of the main complexed fraction whole system normalised to 12°C = 0.55 – 1.14 days. DT50 of sum of complexed fractions in whole system normalised to 12°C = 0.59 – 0.97 days. No partitioning of mancozeb to either sediment. Mineralisation 17.6 – 47.1% AR at 105 days. Unextracted residues 39.5 – 43.6% AR at 105 days. Significant formation of degradants: EBIS – up to 12.7% AR total system; ETU – up to 51.6% AR total system; EU – up to 43.5%

AR total system; hydantoin – up to 11.7% AR total system; unknown degradant 2 - up to 15.2% AR total system. Study is reliable.

11.1.4.4 Photochemical degradation

Yeh 1985; the study was not conducted to a specified guideline and predates GLP. ^{14}C-mancozeb declined rapidly in the dark at 25°C as might be expected from hydrolysis data; at time zero sample time, mancozeb concentrations had already declined to 6.8% AR in light exposed samples and 8.7% AR in dark control. There was very little effect of light exposure on the degradation of mancozeb, however, the lamp was a mercury vapour lamp which may not have a spectral distribution sufficiently similar to sunlight. The degradation of the major degradant EBIS appeared to be enhanced by light exposure. The use of a mercury vapour lamp makes the study of questionable reliability, however the rapidity of hydrolysis suggests that aqueous photolysis will be of limited relevance to mancozeb.

Summary and discussion of degradation

Mancozeb degrades rapidly in hydrolysis studies; three of four studies show DT50 values normalised to 12°C of 0.6 hours – 1.5 days. One hydrolysis study gave longer DT50 values normalised to 12°C of 2.7 – 6.0 days. Degradation varied with pH, although a clear pH tendency was not clear across the four studies. Two of the studies indicated that degradation was faster under acid conditions and slower under alkaline conditions. Degradant occurrence in the hydrolysis studies was pH related. ETU was formed at high levels in all pH tested with >90% at pH 4-5, 57 – 87% at pH 7 and 57-90% at pH 9. EU was found up to 6% at pH 4, 13% AR at pH 7 and 11-55% at pH 9. EBIS was potentially less affected by pH with maximum 33% at pH 4, 41% at pH 7 and 30% at pH 9.

A single aqueous photolysis study using mancozeb was available. This old study dating to 1985 was conducted using a mercury vapour lamp. The RMS is of the understanding that such lamps are not recommended for use in sunlight simulation tests. However, it was noted that decline of mancozeb in the dark control was very rapid due to hydrolysis and that there was no influence of illumination on rate of decline.

A single ready biodegradability study was submitted using a simple dispersion in water formulation containing 80% w/w mancozeb. This study indicated that the mancozeb formulation was not classified as readily biodegradable.

A study on aerobic mineralisation of mancozeb in surface water was submitted. A pelagic system from a natural fresh water body was chosen for this study. Given the expected rapid degradation of mancozeb, the study authors decided to concentrate attention on degradant behaviour rather than mancozeb and therefore the second sample time was three days after application rather than attempting to sample more frequently within the first three days. As expected, degradation of mancozeb was rapid with no mancozeb detected at 3 DAT. No kinetic parameters could be calculated, thus it can be concluded that mancozeb in this study had a DT90 <3 days at 20°C (DT90 <5.7 days normalised to 12°C). There was maximum of 16.8% AR as CO_2 at 60 days (study end) suggesting relatively little mineralisation in this study. Given the frequency of initial sampling, it is possible that peak formation of the rapidly formed and degraded degradant EBIS (as seen in the aerobic water/sediment studies) may have been missed. However, other degradants were also found to be major in this study. ETU was found at a maximum of 35% AR at 3 DAT and EU at 41% AR at 60 DAT (study end). Four other major degradants were also found which in some cases were tentatively identified. These were M1 (unidentified, 11% at 60 DAT), ethanolamine (15.4% at 14 DAT), glycolic acid (15.2% at 14 DAT), ethylene glycol (24.7% at 49 DAT).

Two water/sediment studies were submitted; the studies were very similar to each other, having been conducted by the same laboratory and using nominally the same two water/sediment systems and methodology, but separated in time. As in other water studies, mancozeb degraded rapidly. DT50 values expressed as the main complexed fraction (i.e. nabam, the sodium salt of EBDC) were in the range 0.17 – 1.14 days (normalised to 12°C) for the whole water/sediment systems. When expressed as the sum of complexed fractions, the DT50

range (at 12°C) was 0.59 – 3.41 days. Mancozeb was not detected in sediment. There were three major identified degradants:

EBIS - maximum in total system 8.9 – 30.9% AR after 0 – 0.25 d.

ETU - maximum in total system 33.6 – 51.6 % AR after 2 days.

EU - maximum in total system 30.7 – 43.5% AR after 7 - 59 days.

A number of other degradants were also found at >10% AR. Mineralisation ranged from 17.6 – 57.8% AR at study termination, i.e. 105 – 106 days after treatment. Unextracted residues were at significant levels at study termination, ranging from 35.4 – 43.6% AR.

Overall, the range of degradation studies indicate that mancozeb undergoes rapid primary degradation in water. A range of degradants are formed at high levels. However, ultimate degradation in biologically active water systems is relatively slow with 16.8% AR as CO_2 at 60 days in the aerobic mineralization in surface water study and 17.6 – 57.8% AR at 105 – 106 days after treatment in water/sediment studies. This suggests that mancozeb would not meet the criteria to be classified as rapidly degradable.

11.2 Environmental transformation of metals or inorganic metals compounds

Not applicable.

11.3 Environmental fate and other relevant information

11.3.1 Adsorption/Desorption

A single adsorption/desorption study on mancozeb in four soils (pH range 5.7 – 7.4, organic carbon range 0.5 – 2.0) is available performed to US EPA guideline 163-1 (similar to OECD 106). However, the study was unable to reliably determine soil adsorption coefficients as mancozeb was unstable during the 24 hour equilibrium period. As a result, the calculated adsorption coefficients represent mancozeb and a range of its degradation products. Koc values ranged from 363 – 2334 ml/g, 1/n 0.686 – 0.777. Whilst not ideal, it would be very difficult to obtain an accurate and reliable estimate of soil adsorption for mancozeb using the OECD 106 test method due to its very rapid degradation in soil.

Whilst not directly indicative of adsorption/desorption or distribution coefficients, in the two water/sediment studies performed with mancozeb (Müller-Kallert 1994 and Völkel 1995), mancozeb degraded rapidly and no mancozeb was detected in sediment. This suggests that if mancozeb were to reach natural water surface water systems, partitioning to sediment would be unlikely to take place to a significant extent, most probably due to rapid degradation. In addition, rapid degradation of mancozeb was also seen in the aerobic mineralisation in surface water study (Dobson 2015). This further suggests that mancozeb would typically exist transiently in water with little or no partitioning to sediment.

11.3.2 Volatilisation

Mancozeb has a vapour pressure of is $<5.6 \times 10^{-5}$ Pa at 25°C and Henry's Law constant calculated from vapour pressure and solubility of $< 6.17 \times 10^{-2}$ $Pa.m^3.mol^{-1}$. This suggests that mancozeb is of low volatilisation potential and is unlikely to partition from water to the air.

11.4 Bioaccumulation

A study to assess the bioaccumulation of mancozeb in fish has not been performed as the log Kow was concluded to be 2.3 (at 20-25°C and pH 6-10) from new chemistry data submitted for the renewal of mancozeb. This is below the EFSA aquatic guidance (2013) trigger of 3 above which a BCF study must be performed for pesticide assessment. It is also below the cut-off value of 4 given in the CLP Regulation and discussed in the

ECHA Guidance on the Application of the CLP Criteria (2015) and it indicates a low potential for bioaccumulation of mancozeb. Information on the aquatic bioaccumulation of the degradant ETU is presented in Annex 1.

Table 24: Summary of relevant information on bioaccumulation

Method	Test Substance	Results	Remarks	Reference
OECD 107 (shake flask method, concentration of test item in layer by CIPAC MT 165) GLP	Mancozeb pure, 92.3% Lot FL13-068-6E	At 20 – 25 ºC: Log Kow = 2.3 (pH 6 – 8) Log Kow = 2.3 (pH 9 - 10) Log Kow = ∞ (pH 4 - 5) Mancozeb has very low solubility in water and at a low pH mancozeb does not tend to migrate into the water layer.	Acceptable. This indicates that the substance is unlikely to bioaccumulate.	Bos MWS, 2014 Study DL 14-006
Predicted value (ACD Labs version 12.01) Non-GLP	Ethylenethiourea (ETU)	Log Kow = -0.66 (pH 5.5) Log Kow = -0.66 (pH 7.0) Log Kow = -0.66 (pH 9.0)	Acceptable. ETU is included in the residue definition, therefore these data are required.	Anonymous (2014) Study 2014/1321435

11.4.1 Estimated bioaccumulation

Not estimated as log Kow < 4.

11.4.2 Measured partition coefficient and bioaccumulation test data

No data are available as log Kow < 3.

Summary and discussion of aquatic bioaccumulation

The low log Kow of 2.3 indicates no significant potential for bioaccumulation of mancozeb in the aquatic environment, as it falls below the CLP value for concern (log Kow ≥ 4). It is considered to have a low potential for bioaccumulation.

11.5 Acute aquatic hazard

Background

Mancozeb was first included in Annex I of Dir. 91/414/EEC on 1st July 2006. The UK is the RMS for the AIR 3 renewal of the active under the latest pesticide legislation (EC Reg. no. 1107/2009) and Greece is the Co-RMS. Italy is the RMS for the active metiram, and has evaluated the data on the common degredants of the two actives (see Annex III). Aquatic data were submitted and assessed for the original authorisation. These data have been used for the renewal (for the most part without additional assessment), along with newly submitted aquatic data which have been assessed and summarised for the renewal. Where old endpoints were reported as nominal values, studies were briefly re-assessed to determine if these endpoints are valid in the light of current guidance. Where this could not be confirmed, the studies were considered not acceptable for use in the risk assessment and the classification. This is with the exception of endpoints which are the lowest of the respective taxonomic/trophic group, which have been included in the hazard assessment as a conservative approach. Some of the old studies submitted for the DAR were deemed not valid in additional addenda to the DAR. The endpoints of these studies have not been considered for classification and labelling purposes. This is with the exception of the algal study Forbis (1990) which was considered not valid in addendum 1 to the original DAR. However, as no valid algal studies were available at the time, the endpoint from this study was used in the risk assessment for the DAR. The RMS requested of the applicant that reviewed, mean-measured endpoints be calculated for this and the *Lemna* study. These were provided and have been presented in the summaries below, as well as in table 25. In addition, the applicant later submitted a new algal study on the species *P. subcapitata* which has been evaluated and the results presented in table 25.

Both active and formulation study endpoints have been included in this assessment, as valid mancozeb technical studies were not available to determine the chronic hazard and risk to aquatic invertebrates and aquatic macrophytes. However, this is justified in the case of mancozeb due to the simplicity of the formulations and the high percentage of active substance in the formulations (formulations contain roughly 80% mancozeb w/w with inert/less toxic co-fomulants). A confidential Annex has been provided as a separate document which provides details of those formulations used in studies which were critical for the classification of mancozeb. The lowest acute endpoint was from a formulation study, and there is no technical substance study available for chronic invertebrates (the surrogate method was required). Therefore, the acute and chronic classifications using both the lowest endpoints for technical mancozeb and for the formulations have been presented, which result in the same outcome. The identical classifications support the assertion that low levels of co-formulants present in mancozeb formulations that have been tested are unlikely to have an effect on the resulting endpoint, and therefore on the environmental classification. Regardless of whether individual studies were performed with the active or a formulation, all endpoints have been reported in terms of active substance. The degradants of mancozeb for which toxicity data are available are not relevant to the classification of the active substance. Therefore these endpoints have not been presented in the classification assessment, but are summarised in Annex III to the report for completeness. The degradant endpoints were assessed by Italy. . Finally, all the studies summarised in this document are first tier studies on standard species. Higher tier studies are not considered for classification purposes. In cases where sediment-dwelling species have been used, the study endpoints have only been considered if they are available in the form of mg/L (i.e. measurements of test substance were made in the water phase).

Table 25: Summary of relevant information on acute aquatic toxicity

Guideline and GLP status	Substance (purity in % w/w)	Species	Endpoint Effect	Exposure		Results		Reference	Submitted for original approval (O) or renewal (N)
				Design	Duration	Endpoint	Toxicity (mg a.s./L)		
Fish									
OECD 203 GLP	**Mancozeb Tech. (purity > 90%)**	***Oncorhynchus mykiss***	**Mortality**	**Semi-static**	**96h**	**LC_{50}**	**0.074 (mm)**	**Anonymous, 1987**	**O**
OECD 203 GLP	Mancozeb Tech. (purity > 90%)	*Lepomis macrochirus*	Mortality	Semi-static	96h	LC_{50}	0.083 (mm)	Anonymous, 1987e	O
OECD 203 GLP	Mancozeb Tech. (purity > 90%)	*Oncorhynchus mykiss*	Mortality	Semi-static	96h	LC_{50}	0.088 (mm)	Anonymous, 1997e	O
OECD 203 GLP	Penncozeb 80 WP (Mancozeb: 82.0%)	*Oncorhynchus mykiss*	Mortality	Flow-through	96h	LC_{50}	0.15 (mm)	Anonymous, 1993a	O
OECD 203 GLP	Dithane M-45 (Mancozeb: 81.3%)	*Oncorhynchus mykiss*	Mortality	Flow-through	96h	LC_{50}	1.0 (nom)	Anonymous, 2000a	O
OECD 203 GLP	Penncozeb 80 WP (Mancozeb: 82.0%)	*Cyprinus carpio* (Mirror carp)	Mortality	Flow-through	96h	LC_{50}	1.84 (nom)	Anonymous, 1993b	O
OECD 203 GLP	Dithane M-45 (Mancozeb: Not stated)	*Lepomis macrochirus*	Mortality	Flow-through	96h	LC_{50}	> 3.6 (mm)	Anonymous, 2000b	O

Invertebrates									
OECD 202 GLP	Mancozeb Tech. (purity > 90%)	*Daphnia magna*	Immobilisation	Static	48h	EC_{50}	0.073 (mm)	Douglas *et al*., 1988	O
OECD 202 GLP	Sancozeb 800 WP (Mancozeb: 80%)	*Daphnia magna*	Immobilisation	Static	48h	EC_{50}	0.9 (nom)	Brouwers, 1995	O
OECD 202 GLP	**Mancozeb 80% WDP (Mancozeb: 80%)**	***Daphnia magna***	**Immobilisation**	**Static**	**24h**	**EC_{50}**	**0.0112 (nom)**	**Rakesh M. Patel, 1998**	**O**
OECD 202 GLP	Penncozeb 80 WP (Mancozeb: 82.0%)	*Daphnia magna*	Immobilisation	Static	48h	EC_{50}	0.39 (nom)	Wüthrich, 1993b	O
OECD 202 GLP	Fortuna 800 WP (Mancozeb: 79.4%)	*Daphnia magna*	Immobilisation	Static	48h	EC_{50}	1.10 (nom)	Sesso, J.N. 2007a	N
OECD 202 GLP	Dithane M-45 (Mancozeb: 81.3%)	*Daphnia magna*	Immobilisation	Flow-through	48h	EC_{50}	3.8 (mm)	Rhodes, 2000b.	O
Algae									
OECD 201 GLP	Mancozeb Tech. (purity: 86.1%)	*Pseudokirchneriella subcapitata***	Growth rate	Static	72h	E_rC_{50} E_rC_{10} NOEC	0.0509 (Geomean-measured) 0.016 (Geomean-measured) 0.00201 (Geomean-measured)	Börschig, & Sonntag, 2017	N

Similar to OECD 201 GLP	**Dithane M-45 (Mancozeb: 82.4%)**	***Selenastrum capricornutum*****	**Growth rate**	**Static**	**120h**	**E_rC_{50}** **E_rC_{10}** **NOEC**	**0.0322# (Geomean-measured)** **0.00905 (Geomean-measured)** **n.d.**	**Forbis, 1990##**	**O**
Macrophytes									
OECD 221 GLP	**Mancozeb 80 WP (Mancozeb: 80.5%)**	***Lemna minor***	**Growth rate**	**Semi-static**	**7d**	**E_rC_{50} Frond Number** **E_rC_{10} Frond Number** **E_rC_{50} Biomass** **E_rC_{10} Biomass** **NOEC**	**1.811 (Geomean-measured)** **0.0822 (Geomean-measured)** **1.042 (Geomean-measured)** **0.0371 (Geomean-measured)** **0.0246# (Geomean-measured)**	**Dickinson, 2011d**	**N**

n.d. Not determinable
**Currently known as *Raphidocelis subcapitata*
Re-calculated endpoint by the applicant at the request of the RMS.
Study was considered to be not valid in addendum 1 to the original DAR. However, as no valid studies were available, the endpoint from this study was used in the risk assessment for the DAR. Mean measured values from this study are considered acceptable for the RAR.
The most sensitive, valid acute and chronic classification endpoint for each main taxonomic/trophic group is presented in **bold**.

11.5.1 Acute (short-term) toxicity to fish

Short-term fish studies were provided both for the original Annex I approval for mancozeb, and for the AIR 3 renewal of the active. Those considered acceptable at the original approval and those considered acceptable for the current renewal are summarised below.

The acute toxicity of Mancozeb Technical to Rainbow trout (*Oncorhynchus mykiss*) Anonymous (1987d)

The acute toxicity of mancozeb (>90% purity) to the rainbow trout (*Oncorhynchus mykiss*) was assessed in a study performed to OECD guideline number 203 and in compliance with GLP principals. Exposure to the test item was for 96h in semi-static conditions at nominal concentrations of 0 (control), 0.1, 0.18, 0.32, 0.56, and 1.0 mg a.s./L. The mean measured 96h LC_{50} was 0.074 mg a.s./L. It was not stated in the study report whether measurements of test concentrations were made in the fresh or spent media, therefore there is some question over whether the mean measured values used for the endpoints from this study are valid. However, as this is the critical endpoint for the acute lab studies on fish, not including it may mean the hazard assessment is not protective, the mean measured value was concluded as being acceptable for use in the risk assessment during the AIR 3 renewal of mancozeb as a pesticide and also for hazard classification.

The acute toxicity of Mancozerb Technical to Bluegill sunfish (*Lepomis macrochirus*). Anonymous (1987e)

The acute toxicity of mancozeb (>90% purity) to the Bluegill subfish (*Lepomis macrochirus*) was assessed in a study performed to OECD guideline number 203 and in compliance with GLP principals. Exposure to the test item was for 96h in semi-static conditions at nominal concentrations of 0 (control), 0.056, 0.10, 0.18, 0.32, and 0.56 mg a.s./L. The mean measured 96h LC_{50} (of spent media samples) was 0.083 mg a.s./L. Although it was not stated in the study report whether measurements had been made in freshly prepared or spent solutions, actual measured concentrations made after 0h were consistently lower than those made at 0h; implying that measurements made every 24h were made in the spent rather than the fresh media. Considering the data set available, and that it is likely that the endpoint is presented as the mean measured value of spent media samples, the RMS concluded that it is acceptable to include this endpoint in the risk assessment during the AIR 3 renewal of mancozeb as a pesticide and also for hazard classification.

Acute toxicity for freshwater fish *Onchorhynchus mykiss*- 96 hours in a semi-static system. Anonymous (1997e).

The acute toxicity of mancozeb (>90% purity) to the rainbow trout (*Oncorhynchus mykiss*) was assessed on a study performed to OECD guideline number 203 and in compliance with GLP principals. Exposure to the test item was for 96h in semi-static conditions at nominal concentrations of 0 (control), 0.2, 0.4, 1.0, 2.1 and 4.7 mg a.s./L. The mean measured LC_{50} 96h was 0.088 mg a.s./L. Although the raw analytical results of test substance analysis were not presented in the study report, mean measured values were presented and it was stated that they were calculated as the geometric mean of fresh and 24h spent media at each exposure period. It is not known whether measured values were within ±20% of the nominal or initial measured values. However, the geometric mean measured concentration of fresh and spent media is acceptable when measurements are outside of these ranges. Therefore the mean measured LC_{50} of 0.088 mg a.s./L was considered acceptable for use in the risk assessment during the AIR 3 renewal of mancozeb as a pesticide and also for hazard classification.

Penncozeb 80 WP: 96-hours acute toxicity study in the Rainbow Trout under flow-through conditions. Anonymous (1993a).

The acute toxicity of Penncozeb (82% Mancozeb) to the rainbow trout (*Oncorhynchus mykiss*) was assessed in a study performed to OECD guideline number 203 and in compliance with GLP principals. Exposure to the test item was for 96h in flow-through conditions at nominal concentrations of 0 (control), 0.1, 0.17, 0.31, 0.56, and 1.0 mg product/L. The mean measured 96h LC_{50} was 0.18 mg product/L, or 0.15 mg a.s./L. The mean measured NOEC was <0.09 mg product/L. The LC_{50} endpoint was concluded as being reliable and acceptable during the EU Annex I renewal of mancozeb as a pesticide.

Acute Toxicity of Dithane M-45 to the rainbow trout (*Oncorhynchus mykiss*) Detirmined under flow-through test conditions. Anonymous (2000a).

The acute toxicity of Dithane M-45 (81.3% purity) to the Rainbow trout (*Oncorhynchus mykiss*) was assessed in a study performed to OECD guideline number 203 and in compliance with GLP principals. Exposure to the test item was for 96h in flow-through conditions at nominal concentrations of 0 (control), 0.13, 0.25, 0.50, 1.0, and 2.0 mg a.s./L. The nominal 96h LC_{50} was 1.0 mg a.s./L. The nominal NOEC was 0.27 mg a.s./L. The study report demonstrated that measured test substance concentrations were maintained within ±20% of the nominal. Therefore the nominal LC_{50} was considered acceptable for use in the risk assessment during the AIR 3 renewal of mancozeb as a pesticide and also for hazard classification.

Penncozeb 80 WP: 96-hours acute toxicity study in the Mirror Carp under flow-through conditions. Anonymous (1993b)

The acute toxicity of Penncozeb 80 WP (82% Mancozeb) to the Mirror Carp (*Cyprinus carpio*) was assessed in a study performed to OECD guideline number 203 and in compliance with GLP principals. Exposure to the test item was for 96h in flow-through conditions at nominal concentrations of 0 (control), 0.48, 0.86, 1.54, 2.78, and 5.0 mg product/L. The nominal 96h LC_{50} was 2.28-4.1 mg/L. The nominal NOEC was 1.26 mg a.s./L. The RMS used the lowest of these values as a conservative approach and calculated the LC_{50} in terms of active substance using the percentage concentration of mancozeb in the formulation. The LC_{50} in terms of active substance is 1.84 mg a.s./L. The LC_{50} endpoint was concluded as being reliable and acceptable during the EU Annex I renewal of mancozeb as a pesticide.

Acute toxicity of Dithane M-45 to the bluegill sunfish (*Lepomis macrochirus*) determined under flow-through test conditions. Anonymous (2000b).

The acute toxicity of Dithane M-45 (mancozeb % not stated) to the bluegill sunfish (Lepomis macrochirus) was assessed in a study performed to OECD guideline number 203 and in compliance with GLP pricipals. Exposure to the test item was for 96h in flow-through conditions at nominal concentrations of 0 (control), 0.25, 0.50, 1.0, 2.0, and 4.0 mg a.s./L. The mean measured 96h LC_{50} was >3.6 mg a.s./L and the NOEC was 0.89 mg a.s./L. The study report demonstrated that measurements of test substance made at the start and end of the test were within ±20% of the nominal. Nevertheless, mean measured concentrations were used to report the endpoints. This is a more conservative approach than using nominal values and is there considered acceptable by the RMS. The LC_{50} endpoint based on mean measured was therefore considered acceptable for use in the risk assessment during the AIR 3 renewal of mancozeb as a pesticide and also for hazard classification.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Short-term invertebrate studies were provided both for the original Annex I approval for mancozeb, and for the AIR 3 renewal of the active. Those considered acceptable at the original approval and those considered acceptable for the current renewal are summarised below.

The acute toxicity of Mancozeb technical to *Daphnia magna*. Douglas M.T., Handley J.W. and McDonald I.A, (1988)

The acute toxicity of mancozeb (90% purity) to *Daphnia magna* was assessed in a study performed to OECD guideline number 202 and the EPA Pesticide Assessment Guidelines 72-3. The study was performed in compliance with GLP principals. Exposure to the test item was for 48h in static conditions at nominal concentrations of 0 (control), 0.01, 0.018, 0.032, 0.056, 0.1, 0.18, 0.32, 0.56, and 10.0 mg a.s./L. The mean measured 48h EC_{50} was 0.073 mg a.s./L. This endpoint was concluded as being reliable and acceptable during the EU Annex I renewal of mancozeb as a pesticide.

Acute Toxicity of Sancozeb 800 WP for *Daphnia magna*. Study Report No. WE-01-165 of Lisec. Brouwers (1995)

The acute toxicity of Sancozeb 800 WP (80% mancozeb) to *Daphnia magna* was assessed in a study performed to OECD guideline number 202 and in compliance with GLP principals. Exposure to the test item was for 48h

at nominal concentrations of 0 (control), 0.1, 0.2, 0.4, 0.8, and 1.6 mg a.s./L. Mean measured concentrations ranged from 89 to 95% of nominal concentrations. The nominal 48h EC_{50} was 0.9 mg a.s./L. This endpoint was concluded as being reliable and acceptable during the EU Annex I renewal of mancozeb as a pesticide.

24 h EC 50 Acute Immobilisation Study of MANCOZEB 80% WDP in *Daphnia magna*. Rakesh M. Patel (1998).

The acute toxicity of MANCOZEB 80% WDP (80% mancozeb) to *Daphnia magna* was assessed in a study performed to OECD guideline number 202 and in compliance with GLP principals. Exposure to the test item was for 24h in static conditions at nominal concentrations of 0 (control), 0.003, 0.006, 0.012, 0.024, and 0.048 mg product/L. The endpoint as reported in the study summary was a nominal EC_{50} = 0.014 mg test substance/L equivalent to 0.0112 mg a.s./L. As the reported mancozeb content of the formulation used was 80%, this conversion from formulation to active concentration is accurate. The nominal NOEC was 0.024 mg a.s./L. Measurements of test substance at 0 and 24h presented in the study report were below the detectable limit as the detectable limit was greater than the highest concentration of mancozeb tested. However, measurements were also made in a solution with a nominal concentration of 8.0 mg a.s./L which resulted in detected levels of mancozeb of 7.523 mg at 0h (94% of the nominal) and 7.197 at 24h (90% of the nominal). Therefore it may have been possible that the test substance replicates maintained mancozeb concentrations within ±20% of the nominal over the 24 hours. In the absence of any further measurements, the endpoint is therefore reported as a nominal value. It is accepted that there are shortcomings to this study; however it is the lowest endpoint for acute daphnia available. In addition, the study report demonstrated a strong dose-response with mancozeb concentration and immobility. The endpoint is also within a factor of 10 of the next lowest endpoint from an acceptable 48h daphnia study. The RMS has concluded that the endpoint from this study should be considered in the hazard assessment to ensure that the assessment is suitably protective.

48-hour acute toxicity of Penncozeb 80 WP to Daphnia magna (OECD-immobilisation test) (Wuthrich V., 1993)

The acute toxicity of Penncozeb 80 WP (82% mancozeb) to *Daphnia magna* was assessed in a study performed to OECD guideline number 202 and in compliance with GLP principals. Exposure to the test item was for 48h in static conditions at nominal concentrations of 0.063, 0.125, 0.25, 0.5, and 1 mg product/L. Mean measured concentrations were above 80% of the nominal concentrations. The nominal EC_{50} was 0.39 mg a.s./L. The EC_{50} was concluded as being reliable and acceptable during the EU Annex I renewal of mancozeb as a pesticide.

Acute toxicity of FORTUNA 800 WP to *Daphnia magna*. Sponsored by Ameloria Ltd & Zenith Crop Science S.A. Bioagri Report No A0317.206.242.06. Sesso, J.N. (2007a)

The acute toxicity of FORTUNA 800 WP (79.4% mancozeb) to *Daphnia magna* was assessed in a study performed to OECD guideline number 202 and in compliance with GLP principals. Exposure to the test item was for 48h in static conditions at nominal concentrations of 0 (control), 0.65, 1.3, 2.5, 5, and 10 mg product/L. Mean measured concentrations at 48h were 0 (control), 0.55, 1.27, 2.27, 4.63, and 11.82 mg product/L, ranging between 85 and 118% of the nominal concentrations. The nominal 48h EC_{50} was 1.1 mg a.s./L and the NOEC was 0.52 mg a.s./L. The EC_{50} was concluded as being reliable and acceptable during the AIR 3 renewal of mancozeb as a pesticide.

Acute toxicity of Dithane M-45 to the water flea, *Daphnia magna*, determined under flow-through conditions. Rhodes, J.E. (2000).

The acute toxicity of Dithane M-45 (mancozeb content: 81.3%) to *Daphnia magna* was assessed in a study performed to OECD guideline number 202 and in compliance with GLP principals. Exposure to the test item was for 48h in flow-through conditions at nominal concentrations of 0 (control), 0.25, 0.50, 1.0, 2.0, and 4.0 mg a.s./L. Mean measured concentrations ranged from 90 to 116% of the nominal. Despite this, endpoints were reported as mean measured concentrations. This is considered a conservative approach and is therefore considered acceptable by the RMS. The mean measured EC_{50} of 3.8 mg a.s./L was concluded as being reliable and acceptable during the AIR 3 renewal of mancozeb as a pesticide.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

The algal study (Forbis, 1990) was considered not valid in addendum 1 to the original DAR, due to a lack of analytical verification (details on this are vague in the DAR). However, as no other valid algal studies were available at the time the endpoint from this study was used in the pesticide risk assessment and also for hazard classification for the DAR. For the current renewal assessment the RMS requested of the applicant that reviewed, mean-measured endpoints be calculated for this and the new *Lemna* study. These were provided and have been presented in the summaries below, as well as in table 25. They are considered appropriate for use in the risk assessment and hazard classification. In addition, the applicant later submitted a new algal study on the species *P. subcapitata* which has been evaluated and considered acceptable for use in the risk assessment and hazard classification. The results presented in table 25.

Mancozeb TK: Toxicity to *Pseudokirchneriella subcapitata* in an Algal Growth Inhibition Test; Ibacon project No. 121001210 Börschig, & Sonntag, 2017.

A study on the toxicity of Mancozeb Technical (purity: 86.1%) to the algal species *Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata*) was performed to OECD guideline number 201 and in compliance with GLP principals. Exposure to the test item was for 72h in static conditions at nominal concentrations of 100, 320, 100, 32, 10, and 0 (control) µg test item/L. This corresponds to 861, 275.5, 86.1, 27.6, and 8.61 µg a.s./L. Measurements of test substance concentration were made at 0h and every 24h throughout the study. Mean measured concentrations (geometric mean) during the study were 262, 71.5, 20.5, 6.32, and 2.01 µg a.s./L., relating to 6-102% of nominal concentrations (based on actual measured concentrations). Based on geometric mean-measured concentrations, the Growth rate E_rC_{50} was 50.9 µg a.s./L, the E_rC_{10} was 16.0 µg a.s./L, and the NOEC was 2.01 µg a.s./L. These values were considered acceptable for use in the risk assessment for the AIR 3 renewal of mancozeb as a pesticide and will also be used for hazard classification.

Acute toxicity of Dithane® M-45 fungicide to *Selenastrum capricornutum* Printz. Forbis, A. (1990)

A study on the toxicity of Dithane M-45 (82.4% mancozeb) to the algal species *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata*) was performed to 'US.EPA 1984 and ASTM 1984 standard procedures for the acute toxicity testing of freshwater green algae', and in compliance with GLP principals. Exposure to the test item was in static conditions for 120h at nominal formulation concentrations of 0 (control), 0.033, 0.065, 0.13, 0.25, and 0.5 mg a.s./L. Measurements of the test substance were made throughout the study and ranged from 65-75% of the nominal at 0h, and 1.3-7.3% of the nominal at 120h. The initial measured EC50 used for the risk assessment was 0.044 mg a.s./L. The initial measured NOEC was 0.023 mg a.s./L. Although the study was originally considered not valid during the EU Annex I renewal of mancozeb, the endpoints quoted above were used in the risk assessment in addendum 1 to the DAR. The RMS determined that it was possible to calculate a valid (mean measured) endpoint from this study (according to the current EFSA opinion; EFSA technical Report, 2015. Ch. 3.1). The applicant provided these values which were calculated to be: E_rC_{50} = 32.2 µg a.s./L (mm), E_rC_{10} = 9.05 µg a.s./L (mm), E_yC_{50} = 8.79 µg a.s./L (mm), and EyC10 = 3.54 µg a.s./L (mm). These values were considered acceptable for use in the risk assessment for the AIR 3 renewal of mancozeb as a pesticide and will also be used for hazard classification.

A study for the toxicity of mancozeb on higher aquatic plants (*Lemna minor*) was submitted for the AIR 3 renewal of the active. This study was assessed and considered acceptable after mean measured endpoints were provided by the applicant upon the request of the RMS. A summary is presented below.

Mancozeb 80 WP - Higher plant (*Lemna minor*) growth inhibition test; Huntington Life Sciences project No. ARK0004. Dickinson (2011d).

A 7-day study on the toxicity of Mancozeb 80 WP (purity: 80.5% mancozeb) to the aquatic plant *Lemna minor* was performed to OECD guideline number 221 and in compliance with GLP principals. Exposure to the test item was in semi-static conditions at nominal formulation concentrations of 0 (control), 0.13, 0.43, 1.38, 4.44, 14.2, and 45.5 mg Mancozeb 80 WP/L. Mean measured active concentrations in fresh media samples taken on

days 0 and 5 were 0 (control), 0.0828, 0.303, 0.797, 3.00, 9.98, and 31.9 mg a.s./L. These related to 72-88% of nominal concentrations. Mean measured levels in the spent media samples taken on days 3 and 7 were 0 (control), 0, 0, 0.0057, 0.06, 1.16, and 0.52 mg a.s./L, relating to 0-8% of nominal values. The mean measured (fresh media) E_rC_{50} was 9.56 mg a.s./L. The RMS requested endpoints based on mean measured values of fresh and expired media samples. The applicant provided these values which were calculated to be: E_rC_{50} (fronds number) = 1811 µg a.s./L (mm), E_rC_{10} (fronds number) = 82.2 µg a.s./L (mm), E_rC_{50} (biomass) = 1042 µg a.s./L (mm), and E_rC_{10} (biomass) = 37.1 µg a.s./L (mm). These values were considered acceptable for use in the risk assessment for the AIR 3 renewal of mancozeb as a pesticide and also for hazard classification.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No studies on other organisms were considered valid for use in the hazard assessment.

11.6 Long-term aquatic hazard

Table 26: Summary of relevant information on chronic aquatic toxicity

Guideline and GLP status	Substance (purity in % w/w)	Species	Endpoint Effect	Exposure		Results		Reference	Submitted for original approval (O) or renewal (N)
				Design	Duration	Endpoint	Toxicity (mg a.s./L)		
Fish									
OPPTS 850.1500 GLP	**Mancozeb Tech. (purity: 84.7%)**	***Pimephales promelas***	**Reproduction. Life Cycle Study**	**Flow-through**	**215d**	NOEC **EC_{10}**	0.00135 (mm) **0.00127 (mm)**	**Anonymous, 2012**	**N**
OPPTS 850.1400 GLP	Dithane M-45 (Mancozeb: 78.8%)	*Cyprinodon variegatus*	Growth. Early Life Stage.	Flow-through	39d	NOEC EC_{10}	0.000918 (mm) 0.002878 (mm)	Anonymous, 2011a	N
Similar to OECD 210	Dithane M-45 (Mancozeb: 82.4%)	*Pimephales promelas*	Survival. Early Life Stage.	Flow-through	33d	NOEC EC_{10}	0.0052 (mm) 0.00531 (mm)	Anonymous, 1988e	O
Similar to OECD 210 GLP	Mancozeb Tech. (purity 79.3%)	*Pimephales promelas*	Survival. Early Life Stage.	Flow-through	34d	NOEC EC_{10}	0.00219 (mm) 0.002037 (mm)	Anonymous, 1994c	O
Invertebrates									
Similar to OECD 211 GLP	Dithane M-45 (Mancozeb: 82.4%)	*Daphnia magna*	Reproduction	Flow-through	21d	NOEC EC_{10}	0.0073 (mm) 0.0109 (mm)	Burgess, 1988	O

Guideline and GLP status	**Substance (purity in % w/w)**	**Species**	**Endpoint Effect**	**Exposure**		**Results**		**Reference**	**Submitted for original approval (O) or renewal (N)**
				Design	**Duration**	**Endpoint**	**Toxicity (mg a.s./L)**		
OPPTS 850.1350 GLP	**Dithane M-45 (Mancozeb: 78.8%)**	***Americamysis bahia***	**Survival**	**Flow-through**	**39d**	NOEC **EC_{10}**	0.00164 (mm) **0.00171 (mm)**	**Hicks, 2011b**	**N**
Algae									
OECD 201 GLP	Mancozeb Tech. (purity: 86.1%)	*Pseudokirchneriella subcapitata***	Growth rate	Static	72h	E_rC_{50} E_rC_{10} NOEC	0.0509 (Geomean-measured) 0.016 (Geomean-measured) 0.00201 (Geomean-measured)	Börschig, & Sonntag, 2017	N
Similar to OECD 201 GLP	**Dithane M-45 (Mancozeb: 82.4%)**	***Selenastrum capricornutum*****	**Growth rate**	**Static**	**120h**	**E_rC_{50}** **E_rC_{10}** **NOEC**	**0.0322# (Geomean-measured)** **0.00905 (Geomean-measured)** **n.d.**	**Forbis, 1990##**	**O**

Macrophytes									
OECD 221 GLP	**Mancozeb 80 WP (Mancozeb: 80.5%)**	***Lemna minor***	**Growth rate**	**Semi-static**	**7d**	**E_rC_{50} Frond Number** **E_rC_{10} Frond Number** **E_rC_{50} Biomass** **E_rC_{10} Biomass** **NOEC**	**1.811 (Geomean-measured)** **0.0822 (Geomean-measured)** **1.042 (Geomean-measured)** **0.0371 (Geomean-measured)** **0.0246# (Geomean-measured)**	**Dickinson, 2011d**	**N**

n.d. Not determinable
**Currently known as *Raphidocelis subcapitata*
Re-calculated endpoint by the applicant at the request of the RMS.
Study was considered to be not valid in addendum 1 to the original DAR. However, as no valid studies were available, the endpoint from this study was used in the risk assessment for the DAR. Mean measured values from this study are considered acceptable for the RAR.
The most sensitive, valid acute and chronic classification endpoint for each main taxonomic/trophic group is presented in **bold**.

11.6.1 Chronic toxicity to fish

Long-term fish studies were provided both for the original Annex I approval for mancozeb, and for the AIR 3 renewal of the active. Those considered acceptable at the original approval and those considered acceptable for the current renewal are summarised below.

Mancozeb: Life-Cycle Toxicity Test with the Fathead Minnow, *Pimephales promelas* under Flow-Through Conditions, Anonymous, (2012)

The chronic toxicity of Mancozeb technical (84.8% purity) to the fathead minnow (*Pimephales promelas*) was assessed in a full life-cycle study performed to US EPA OPPTS guideline 850.1500 and in compliance with GLP principals. The parental and offspring generations were exposed to the test item for a total period of 215 days under flow-through conditions at nominal test concentrations of 0 (control), 0.50, 1.0, 2.0, 4.0, and 8.0 µg a.s./L. Mean measured concentrations throughout the test were <MQL (control), 0.382, 0.694, 1.35, 2.58, and 5.05 µg a.s./L. These were within the range of 63-76% of nominal concentrations. Two hundred F0 embryos were used per treatment group. These embryos were allowed to hatch, grow and spawn in the test-substance treated water. Measurements were made of survival, length, wet weight, sublethal effects compared to the control (incl. spinal curvature, resting on bottom, erratic swimming, haemorrhagic, discolouration, and unusual respiration), days till first spawn, number of spawns, and number of eggs at various points throughout the study. F0 female growth was mostly unaffected by treatment concentration. The mean measured NOEC for spinal curvature was 2.58 µg a.s./L, as was the NOEC for the day 167 spawning group. F0 male weight and length on days 118-119 was negatively affected by mancozeb concentration with a NOEC of 2.58 µg a.s./L. This was also the NOEC of F1 survival. Reproduction-related effects were the most sensitive. The number of spawns and percentage of fertile eggs a female produced were negatively affected by mancozeb concentration with mean measured NOECs of 2.58 µg a.s./L. The most sensitive endpoints were the number of eggs per F0 female per day and the cumulative number of eggs produced by F0 minnows, with NOECs of 1.35 µg a.s./L in both cases. The most sensitive mean measured EC_{10} was calculated for reproduction with a value of 1.27 µg a.s./L. The EC_{10} was concluded as being reliable and acceptable during the AIR 3 renewal of mancozeb as a pesticide.

Mancozeb: Early Life-Stage Toxicity Test with the Sheepshead Minnow, *Cyprinodon variegatus*, Under Flow-Through Conditions; Anonymous, (2011a).

The chronic toxicity of Dithane M-45 (78.8% purity) to the Sheepshead Minnow (*Cyprinodon variegatus*) was assessed in an early life stage study performed to US EPA OPPTS guideline 850.1400 and in compliance with GLP principals. 80 embryos per test treatment were exposed to the test substance for 39 days (29 days post-hatch) in flow-through conditions. The nominal test concentrations were 0 (control), 1.9, 3.8, 7.5, 15, and 30 µg a.s./L. Mean measured concentrations were <MQL (control), 0.918, 2.13, 4.46, 9.04, and 19.5 µg a.s./L. Individual measurements ranged from 20-95% of the nominal. Hatchability, survival, length, and wet weight of fish were measured during the test. The mean measured NOECs for hatchability and survival were both 19.5 µg a.s./L. The most sensitive NOEC was growth with a mean measured NOEC of 0.918 µg a.s./L. The most sensitive mean measured EC_{10} was calculated for growth with a value of 2.878 µg a.s./L. This EC_{10} was concluded as being reliable and acceptable during the AIR 3 renewal of mancozeb as a pesticide.

Early life stage toxicity of Dithane® M-45 to Fathead Minnow (*Pimephales promelas*) in a flow-through system. Anonymous. (1988e)

The chronic toxicity of Dithane M-45 (82.4% mancozeb) to the fathead minnow (*Pimephales promelas*) was assessed in an early life stage study performed to 'ASTM (1983) and the Committee on Methods for Toxicity Testing with Aquatic Organisms (1975) protocol ABC 7809' (similar to OECD 210) and in compliance with GLP principals. 80 eggs per test treatment were exposed to the test substance for 33 days in flow-through conditions. The nominal test concentrations were 0 (control), 3.1, 6.2, 12, 25, and 50 µg a.s./L. Mean measured concentrations were 0 (control), 3.5, 5.3, 10, 20, and 43 µg a.s./L. Survival, length, and wet weight of fish were measured during the test. The most sensitive NOEC was survival with a mean measured NOEC of 5.2 µg a.s./L. The most sensitive EC_{10} was calculated for survival with a mean measured value of 5.31 µg a.s./L. The

EC_{10} was concluded as being reliable and acceptable during the EU Annex I renewal of mancozeb as a pesticide.

Early Life-stage Toxicity of Mancozeb to the Fathead Minnow (*Pimephales promelas*) Under Flow-Through Conditions. Anonymous (1994c).

The chronic toxicity of mancozeb technical (79.3% purity) to the fathead minnow (*Pimephales promelas*) was assessed in an early life-stage study performed to 'ABC protocol n° FIFRA 72-4 based on that described in the Proposed Recommended Bioassay Procedure for Egg and Fry stages of Freshwater Fish (USEPA 1972) and Standard Guide for Conducting Early Stage Toxicity Tests for Fish (ASTM 1988)', similar to OECD 210, and in compliance with GLP principals. 120 eggs per test treatment were exposed for 34 days (28 days post-hatch) in flow-through conditions. The nominal test concentrations were 0 (control), 0.30, 0.60, 1.3, 2.5, 5.0, 10, and 20 µg a.s./L. Mean measured concentrations were 0.592, 1.07, 2.19, 4.56, an 7.97 µg a.s./L in the 1.3, 2.5, 5.0, 10, and 20 µg a.s./L test treatments as measured by GC analysis. Measurements were not made of the first two levels as they were reportedly below the Limit of Detection for the analysis. Measurements were also made by LSC analysis in all levels and resulted in test concentrations of 0.236, 0.535, 1.08, 2.37, 4.65, 9.57, and 19.0 µg a.s./L in the 0.3-20 µg a.s./L nominal treatments, respectively. Egg hatchability, survival, growth, and morphological and behavioural effects were measured throughout the study. There was no statistically significant effect of mancozeb concentration on growth or egg hatchability. The most sensitive NOEC was fry survival with a with a mean measured (by GC analysis) NOEC of 2.19 µg a.s./L, or 4.65 µg a.s./L by LSC analysis. The most sensitive EC_{10} was calculated for survival with a value of 2.037 µg a.s./L (by GC analysis). The EC_{10} was concluded as being reliable and acceptable during the AIR 3 renewal of mancozeb as a pesticide.

11.6.2 Chronic toxicity to aquatic invertebrates

Long-term invertebrate studies were provided both for the original Annex I approval for mancozeb, and for the AIR 3 renewal of the active. Those considered acceptable at the original approval and those considered acceptable for the current renewal are summarised below.

Chronic toxicity of Dithane® M-45 to *Daphnia magna* under flow-through conditions. Burgess, D. (1988).

The chronic toxicity of Dithane M-45 (82.4% mancozeb) to *Daphnia magna* was assessed in a study performed to US EPA FIFRA 72.4 (similar to OECD guideline number 202) and in compliance with GLP principals. Exposure to the test item was for 21 days in flow-through conditions at nominal concentrations of 0 (control), 3, 5.9, 12, 25, and 50 µg a.s./L. Mean measured concentrations during the study were 0 (control), 2.9, 7.3, 12, 26, and 53 µg a.s./L. 40 daphnids were used per test concentration. Observations were made of survival, occurrence of first brood, fecundity of adults, and growth (as adult length) throughout the study. The mean measured 21 day EC50 for survival was >53 µg a.s./L and the NOEC was 26 µg a.s./L. This was also the case for daphnid length. There was no significant effect of test treatment on time to first brood. The most sensitive NOEC was for fecundity as number of young produced per adult per day, at 7.3 µg a.s./L. The most sensitive EC_{10} was calculated for reproduction with a mean measured value of 10.9 µg a.s./L. The EC_{10} was concluded as being reliable and acceptable during the EU Annex I renewal of mancozeb as a pesticide.

Mancozeb: Life-Cycle Toxicity Test of the Saltwater Mysid, *Americamysis bahia*, Conducted under Flow-Through Conditions, Mancozeb Task Force Study No. 2010-1, ABC Study No. 66201. Hicks, S.L. (2011b).

The chronic toxicity of Dithane M-45 (78.8% mancozeb) to the saltwater mysid crustacean *Americamysis bahia* was assessed in a study performed to U.S. EPA Ecological Effects Testing Guidelines OPPTS 850.1350 and in compliance with GLP principals. Exposure to the test item was for 28 days in flow-through conditions at nominal concentrations of 0 (control), 0.33, 0.65, 1.3, 2.5, and 5.0 µg a.s./L. Mean measured concentrations during the study were 0 (control), 0.217, 0.625, 0.882, 1.64, and 3.25 µg a.s./L, relating to 66-96% of the nominal concentrations. 90 <24h old mysids were used per test concentration. Observations were made of survival, sublethal effects compared to the control, time to first brood, and body length in the F0 generation. Brood were collected from this generation, which was terminated at 28 days. Body length was measured for the F1 generation, which was terminated at 11 days after the termination of the F0. There was no statistical effect of test concentration on F0 mysid survival at 0-21 days. The mean measured NOEC was 3.25 µg a.s./L.

This was also the case for male and female mysid length for both the F0 and F1 generations. The most sensitive endpoints were F0 survival at 28 days and F1 survival on all days. The mean measured NOECs based on these effects were 1.64 µg a.s./L. The most sensitive EC_{10} was calculated for F0 survival with a mean measured value of 1.71 µg a.s./L. The EC_{10} was concluded as being reliable and acceptable during the AIR 3 renewal of mancozeb as a pesticide.

11.6.3 Chronic toxicity to algae or other aquatic plants

See study summaries for algae and aquatic plants provided under section 11.5.3.

11.6.4 Chronic toxicity to other aquatic organisms

No studies on other organisms were considered valid for use in the hazard assessment.

11.7 Comparison with the CLP criteria

Data on the degradability of mancozeb has been considered in section 11.1 and it is proposed that the active is considered 'not rapidly degradable' for classification purposes (See Table 23). It was also concluded that mancozeb has a low bioaccumulation potential.

The most sensitive acute and chronic endpoints for each main taxonomic/trophic group are presented in Table 25 and 26 in bold.

11.7.1 Acute aquatic hazard

Acute aquatic toxicity data on technical mancozeb are available for fish, invertebrates, and algae. Using the technical endpoints only, the lowest LC/EC_{50} value is the 72h E_rC_{50} of 0.0509 mg a.s./L for *P. subcapitata.* This is > 0.01 but ≤ 0.1 mg/L, and therefore mancozeb should be classified as Aquatic Acute category 1 with an acute M-factor of 10. In addition, acute aquatic toxicity data on ~80% w/w wettable powder formulations with mancozeb are available for fish, invertebrates, algae and aquatic plants. The lowest reliable acute endpoint is the 24h nominal EC_{50} of 0.0112 mg a.s./L for *Daphnia magna*. This is > 0.01 but ≤ 0.1 mg/L, and therefore mancozeb should be classified as Aquatic Acute category 1 with an acute M-factor of 10.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Bioaccumulation

For pesticide approval under EC Reg. 1107/2009, a Log Kow >3 triggers the requirement for a bioconcentration study. Since the Log Kow of mancozeb is < 3 a bioconcentration study is not required. For classification and labelling purposes a substance with Log Kow < 4 may be considered unlikely to bioaccumulate in aquatic organisms. In conclusion, according to the CLP criteria, mancozeb has a low potential for bioaccumulation.

Degradation

Mancozeb does not meet the criteria for 'rapidly degradable' as whilst its primary degradation is very fast, it does not meet the criteria for ultimate degradation, i.e. the formation of CO_2. It is considered that mancozeb meets the criteria for primary degradation. According to The Guidance on the Application of the CLP Criteria (ECHA, 2015); "Data on primary degradation can only be used where it is demonstrated that the degradation products shall not be classified as hazardous to the aquatic environment, i.e. that they do not fulfil the classification criteria." Experimental toxicity endpoints are only available for the mancozeb degradants ETU and EU. Theoretical QSAR endpoints are available for the degradant EBIS. These are summarised in Annex III, Table A3-2.

The lowest acute endpoint for ETU is the *A. bahia* EC_{50} of 11 mg a.s./L which is greater than 1 mg/L, Therefore ETU is not classified as acute category 1 based on this data. The lowest acute endpoint for EU is *O. mykiss*

LC_{50} of >122 mg a.s./L which is greater than 1 mg/L. Therefore EU is not classified as acute category 1 based on this data.

For ETU, adequate chronic data are available for three trophic levels. The lowest chronic endpoint for ETU is the *D. magna* NOEC of 2 mg a.s./L which is greater than 1 mg/L. Therefore ETU does not fulfil the criteria for chronic classification. For EU, chronic data is only available for algae. However, the algal ErC50 and all other available acute endpoints are greater than 100 mg/L. Therefore EU does not fulfil the criteria for chronic classification.

For the other relevant degradants, a request was made to the applicant to provide classification information based on any available, relevant data. Harmonised classifications were available for all relevant degradants with the exception of glycolic acid, for which the applicant performed a calculation of classification based on available lab data. The full response from the applicant is provided in Annex III, and the classification of the degradants is summarised in the table below.

Table 11.7.2: **Acute and chronic classification of the relevant degradants of mancozeb**

Degradant (CAS No.)	Study type	Findings [mg/L]	DT_{50} (or DT_{90}/3.322 where behaviour is biphasic)	**Triggered classification and labelling**	Reference
EBIS (33813-20-6)	--	--	0.5 – 10.9 days[1]	**Aquatic acute hazard cat. 1 (H400)**	legal classification of EBIS in Annex VI of (EC) No 1272/2008 (CLP)
	--	--		**Aquatic chronic hazard cat. 1 (H410)**	
EDA (1,2-Ethanediamine) (107-15-3)	--	--	Not calculable	**No aquatic acute hazard cat.**	legal classification of EDA in Annex VI of (EC) No 1272/2008 (CLP), however self classification as Chronic 3 is included in CLP Inventory.
	--	--		**No aquatic chronic hazard cat.**	
Ethanolamine (2-Aminoethanol) (141-43-5)	--	--	20.2 days	**No aquatic acute hazard cat.**	legal classification of ethanolamine in Annex VI of (EC) No 1272/2008 (CLP)
	--	--		**No aquatic chronic hazard cat.**	
Glycolic acid (79-14-1)	48-h EC_{50}	44.0	2060 days	**No aquatic acute hazard cat.**	Japanese Environment Agency (NR), Acute Immobilization Test of Glycolic acid to Daphnia magna Report Nr. 1998-生22
	21-d NOEC	4.38		**No aquatic chronic hazard cat.**	Japanese Environment Agency (NR), Reproduction Inhibition Test of Glycolic acid to Daphnia magna Report No: 1998-生23
Ethylene glycol (107-21-1)	--	--	11.9 days[2]	**No aquatic acute hazard cat.**	legal classification of ethylene glycol in Annex VI of (EC) No 1272/2008 (CLP)
	--	--		**No aquatic chronic hazard cat.**	
Oxalic acid (possibly M1) (144-62-7)	--	--	37.8 days[3]	**No aquatic acute hazard cat.**	legal classification of oxalic acid in Annex VI of (EC) No 1272/2008 (CLP)
	--	--		**No aquatic chronic hazard cat.**	

[1] = Decline calculated on total amount in water + sediment phase
[2] = DT50 calculated tentatively on 2 sample points
[3] = Study had two incubations; maximum occurred in one incubation at study end, but in the other incubation the substance declined before end to allow tentative DT50 calculation (three sample points from study end)

As demonstrated, the degradant EBIS is classified as hazardous to the aquatic environment, with a classification of Aquatic Acute category 1 and Aquatic Chronic category 1. The applicant provided further case that the classification of EBIS is 'covered by the classification of mancozeb and therefore mancozeb can be assessed as rapidly degradable'. However, the CLP guidance does not provide any information as to whether the degradants have to be classified as more hazardous to the aquatic environment than the parent compound in order to preclude consideration as 'rapidly degradable'. Nor is there guidance to determine if the case provided by the applicant is sufficient to discount the classification of EBIS. Therefore, as a conservative approach, mancozeb will not be classified as 'rapidly degradable' due to the classification of the major metabolite EBIS as hazardous to the aquatic environment. It is also noted that another aquatic degradant, EDA, is self-classified as Chronic 3 according to information on the ECHA dissemination website (349 Notified classifications and labelling according to CLP criteria).

Chronic/long-term toxicity data on technical mancozeb are available for fish and algae. Data on ~80% w/w wettable powder formulations are also available on invertebrates and aquatic plants. The lowest NOEC of all the reliable studies is 0.000918 mg a.s./L from the early life stage study on *Cyprinodon variegates*, however, according to page 490 of ECHA (2015)[1] if EC_{10} values are available for chronic data then these should be used preferentially over the NOEC for the purpose of hazard classification. The lowest reliable chronic EC_{10} is the Full Life Cycle mean measured growth EC_{10} of 0.00127 mg a.s./L for *Pimephales promelas*, using technical mancozeb. This is > 0.001 but ≤ 0.01 mg/L, and therefore since it is considered 'not rapidly degradable', mancozeb should be classified as Aquatic Chronic category 1 with an M-factor of 10.

Chronic data on the technical material are not available on inverbtebrates or aquatic plants, the lowest formulation endpoint is 39-day EC_{10} of 0.00171 mg/L for *Americamysis bahia*. Which would also result in a Chronic 1 classification with a Chronic M-factor of 10. Although this was a relatively simple 80% w/w wettable powder formulation, if the use of formulation data are not appropriate, then using the surrogate approach and the lowest invertebrate active substance EC_{50} of 0.073 mg active substance/L for *D. magna*, would also result in a Chronic 1 classification and Chronic M-factor of 10 for a non-rapidly degradable substance.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Aquatic Acute 1; H400: Very toxic to aquatic life

Acute M-factor = 10

Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects

Chronic M-factor = 10

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Mancozeb is a fungicide with an existing classification in Annex VI of the CLP Regulation as Aquatic Acute1; H400; M-factor 10.

The DS proposed to confirm the existing entry for aquatic acute toxicity and to add classification for aquatic chronic toxicity. Aquatic acute toxicity data on technical mancozeb are available for fish, invertebrates, and algae. The lowest reliable acute value is a 72h E_rC_{50} of 0.0509 mg a.s./L for algae (*Pseudokirchneriella subcapitata)* resulting in a classification as Aquatic Acute 1 (H400) with an M-factor of 10. In addition, aquatic acute toxicity data on ~80% wettable powder formulation with mancozeb are available for all three trophic levels with the lowest 24-h nominal E_rC_{50} value of 0.0112 mg a.s./L for *Daphnia magna* resulting in a classification as Aquatic Acute 1 (H400) with an M-factor of 10.

Chronic aquatic toxicity data on technical mancozeb for invertebrates are not available and a chronic EC_{10} of 0.00127 mg a.s./L for fish (*Pimephales promelas)* results in classification as Aquatic Chronic 1 (H410) with an M-factor of 10. The surrogate approach was applied by the DS with the lowest invertebrate active substance EC_{50} of 0.073mg a.s./L for *Daphnia magna*. Mancozeb is not considered rapidly degradable for classification purposes; consequently, the chronic hazard classification would result in Aquatic Chronic 1 (H410) with an M-factor of 10.

The measured water solubility of mancozeb is 0.2 mg/L (at 20°C and pH 4-5, 6-8), and 0.3 mg/L (at 20°C and pH 9-10), which indicate that mancozeb is poorly soluble in water. Mancozeb is not anticipated to dissociate in water (confirmed by conductometric method).

Degradation

The hydrolysis of mancozeb was tested in four studies - two GLP studies conducted according to OECD TG 111, and two according to US EPA subdivision N guideline 161-1 (similar to OECD TG 111). The studies showed rapid degradation of mancozeb. Three of the four studies showed DT_{50} values of 0.6 hours to 1.5 days (normalised to 12°C), while one of the studies demonstrated a DT_{50} value of 2.7 – 6.0 days (normalised to 12°C). Mancozeb degradation seems to be pH dependent in two of four studies (faster degradation at acid pH, slower degradation at alkaline pH), but in the two other studies, either pH dependence was not demonstrated, or a slower alkaline degradation was not found. The occurrence of metabolites in the hydrolysis studies was pH dependent. Ethylenethiourea (ETU) was formed at high levels at all pH tested with >90% at pH 4-5, 57- 87% at pH 7 and 57 – 90% at pH 9. N, N´-Ethyleneurea (EU) was formed up to 6% AR at pH 4, 13% AR at pH 7 and 11-55% AR at pH 9. Ethylenebisisothiocyanate sulfide (EBIS) was less affected by pH, with a maximum of 33% AR at pH4, 4.41%AR at pH 7 and 30%AR at pH 9.

In an aqueous photolysis study (guidelines not stated, predates GLP), mancozeb decomposed completely within 3 hours in pH 8.8 buffer. Irradiated and dark control

samples showed a similar behaviour, indicating that the major decomposition routes were hydrolysis and oxidation, not photolysis.

A ready biodegradability test (OECD TG 301B - CO2 Evolution Test, GLP) conducted on a commercial formulation containing 80% w/w of mancozeb resulted in a degradation of 5-6% in 36 days. This study indicated that the mancozeb formulation is not readily biodegradable.

A study on an aerobic mineralisation of mancozeb in surface water (OECD TG 309, GLP) was conducted in fresh water from pelagic water system at 20°C and pH of 8.81 in the dark using two dose rates of ^{14}C-mancozeb (10 and 100 µg/L). The choice of sampling interval at the beginning of the study, i.e. the second sample was 3 days after treatment (DAT). It is stated by the study´s author, that the main focus of the study was to investigate the behaviour of the metabolites, rather than to quantify a decline of mancozeb. Therefore the second sampling time was 3 DAT. As expected, mancozeb declined by >90% and was not detected. It was concluded that Mancozeb in this study had a DT_{90} <3 days at 20°C (DT_{90} <5.7 days normalised to 12°C). There was a maximum of 16.8% AR as CO_2 at 60 days (study termination) suggesting a little mineralisation in this study. Due to lack of frequency of initial sampling, it was possible that peak formation of the rapidly formed metabolite EBIS (as seen in the aerobic water/sediment study) may had been missed, given that the peak occurrence was approximately 6% at 3 DAT but it did not trigger the criteria of >5% at two consecutive times. ETU was formed at maximum 35% AR at 3 DAT; EU at 41.2% AR at 60 DAT. The other metabolites were also found - ethanolamine (max 15.4% AR at 14 DAT); glycolic acid (15.5% AR at 28 DAT); ethylene glycol (24.69% AR at 49 DAT) and unidentified M1 (max 11% AR at 60 DAT).

There were two aquatic water/sediment studies available for mancozeb which were conducted according to EPA guideline 162-3 & 162-4 (similar to OECD TG 308) and GLP by the same laboratory. The metabolism of [^{14}C]-Mancozeb was studied at 20±1°C in the dark, in a river and a pond aquatic system for 105 – 106 days. Mancozeb declined rapidly in the whole systems. To aid the analysis of mancozeb, the water samples for HPLC analysis were subject to a complexation step using EDTA/TBAH (ethylenediamintetraacetic acid/tetrabuthylammonium hydroxide), which converts mancozeb to nabam, the sodium salt of ethylenebisdithiocarbamate and a number of other peaks being termed the "complexed fractions". Degradation of mancozeb can be expressed as the degradation of the main complex fraction (i.e. nabam, the sodium salt of ethylenebisdithiocarbamate) or as the sum of the complexed fractions:

- DT_{50} values expressed as the main complexed fraction were in the range of 0.17 – 1.14 days (normalised to 12°C) for the whole water sediment system.
- DT_{50} values expressed as the sum of complexed fractions were in range of 0.59 – 3.41 days (normalised to 12°C).

Three major metabolites were identified:

- EBIS: max total system 8.9 – 30.9% AR after 0 – 0,25 days,
- ETU: max total system 33.6 – 51.6% AR after 2 days,
- EU: max total system 30.7 – 43.5% AR after 7 -59 days.

Several other metabolites at >10% AR were also detected:

- Hydantoin: max total system 11.7% AR at 14 days
- Unknown degradant 2: max total system 15.2% AR at 14 day

Ultimate degradation was slower with mineralisation in the range of 17.6 – 57.8% AR at 105/106 DAT in water sediment studies. Unextracted residues were in range of 35.4 - 43.6% AR at study termination.

Overall, the DS concluded that mancozeb does not meet the criteria for rapid degradation. Although mancozeb undergoes rapid primary degradation with a range of degradants formed at high levels, an ultimate degradation in biologically active water systems is relatively slow with 16.8% AR as CO_2 after 60 days in a study on aerobic mineralisation in surface water and with 17.6 – 57.8% AR at 105/106 DAT in two water/sediments studies.

Bioaccumulation

The experimentally derived log K_{ow} for mancozeb is 2.3 (pH 6-10); this is less than the trigger value of 4 given in the CLP Regulation. No experimental bioconcentration data was available. The DS concluded that mancozeb has a low bioaccumulation potential in the aquatic environment.

Aquatic toxicity

The DS considered that toxicity endpoints concluded from formulations studies (reported in terms of active substance concentrations) could be used in support of the classification of the active substance. Studies conducted with technical mancozeb are available for three taxa with respect to acute ecotoxicity and for fish and algae with respect to chronic toxicity. Studies conducted with ~80% wettable powder formulation of mancozeb are available for all three taxa with respect to acute ecotoxicity, and for aquatic invertebrates and aquatic plants for chronic ecotoxicity. A summary of the relevant information on aquatic toxicity is presented the table below.

Summary of the most relevant information on aquatic toxicity on technical mancozeb and ~80% wettable powder formulation of mancozeb (in grey). The key data are highlighted in bold.

Test substance and guideline	**Species, test conditions**	**Endpoint**	**Results**	**Reference**
Fish				
Mancozeb Tech. Purity: > 90% OECD TG 203, GLP	*Oncorhynchus mykiss* Acute, semistatic, 96 h	Mortality	96-h LC_{50}: 0.074 mg a.s./L (mm) Nominal concentration: 0, 0.18, 0.32, 0.56 and 1.0 mg a.s./L Measured concentration : 22 – 53% of the nominal	Anonymous, 1987d
Mancozeb Tech. Purity: > 90% OECD TG 203, GLP	*Lepomis macrochirus* Acute, semistatic, 96 h	Mortality	96-h LC_{50}: 0.083 mg a.s./L (mm) Nominal concentration: 0, 056, 0.1, 0.18, 0.32 and 0.56 mg a.s./L Measured concentration : 14 – 44.5% of the nominal	Anonymous, 1987e
Mancozeb Tech. Purity: > 90%	*Oncorhynchus mykiss* Acute, semistatic, 96 h	Mortality	96-h LC_{50}: 0.088 mg a.s./L (mm)	Anonymous, 1997e

OECD TG 203, GLP			Nominal concentration: 0, 0.2, 0.4, 1.0, 2.1 and 4.7 mg a.s./L Mean measured concentration calculated as geometric mean of both fresh and 24h spent media	
Penncozeb 80 WP (mancozeb:82%) OECD TG 203, GLP	*Oncorhynchus mykiss* Acute, flow-through, 96 h	Mortality	96-h LC_{50}: 0.15 mg a.s./L (mm) Nominal concentration: 0, 0.1, 0.17, 0.31, 0.56 and 1.0 mg product/L	Anonymous, 1993a
Mancozeb Tech. Purity: 84.7% US EPA OPPTS 850.1500, GLP	***Pimephales promelas*** **chronic**, flow-through, 215 d	Reproduction. Life Cycle Study	NOEC: 0.00135 mg a.s./L (mm) **EC_{10}: 0.00127 mg a.s./L (mm)** Nominal concentration: 0, 0.50, 1.0, 2.0, 4.0 and 8.0 µg a.s./L Mean measured concentration: within the range of 63-76% of nominal concentration	Anonymous, 2012
Mancozeb Tech. Purity: 79.3% similar to OECD TG 210, GLP	Fathead Minnow *(Pimephales promelas)* chronic, flow-through, 34 d	Survival. Early Life Stage	NOEC: 0.00219 mg a.s./L (mm) EC_{10}: 0.002037 mg a.s./L (mm) Nominal concentration: 0, 0.30, 0.60, 1.3, 2.5, 5.0, 10, and 20 µg a.s./L	Anonymous, 1994c
Invertebrates				
Mancozeb Tech. Purity: > 90% OECD TG 202, GLP	***Daphnia magna*** **Acute**, *static, 48h*	Immobilisation	**48-h EC_{50}: 0.073 mg a.s./L (measured)** Nominal concentration: 0, 0.01, 0.018, 0.032, 0.56, 0,1, 0.18, 0,32, 0,56 and 10 mg a.s./L	Douglas et al., 1988
Mancozeb 80% WDP (Mancozeb:80%) OECD TG 202, GLP (**strictly not valid, short study duration)	*Daphnia magna* Acute, static, 24h	Immobilisation	24-h EC_{50}: 0.0112 mg a.s./L (nominal) Nominal concentration: 0, 0.003, 0.006, 0.012, 0.024, and 0.048 mg a.s./L Measurement at 0, 24h bellow the detectable limit	Rakesh M., Patel, 1988

Dithane M-45 (Mancozeb: 82.4%) Similar to OECD TG 211, GLP	*Daphnia magna* Chronic, flow-through, 21d	Reproduction	NOEC: 0.0073 mg a.s./L (mm) EC_{10}: 0.0109 mg a.s./L (mm) Nominal concentration: 0, 3, 5.9, 12, 26 and 53 µg a.s./L	Burgess, 1988
Dithane M-45 (Mancozeb: 78.8%) US EPA OPPTS 850.135, GLP	*Americamysis bahia* Chronic, Flow-through, 39d	Survival	NOEC: 0.00164 mg a.s./L (mm) EC_{10}: 0.00171 mg a.s./L (mm)	Hicks, 2011b
Algae/Macrophytes				
Mancozeb Tech. Purity: 86.1% OECD TG 201, GLP	***Pseudokirschneriella subcapitata**** **short-term**, static, 72h	Growth rate	**72-h E_rC_{50}: 0.059 mg a.s./L (geomean measured)** 72-h E_rC_{10}: 0.016 mg a.s./L (geomean measured) 72-h NOEC: 0.00201 mg a.s./L (geomean measured) Nominal concentration: 0, 10, 32, 0.032, 0.56, 10, and 20 mg a.s./L Measured concentration: 6-102% of the nominal	Börschig, & Sonntag, 2017
Mancozeb 80 WP (Mancozeb 80.5%) OECD TG 221, GLP	*Lemna minor* Chronic, semi-static, 7d	Growth rate	E_rC_{50} (grows rate): 1.811 mg a.s./L (geomean measured) E_rC_{10} (grows rate): 0.0822 mg a.s./L (geomean measured) NOEC: 0.024.6 mg a.s./L (geomean measured) Nominal product concentrations: of 0.13, 0.43, 1.38, 4.44, 14.2 and 45.5 mg Mancozeb 80 WP/L	Dickinson, 2011d

*Currently known *as Raphidocelis subcapitata*

**EU Mancozeb TF comment during PC

Acute toxicity

Three acute fish studies on mancozeb (purity > 90%) were performed according to OECD TG 203 and in accordance with the principles of GLP. Two studies using Rainbow trout (*Oncorhynchus mykiss)* and one study using Bluegill Sunfish (*Lepomis macrochirus)* were conducted under semi-static conditions over a period of 96 hours. Measured concentration

(every 24 hours) indicated instability of the test substance in water and showed a deviation of more than 20% over the nominal value. The reported 96-h LC_{50} values were 0.074 mg a.s./L (mean measured) for *Oncorhynchus mykiss* in the first test, 0.083 mg a.s./L (mean measured) for *Lepomis macrochirus* in the second test, and 0.088 mg a.s./L (geomean measured) for *Oncorhynchus mykiss* in the third test. The results of three acute toxicity studies on fish indicated that the sensitivity of both species tested is very similar, with the lowest 96-h LC_{50} value of 0,074 mg a.s./L (mean measured) for *Oncorhynchus mykiss*.

An acute toxicity study on mancozeb (purity > 90%) with *Daphnia magna* was conducted under static conditions following OECD TG 202 and according to GLP principles, resulting in 48-h E_rC_{50} of 0.073 mg a.s./L (mean measured).

A static algal growth inhibition study on mancozeb (purity 86.1%) with *Pseudokirschneriella subcapitata* was conducted following OECD TG 201 and according to GLP principles. The 72-h E_rC_{50} was reported to be 0.059 mg a.s./L (geomean measured), the 72-h E_rC_{10} of 0.016 mg a.s./L (geomean measured) and the 72-h NOEC of 0.00201 mg a.s./L (geomean measured).

Chronic toxicity

For fish, two chronic flow-through studies on mancozeb were available.

In a 215-day life-cycle toxicity test on mancozeb (purity 84.7%) performed according to US EPA OPPTS guideline 850.1500 and GLP, fathead minnow (*Pimephales promelas*) hatchability, survival, growth and morphological and behavioural effects in fish in parental generation (F0) and F1 generation were evaluated.

Conclusion: Growth and survival of F0 female was mostly not influenced by the treatment, except for cases of spine curvature and the survival the study day 167 spawning group (NOEC: 0.00258 mg a.s./L). Negative effects of the treatment with test substance were observed in F0 males weight and length on study days 118-119; F_1 survival; reproduction-related endpoints (number of spawns and the percentage of fertile eggs a female produced (NOEC: 0.00258 mg a.s./L). The most sensitive endpoints were number of eggs per F0-female minnow per day and a cumulative number of eggs produced by the F0-female minnows, with the lowest NOEC of 0.00135 mg a.s./L (mm) for both endpoints. The most sensitive EC_{10} value of 0.00127 mg a.s./L (mm) was calculated for the reproduction from the number of eggs per female per day.

In a 34-day fish early life stage flow-through test on mancozeb (purity 79.3%) performed according to the methodology similar to OECD TG 210 and GLP, fathead minnow *(Pimephales promelas)* hatchability, survival, growth and morphological and behavioural effects in early life stages were evaluated. There was no statistically significant effect on growth and egg hatchability. The most sensitive endpoint was fry survival, with a 34-d NOEC of 0.00219 mg a.s./L (mm, by GLC analysis) and 34-d EC_{10} of 0,002037 mg a.s./L (mm by GLC analysis).

One algae test was described in relation to short-term toxicity. In the *Pseudokirchneriella subcapitata* study, the 72-h E_rC_{10} was 0.016 mg a.s./L (geomean measured) and the 72-h NOEC was 0.00201 mg a.s./L (geomean measured).

Comments received during public consultation

Comments were received during public consultation (PC) from 5 MSCAs and 1 company - EU Mancozeb TF. All 5 MSCAs were in support of proposed classification and labelling regarding aquatic hazards (acute and chronic) and M-factors, and three of them have stated explicitly that mancozeb cannot be considered as rapidly degradable.

The EU Mancozeb Task Force pointed out that the Patel (1998) aquatic toxicity study should be disregarded as strictly not valid (not according to OECD TG 202) and described uncertainties of this study due to short duration (24 hours instead of 48 hours) and no analytical validation of the test substance concentration. They had noted the Patel (1998) study should not be used in the CLH assessment and that the Douglas (1988) study should be used with the most sensitive endpoint (LC50 = 0.073 mg a.s./L).

The EU Mancozeb Task Force expressed the view that Mancozeb should be assessed as rapidly biodegradable. The argumentation was as follows:

"In conclusion, only the metabolite EBIS must be considered as environmentally hazardous in the evaluation of ready biodegradability of the parent compound. Regarding its environmental behaviour, EBIS is rapidly formed from mancozeb and maximum half-lives of < 1 day are reported for the water phase before the metabolite will be converted to the non-toxic ETU. Longer half-lives, as given in the table above, are related to the total water-sediment system. For the compartment water EBIS can therefore be classified as rapidly degradable too.
Moreover, looking at the known mode of action of EBDCs like mancozeb, this is explained in the literature by the transient formation of early intermediate products like EBIS (often named DIDT, forming other intermediates like ethylene diisothiocyanate) and their unspecific reaction with cell constituents such as thiol-containing enzymes (Ludwig & Thorn, 1960; Kaars Sijpesteijn, 1984).
Hence, as EBDCs like mancozeb act via their early intermediate products, the classification of mancozeb into Aquatic Acute Hazard Cat. 1 (H400) and Aquatic Chronic Hazard Cat. 1 (H410) already considers the aquatic toxicity of EBIS (which has the same classification). Therefore, the consideration of the classification of both molecules (mancozeb and EBIS) within the evaluation of the rapid degradability of mancozeb would overestimate the risk for the environment.
In conclusion, apart from EBIS the aquatic metabolites of mancozeb are not hazardous to aquatic environment. The classification of EBIS is already covered by the classification of mancozeb and therefore mancozeb can be assessed as rapidly degradable."
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Ludwig, R.D. & Thorn, G.D. (1960). Chemistry and mode of action of Dithiocarbamate fungicides. Adv. Pest Control Res. 30, 219-252."

As for the Patel study, the DS responded that the relevance of this study is open for interpretation. For the purpose of CLH, full consideration of this study had been provided by the DS including short-comings of this study and the support for use in the risk assessment. It was noted that this will have no outcome on the final acute classification or M-factor.

Regarding degradation of EBIS, the DS pointed out that EBIS was found in the sediment in the OECD 308 water/sediment studies, although at relatively low levels. Sediment analysis was not performed during the first day of the study when the peak concentrations were detected in the water phase. The DS concluded that the whole system half-lives were more representative of degradation and water phase values were strictly representative of dissipation from the water phase, not degradation in the water phase. For the purpose of classification, degradation rather than dissipation was considered.

Regarding biodegradation, the DS considered that according to the guidance on application of the CLP criteria (ECHA, 2015), mancozeb should not be classified as rapidly degradable based on the classification of degradant EBIS.

Assessment and comparison with the classification criteria

Degradation

Mancozeb is not readily biodegradable (5-6% degradation in 36 days). Hydrolysis is rapid, ranging from 0.6 hour to 6.0 days. Several hydrolysis products were detected, including EBIS with maximum concentration of 33% at pH 4, 41% at pH 7 and 30% at pH 9. EBIS has a harmonized classification as Aquatic Acute category 1 and Aquatic Chronic category 1. Although the available results showed that mancozeb undergoes rapid hydrolysis with a half-life <16 days, the degradation product EBIS fulfils the criteria for classification as hazardous for the aquatic environment.

Rapid primary degradation of mancozeb was observed in surface water (OECD TG 309) with DT_{90} <5.7 days normalised to 12°C. Mineralization reached a maximum of 16.8% AR as CO_2 at 60 days. In two water sediment simulation studies, DT_{50} normalised to 12°C ranged from 0.59 to 3.41 days and the mineralisation ranged from 17.6 AR to 57.8% AR at 105/106 days. Three major degradants were identified (EBIS - max total system 8.9 - 30.9% AR after 0 - 0.25 days; ETU- max total system 33.6 – 51.6% AR after 2 days and EU - max total system 30.7 – 43.5% AR after 7 -59 days), but also other degradants were found at levels >10% AR including one unknown degradant. The degradation data showed that mancozeb does not ultimately degrade sufficiently either to CO_2 or to non-hazardous degradants in whole water sediment systems.

Overall conclusion on degradation: Based on available data, mancozeb is not degraded (abiotically and/or biotically) in the aquatic environment to a level of > 70% within a 28 day window or transformed to non-classifiable product. Consequently, mancozeb is considered not rapidly degradable for the purpose of classification and labelling.

Aquatic Bioaccumulation

Mancozeb had a low potential to bioaccumulate. There was no experimental BCF for fish available. The experimental log K_{ow} of 2.3 is below the CLP cut-off value of 4.

Aquatic toxicity

Aquatic acute toxicity data on mancozeb are available for fish, invertebrates and algae (table X). Acute endpoints for fish, invertebrates and algae lie in the range of $0.01 < L(E)C_{50} \leq 0.1$. The lowest acute toxicity value is a 72-h E_rC_{50} of 0.059 mg/L in algae (*Pseudokirschneriella subcapitata,* currently known *as Raphidocelis subcapitata).* According to Tables 4.1.0(a) and 4.1.3 of the CLP guidance, mancozeb should be classified as Aquatic Acute 1 with an acute M-factor of 10.

Aquatic chronic toxicity data on Mancozeb is available for two trophic levels, fish and algae. In the absence of adequate long–term toxicity data for aquatic invertebrates, the surrogate method is applied as recommended in the CLP guidance sections 4.1.3.3 and table 4.1.0. The substance is considered not rapidly degradable and does not fulfil the criteria for bioaccumulation potential.

Classification based on adequate chronic toxicity data: Algae long-term testing provides a 72-h NOEC of 0.00201 mg a.s./L. There are two long term studies in fish (*Pimephales promelas*) available that provide a 215-d EC_{10} of 0.00127 mg/L and 34-d EC_{10} of 0.002037 mg/L. The lowest chronic toxicity value 215-d EC_{10} of 0.00127 mg/L for fish is between 0.001 and 0.01 mg/l and the substance is not rapidly degradable. According to Tables 4.1.0(b)(i) and 4.1.3 of the CLP guidance, mancozeb should be classified as Aquatic Chronic 1 with a chronic M-factor of 10.

Classification based on surrogate data for aquatic invertebrates: The lowest acute toxicity value is a 48-h EC_{50} of 0.073 mg/L for *Daphnia magna*. This is in the range of $0.01< L(E)C_{50} \leq 0.1$ and the substance is not rapidly degradable. According to Tables 4.1.0(b)(iii) and 4.1.3 of CLP guidance, mancozeb should be classified as Aquatic Chronic 1 with an acute M-factor of 10. In case where chronic data are not available and Table 4.1.0(b)(iii) is used for defining long-term aquatic hazard, the resulting M factor derived for acute aquatic hazard classification is also applied to the long – term aquatic hazard classification.

Overall, RAC agrees with the DS proposal to confirm the current classification for aquatic acute toxicity as **Aquatic Acute 1 with an acute M-factor of 10 and to add a classification for aquatic chronic toxicity as Aquatic Chronic 1 with a chronic M-factor of 10**.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

Not considered in this report.

12.1.2 Comparison with the CLP criteria

Not considered in this report.

12.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Not evaluated. No classification proposed.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON MANCOZEB (ISO); MANGANESE ETHYLENEBIS(DITHIOCARBAMATE) (POLYMERIC) COMPLEX WITH ZINC SALT

13 ADDITIONAL LABELLING

Additional labelling is not required.

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15 ANNEXES

ANNEX I: MANCOZEB AND THYROID CARCINOGENICITY: MODE OF ACTION ANALYSIS USING THE WHO/IPCS MOA FRAMEWORK

ANNEX II: EVALUATION OF DEVELOPMENTAL TOXICITY, POSSIBLE MODES OF ACTION AND RELEVANCE TO HUMANS

ANNEX III: SUPPLEMENTARY DATA ON THE DEGRADANTS OF MANCOZEB

ANNEX IV: INFORMATION ON THE FORMULATION USED IN STUDIES WHICH WERE CRITICAL TO THE ENVIRONMENTAL CLASSIFICATION (SEPARATE DOCUMENT)

ANNEX V: CONFIDENTIAL REFERENCES (SEPARATE DOCUMENT)

ANNEX I: MANCOZEB AND THYROID CARCINOGENICITY: MODE OF ACTION ANALYSIS USING THE WHO/IPCS MOA FRAMEWORK

Problem formulation

Administration of mancozeb to rats for 2 years in a carcinogenicity study, resulted in an increased incidence of thyroid follicular tumours (adenomas and carcinomas) at the highest dose of 750 ppm (30 to 40 mg/kg bw/day) (Anonymous, 1990a). Another carcinogenicity study conducted at lower doses (highest dose of 454 ppm; 16 to 20 mg/kg bw/day) did not produce thyroid tumours in rats (Anonymous, 1992a). The tumour incidences are shown in Table A1-1. A carcinogenicity study in mice produced no thyroid tumours (Anonymous, 1991a). The thyroid gland and thyroid hormonal system are the target for mancozeb in short-term and long-term toxicity studies in rats, mice and dogs. The effects on the thyroid hormonal system are caused by the metabolite of mancozeb, ethylenethiourea (ETU). In mammals metabolism of mancozeb to ETU is approximately 7% by weight. Exposure of different species to ETU may differ due to differences in the down-stream metabolism of ETU, although there is some contradictory evidence on this. This leads to quantitative differences in effects on the thyroid across different species, rats being the most sensitive.

Perturbation of the thyroid hormonal system is a common chemically-induced effect and there are several possible mechanisms by which this may occur. The mode of action (MoA) for ETU is via inhibition of the thyroperoxidase enzyme (TPO) in the thyroid gland. TPO is a key enzyme involved in the synthesis of thyroid hormones, thus inhibition results in decreased production of the thyroid hormones T4 (thyroxine) and T3 (triiodothyronine). This in turn, results in disturbance of the hypothalamus-pituitary-thyroid axis (HPT) as the hormonal feedback control mechanisms attempt to adjust thyroid hormone concentrations to normal endogenous levels.

Humans have qualitative and quantitative differences in thyroid physiology and response compared to rodents and therefore the carcinogenic effects of mancozeb on the thyroid gland in rats may have low relevance for humans exposed to mancozeb. In addition, exposure of humans to ETU from mancozeb may be lower than rats because of greater metabolism of ETU to inactive products. This analysis uses the Human Relevance Framework (HRF) to help understand whether mancozeb-induced thyroid tumours seen in rats indicate a risk to humans. The analysis includes evidence for the MoA, qualitative and quantitative differences in thyroid physiology between experimental animals and humans, the role of metabolism and possible alternative MoAs. It will provide an answer to the question "**Are thyroid tumours in rats caused by mancozeb, relevant for humans who may be exposed to mancozeb?"**

Table A1-1: Thyroid follicular tumour incidences in rats administered mancozeb

Reference	Dose mancozeb ppm (mg/kg bw/day; males/females)	Tumour incidence (% tumour bearing animals, day 365 -734)	
		Males	Females
Anonymous (1990a)	0	0A, 0C [a]	1.6A, 0C
	20 (0.7/1.1)	1.6A, 0C	1.7A, 0C
	60 (2.3/3.1)	1.7A, 3.3C	1.6A, 0C
	125 (4.8/6.7)	0A, 3.4C	1.6A, 1.6C
	750 (30.9/40.2)	32.8A*, 23.0C*	9.8C*, 6.6C*
Hooks *et al* (1992a)	0 to 454 (0-16.8/20.8)	No tumours observed at any doses	

[a]A=adenomas, C=carcinomas

*Statistically significant (P ≤0.05 as described in the study report).

Hypothesised mode of action for the induction of thyroid follicular tumours in rats by mancozeb

A large number of chemicals cause disturbance of the thyroid hormonal system in experimental animals via several MoAs including both direct and indirect effects on thyroid hormones (Howdeshell, 2002; Miller *et al*, 2009). The net effect of MoAs that lead to decreased levels of the thyroid hormones T4 and T3 is the following well-characterised sequence of events. The lowered levels of circulating thyroid hormones are sensed by the hypothalamus and pituitary gland and may result in increased release of TRH (thyrotropin releasing hormone) and TSH (thyroid stimulating hormone, also called thyrotropin) from the hypothalamus and pituitary respectively. TSH acts on thyroid follicular cells in an attempt to stimulate the production of new thyroid hormones. This process may cause thyroid follicular cell hypertrophy, changes in cell shape and loss of colloid from the thyroid follicle. This generally results in increased thyroid gland weight. Under conditions of prolonged exposure, thyroid follicular cell hyperplasia may occur, possibly eventually leading to tumours of the thyroid gland (adenomas and carcinomas). This paradigm has been described for many rodent thyroid perturbants (Hill *et al*, 1998; Hurley, 1998; McClain and Rice, 1999).

The postulated MoA for mancozeb, firstly involves metabolism to ETU, formation is approximately 7% by weight in all mammals studied (DAR, 2000). ETU is the proximate metabolite responsible for the actions of mancozeb on the thyroid hormonal system. The effects of ETU on the thyroid hormonal system, in short and long-term studies mirror those of mancozeb, but occur at lower doses, as would be expected from direct administration of the active entity. ETU is carcinogenic in the rat thyroid and (unlike mancozeb) also in the mouse. The species difference in carcinogenicity is likely to be, at least partly, due to differences in the metabolism of ETU between rats and mice (DAR, 2000).

ETU inhibits the peroxidase activity of TPO within the thyroid, preventing the oxidation of iodide and hence the formation of thyroid hormone precursors on the colloidal protein thyroglobulin. The result of this direct mode of action is that production of T4 and T3 are decreased.

The molecular mode of action of ETU has been extensively investigated. Doerge and Takazawa (1990) showed that ETU inhibited the oxidation of iodine to triiodide. Inhibition of TPO occurred only in the presence of iodide and caused concomitant metabolism of ETU. Inhibition ceased when ETU was completely metabolised. This MoA is similar to that of pharmaceutically-used TPO inhibitors such as propyl thiouracil (PTU) and methimazole but differs in that PTU and methimazole are irreversible inhibitors of TPO (Taurog, 1976; Taurog and Dorris, 1989) whilst ETU is a reversible inhibitor. Covalent binding of ETU to TPO was negligible. More recently, Freyberger and Ahr (2006) showed that ETU caused a transient inhibition of iodide oxidation followed by resumption of enzyme activity, similar to control values. ETU was metabolised during the reaction and results were similar to those of Doerge and Takazawa (1990). Results confirmed that inhibition of TPO was reversible, unlike when amitrole (another known irreversible inhibitor of TPO) was used.

Summary of data for use in Mode of Action Analysis

Studies that demonstrate that mancozeb causes the events described in the hypothesised mode of action for the induction of thyroid follicular tumours are shown in Table A1-2. These are largely repeated dosing/carcinogenicity studies, but two 2-generation reproduction studies in rats have also been included because parental animals in these studies (dosed for 25 weeks) also developed thyroid tumours and are relevant for consideration of dose-response.

Studies on the metabolism of mancozeb to ETU are not described here but can be found in the DAR (2000) and M-CA Dossier Section 5.1 (2015). The studies used in this MoA analysis were limited to those concerned with oral administration because this provides the most useful database for dose response, temporal effects and species differences. It is acknowledged that human exposure is likely to be via dermal or inhalational routes, however after absorption of mancozeb the end result of systemic exposure will be similar. Only studies that were considered reliable in the DAR (2000) and M-CA Dossier Section 5 (2015) are used. All the studies are described in detail in Sections 10.7 and 10.10 of the CLH Dossier, the DAR (2000) or M-CA Dossier Section 5 (2015).

Table A1-2: Studies used in the MoA analysis. Studies are presented in decreasing order of duration, via oral administration in rats, mice and dogs.

Species/sex	Route/Dose	Findings relevant for the thyroid hormonal system	Comments	Reference
Studies in rats				
Rat/ Male and female	Dietary at 0, 28, 113 or 454 ppm for 104 weeks. The study included interim timepoints at 26, 52 and 78 weeks. Achieved intake of a.i. was 1.0/1.3; 4.0/5.1 or 16.8/ 20.8 mg/kg bw/day for males and females receiving 28, 113 or 454 ppm respectively.	Serum T4 was reduced at 454 ppm at weeks 26 and 52, and in the high dosage females only at weeks 26, 52 and 78. T3 & TSH were unaffected. There were no effects on weight of adrenals, brain, liver, ovaries, testes, thyroid; and no treatment-related histopathological effects on these organs, mammary glands, pituitary or other reproductive organs; with the exception of the thyroid. At 454 ppm there were increases in the height of the thyroid follicular epithelium and prominent micro follicles present in both sexes. There were no increased tumour incidences.	Effects on T4 and the thyroid are consistent with disturbance of the HTP axis at doses ≥ 16.8 mg/kg bw/day. It should be noted that increases in thyroid tumours were not seen. Thyroid weight was not determined. There were no effects on other endocrine-related organs, indicating that other hormonal systems were largely unaffected.	Anonymous (1992a)
Rat/ Male and female	Dietary at 0, 20, 60, 125 or 750 ppm for 104 weeks. The study included interim time points at 3, 6, 12, 18 and 24 months. Achieved intake of a.i. was 0.7/1.1; 2.3/3.1; 4.8/ 6.7 or 30.9/ 40.2 mg/kg bw/day for males and females receiving 20, 60, 125 or 750 ppm respectively.	Serum T4 was reduced and TSH was increased at 750 ppm at intervals, in both sexes through the study. There was no consistent pattern to effects on T3. Thyroid weight and thyroid follicular cell hypertrophy/hyperplasia increased at 750 ppm in both sexes. Thyroid follicular hypertrophy/ hyperplasia was seen at 52 weeks and increased thyroid weight was seen at 104 weeks at this dose. An increased incidence of thyroid follicular cell carcinomas, adenomas, nodular hyperplasia and hypertrophy/hyperplasia occurred at 750 ppm. The incidence of carcinomas and adenomas was higher in males (14/61 and 20/61 respectively) than in females (4/61 and 6/61). In control rats, the incidence of both carcinomas and adenomas was 0 in males and 0 and 1 in females.	Effects on T4, TSH and the thyroid are consistent with disturbance of the HTP axis at doses ≥ 30.9 mg/kg bw/day. Thyroid follicular tumours resulted from this disturbance. There were no effects on other endocrine-related organs, indicating that other hormonal systems were largely unaffected.	Anonymous (1990a)

Species/sex	Route/Dose	Findings relevant for the thyroid hormonal system	Comments	Reference
Rat/ Male and female	2-generation reproduction study: parental animals in F0 and F1 phases. Dietary at 0, 30, 120 or 1200 ppm for approximately 25 weeks. Achieved intake of a.i. was 1.73-2.49, 6.95-10.52 or 68.9-114.26 mg/kg bw/day for males and females receiving 30, 120 or 1200 ppm respectively.	In both F0 and F1 parental males and females at the highest dose, thyroid weight was increased and most rats showed thyroid follicular hypertrophy & hyperplasia. Thyroid follicular adenomas were increased: F0: 3/25 males, none in females or controls; F1: 4/24 males, none in females or controls. A low incidence of hypertrophy/vacuolation of the pituitary adenohypophysis occurred in males. There were no thyroid effects at the lower doses.	Effects on thyroid weight and histopathology are consistent with disturbance of the HTP axis at doses 68 mg/kg bw/day. Thyroid follicular adenomas were seen in male rats. The sex specificity and the doses at which the tumours occurred are consistent with the carcinogenicity studies in rats.	Anonymous (1988b)
Rat/ Male and female	2-generation reproduction study: parental animals in F0 and F1 phases. Dietary at 0, 25, 150 or 1100 ppm for approximately 25 weeks. Achieved intake of a.i. was 1.7, 10 or 74 mg/kg bw/day for males and females receiving 25, 150 or 1100 ppm respectively.	In both F0 and F1 parental males and females at the highest dose, thyroid weight was increased and most rats showed thyroid follicular hypertrophy & hyperplasia. Thyroid follicular adenomas were increased: F0: 5/25 males, 1/25 females, none in controls; F1: 11/25 males, none in females or controls. No effects on thyroid weight at 150 or 25 ppm (10 or 1.7 mg/kg/bw/day). Thyroid histopathology was not done.	Effects on thyroid weight and histopathology are consistent with disturbance of the HTP axis at doses 74 mg/kg bw/day. Thyroid follicular adenomas were seen in male rats. The sex specificity and the doses at which the tumours occurred are consistent with the carcinogenicity studies in rats.	Anonymous (1992c)
Rat/ Male and female	Dietary at 0, 28, 113 or 454 ppm for 90 days (13 weeks). Rats were killed after 90 days or after a 4 week recovery period. Achieved intake of a.i. was 1.7/2.1; 6.8/8.5 or 27/ 34 mg/kg bw/day for males and females receiving 28, 113 or 454 ppm respectively.	After 90 days of treatment Mancozeb reduced plasma T4 levels at 454 ppm (27 mg/kg bw/day) in males and 113 ppm (8.5 mg/kg bw/day) and above in females. Changes in females were not-statistically significant at any dose. Effects on T3 were equivocal. The effects on T4 were reversed after the recovery period.	Disturbance of the HTP axis was indicated by effects on T4 at 8.5 mg/kg bw/day, although there were no effects on thyroid weight or histopathology. Effects were reversible. TSH was not measured.	Anonymous (1989)
Rat/ Male and female	Dietary at 0, 30, 60, 125, 250 or 1000 ppm for 90 days (13 weeks). Achieved intake of a.i. was 1.7/2.2; 3.5/4.4, 7.4/9.2, 15/18 or 57/75 mg/kg bw/d for males and females receiving 30, 60,	Mancozeb: Serum T4 was decreased in both sexes at 1000 ppm (57 mg/kg bw/d) and TSH was increased. At 250 ppm (15 mg/kg bw/day), T4 was decreased in females and TSH was increased in males (36%) and females (50%). Thyroid weight and thyroid follicular hyperplasia were increased at 1000 ppm. Relative liver weight was	Mancozeb caused disturbance of the HTP axis, indicated by effects on T4 & TSH at 15 mg/kg bw/day. At higher doses further changes were thyroid weight increases and histopathological findings. A similar pattern of changes was caused by ETU (14 mg/kg bw/day) indicating that the effects caused by	Anonymous (1986b)

Species/sex	Route/Dose	Findings relevant for the thyroid hormonal system	Comments	Reference
	125, 250 or 1000 ppm respectively.	increased and mixed function oxidase (MFO) activity was decreased at 1000 ppm.	Mancozeb are attributable to ETU. ETU also affected the pituitary.	
Rat/ Male	Dietary at 0, 10, 50, 75, 113, 169, 253 or 379 mg/kg bw/day in the diet for 12 weeks.	Relative thyroid weight was increased at 75 mg/kg bw/day and above. MFO activity was decreased whilst P450 levels were unchanged). Dose dependent thyroid hyperplasia occurred from 113 mg/kg bw/day.	Effects on the thyroid are consistent with disturbance of the HTP axis at doses from 75 mg/kg bw/day. Thyroid hormones and TSH were not measured.	Szepvolgyi *et al* (1989)
Rat/ Female	Dietary at 0, 75, 150, 300, or 600 mg/kg bw/day for 6 weeks.	Thyroid weight, iodine concentrations in serum and protein bound iodine were increased at all doses.	Effects on the thyroid and iodine levels, are consistent with disturbance of the HTP axis at doses from 75 mg/kg bw/day. Thyroid hormones, TSH and thyroid histopathology were not were not determined.	Horing *et al* (1990)
Rat/ Female	Oral by gavage at 0, 3.9, 7.8, 15.6, 31.3, 62.5, 125, 250, 500 or 1000 mg/kg bw/day for 4 days.	Mancozeb reduced serum T4 with 31.3 mg/kg bw/day giving a 20% decrease and an ED50 (the dose eliciting a 50% decrease in T4) of 259 mg/kg bw/day.	Effects on serum T4 are consistent with disturbance of the HTP axis at doses from 31 mg/kg bw/day. TSH, thyroid weight and histopathology were not determined.	Flippin *et al* (2009)
Studies in mice				
Mouse/ Male and female	Dietary at 0, 30, 100 or 1000 ppm for 18 months. The study included an interim timepoint at 12 months. Achieved intake of a.i. was 4/5; 13/18; or 131/180 mg/kg bw/day for males and females receiving 30, 100 or 1000 ppm respectively.	T4 was decreased at 1000 ppm in both sexes at 12 & 18 months. Effects on T3 were equivocal and TSH was unaffected. No other treatment-related findings (including thyroid histopathology) were reported and there was no evidence of carcinogenicity.	Mancozeb caused disturbance of the HTP axis, indicated by effects on T4 at doses of 131 mg/kg bw/day. Thyroid weight was not determined.	Anonymous (1991a)
Mouse/ Male and female	Dietary at 0, 10, 100, 1000 or 10,000 ppm for 90 days (13 weeks). Achieved intake of a.i. was approximately 1.8/2.2; 18/22; 180/220 or 1800/2200 mg/kg bw/day for males and females receiving 10, 100, 1000 or 10,000 ppm respectively.	Thyroid weight was increased in both sexes at 10,000 ppm. Thyroid hypertrophy/hyperplasia was seen in both sexes from 1000 ppm. Liver weight was increased at 10,000 ppm, some histopathological changes were seen and MFO activity was reduced.	Mancozeb caused disturbance of the HTP axis, indicated by thyroid weight increases and hypertrophy/hyperplasia at doses ≥ 180 mg/kg bw/day. Liver weight increases occurred at 1800 mg/kg bw/day. Thyroid hormones and TSH were not determined. A similar pattern of changes was caused by ETU (from 17 mg/kg bw/day) indicating that the effects caused by	Anonymous (1985c)

Species/sex	Route/Dose	Findings relevant for the thyroid hormonal system	Comments	Reference
			Mancozeb are attributable to ETU.	
Mouse/ Male and female	Dietary at 0, 1, 10, 100, 1000 or 10,000 ppm for 4 weeks. Achieved intake of a.i. was approximately 0.15, 1.5, 15, 150 or 1500 mg/kg bw/day for mice receiving 1, 10, 100, 1000 or 10,000 ppm respectively.	Mancozeb: Thyroid weight was increased at 10,000 ppm in females only. Thyroid hyperplasia was seen in both sexes at 10,000 ppm and in females at 1000 ppm. Liver weight was increased from 1000 ppm but no histopathological changes were seen.	Mancozeb caused disturbance of the HTP axis, indicated by thyroid weight increases and histopathological findings at doses from 150 mg/kg bw/day. Liver weight increases occurred at 1500 mg/kg bw/day. Thyroid hormones and TSH were not determined. A similar pattern of changes was caused by ETU (from 15 mg/kg bw/day) indicating that the effects caused by Mancozeb are attributable to ETU.	Anonymous (1985b)
Studies in dogs				
Dog/ Male and female	Dietary at 0, 50, 200, 800 or 1600 ppm for 52 weeks. Achieved intake of a.i. was 1.8/1.9; 7.6/7.8; 28/29 or 50/56 mg/kg bw/day for males and females receiving 50, 200, 800 or 1600 ppm respectively.	Serum T4 was reduced, thyroid weight was increased and thyroid histopathological changes were observed at 1600 ppm. Liver weight was increased at 800 ppm and above.	Mancozeb caused disturbance of the HTP axis, indicated by effects on T4, thyroid weight and histopathology at 50 mg/kg bw/day. TSH was not determined.	Anonymous (1990c)
Dog/ Male and female	Oral by capsule, at 0, 2.3, 23 or 113 mg/kg bw/day for 52 weeks. [Dogs receiving 113 mg/kg bw/day were killed after 26 weeks].	Serum T4 was decreased at 113 mg/kg bw/day in both sexes and at 23 mg/kg bw/day after 52 weeks in males. T3 was unchanged. Relative liver and thyroid weights were increased at 23 mg/kg bw/day and above. No histopathological changes were seen.	Mancozeb caused disturbance of the HTP axis, indicated by effects on T4 and increased relative thyroid weight at 23 mg/kg bw/day. TSH was not determined.	Anonymous (1991c)
Dog/ Male and female	Oral by capsule, at 0 or 40 mg/kg bw/day for 52 weeks. [Study was conducted following the loss of the high dose group in Broadmeadow, 1991a].	Serum T4 and T3 were decreased and thyroid weight was increased in both sexes. No histopathological changes were seen.	Mancozeb caused disturbance of the HTP axis, indicated by effects on T4, T3 and increased thyroid weight at 40 mg/kg bw/day. TSH was not determined.	Anonymous (1991d)
Dog/ Male and female	Oral by capsule, at 0, 5.7, 34.0 and 340/204 mg/kg bw/day for 13	Serum T4 was decreased at 340/204 mg/kg bw/day in both sexes and at 34 mg/kg bw/day in females. Thyroid weight	Mancozeb caused disturbance of the HTP axis, indicated by effects on T4, thyroid weight and thyroid	Anonymous (1987c)

Species/sex	Route/Dose	Findings relevant for the thyroid hormonal system	Comments	Reference
	weeks. The study included a 6 week reversibility period. [The high dose of 340 mg/kg bw/day was reduced to 204 mg/kg bw/day after 16 days].	was increased at 340/204 mg/kg bw/day in both sexes. Thyroid follicular hyperplasia and accumulation of colloid in the thyroid were seen in all treated groups. Liver weight was increased at 340/204 and 34 mg/kg bw/day. Adrenal weights were increased at 340/204 mg/kg bw/day. After the recovery period, thyroid weight and histopathological changes had reversed and T4 levels had largely recovered.	histopathology at doses ≥ 34 mg/kg bw/day. These changes recovered after 6 weeks without dosing. TSH was not determined.	
Dog/ Male and female	Dietary at 0, 10, 100, 1000 or 5000 ppm for 3 months. Achieved intake of a.i. was 0.3/0.3; 3/3; 29/29 or 102/110 mg/kg bw/day for males and females receiving 10, 100, 1000 or 5000 ppm respectively.	Serum T4 and T3 were decreased at 5000 ppm and enlarged thyroids were noted at 1000 ppm and above. Thyroid follicular cell hyperplasia occurred at 5000 ppm.	Mancozeb caused disturbance of the HTP axis, indicated by effects on T4, T3, thyroid weight and histopathology at doses of 29 mg/kg bw/day and above. TSH was not determined.	Anonymous (1986c)

Listing of key events identified for a specific Mode of Action

The postulated MoA for effects on the thyroid and induction of thyroid follicular tumours in rats by mancozeb can be summarised as follows. Mancozeb is metabolised to ETU (a reversible inhibitor of TPO) resulting in inhibition of thyroid hormone synthesis. The pituitary responds to the decreased blood levels of T4 and T3 by increasing output of TSH. TSH stimulates the thyroid follicular cells in an attempt to increase thyroid hormone production. Stores of thyroid hormone become depleted and prolonged exposure to TSH results in cellular enlargement (hypertrophy) as cellular systems attempt to induce production of thyroid hormones. Continued exposure causes cellular proliferation (hyperplasia) which ultimately progresses to neoplasia if exposure to mancozeb/ETU is chronic.

The initial step of metabolism of mancozeb to ETU is not listed as a Key Event at this stage because the data are not amenable to the dose response and temporal analysis required (see below). Metabolism of mancozeb appears to be linear at a rate of 7% in all species tested (DAR, 2000). However, exposure to ETU also depends upon absorption of mancozeb and metabolism of ETU itself to inactive metabolites. Metabolism of ETU differs across species and is likely to be partly responsible for the differences in lowest effect levels on the thyroid.

The molecular initiating event of TPO inhibition within the thyroid follicular cell is also not amenable to the dose response and temporal analysis required in Section 5a & 5b and therefore is also not listed as a Key Event at this stage. The mechanism of action has been described by Doerge and Takazawa (1990) and Freyberger and Ahr (2006).

The Key Events (shown below) are the onward biological consequences that result from metabolism and the molecular initiating event. These are measurable events that are critical to the induction of the (adverse) effect (Key Event 4: Formation of thyroid follicular tumours). They have been defined by the endpoints measured in the studies in Table A1-2 and are listed in the order in which they occur. Key Events 1 and 2 (Decreased blood T4 levels and Decreased blood TSH levels) have frequently been measured in the studies. Key Event 3

combines increased thyroid follicular cell hypertrophy and hyperplasia as most studies have insufficient time points to distinguish temporally between these, although hypertrophy usually precedes hyperplasia in this MoA. Increased thyroid weight can be viewed as a surrogate for these cellular events, as hypertrophy and hyperplasia contribute to increased thyroid weight. In some studies, thyroid histopathology was not determined whilst thyroid weight was, and therefore Key Event 3 has been described as: Increased thyroid follicular cell hypertrophy and/or hyperplasia (determined as increased thyroid gland weight or histopathologically).

Key events in the mode of action for mancozeb and thyroid carcinogenicity

Metabolism	Metabolism of mancozeb to ETU (activation) and metabolism of ETU (deactivation).
Molecular Initiating Event	Inhibition of TPO by ETU
Key Event 1	Decreased blood T4 levels
Key Event 2	Increased blood TSH levels
Key Event 3	Increased thyroid follicular cell hypertrophy and/or hyperplasia (determined as increased thyroid gland weight or histopathologically)
Key Event 4	Formation of thyroid follicular tumours

Bradford Hill Considerations for Weight of Evidence Analysis of available data/information for Mode of Action Analysis in experimental species

Dose Response Relationships and Temporal Association

The dose response and temporal relationships for the Key Events measured in the studies in rats, mice and dogs in Table A1-2 are presented below (Tables A1-3 to A1-5). The responses for the Key Events 1-4 are shown as positive or negative, quantification (degree of change) is not shown in order to keep the tables clearer. Responses among males and females are not distinguished but were generally similar for Key events 1-3 and are also not shown for reasons of clarity. Key Event 4 (Formation of thyroid tumours) is generally not applicable to subchronic studies and therefore is labelled as "not applicable" in the tables, although the histopathological outcome was measured. A sex difference for Key Event 4 was evident (males having a higher tumour incidence than females), but is also not distinguished in the tables for reasons of clarity. Further details on the studies can found in the main document.

Species: Rat

Table A1-3: Concordance of dose-response and temporal relationships for the Key Events in studies in rats. Data are taken from the studies in Table A1-2.

Dose (mg/kg bw/day)	Key Event 1 Decreased blood T4 levels	Key Event 2 Increased blood TSH levels	Key Event 3 Increased thyroid follicular cell hypertrophy and/or hyperplasia (determined as increased thyroid gland weight or histopathologically)	Key Event 4 Formation of thyroid tumours	Reference
Ordered from low to high dosage	**Key events shown in order from earliest event to later (left to right). Results show the time that the event was observed. Quantitative changes in severity are not shown.**				
0.7/1.1[a]	- [b] (12 to 104 weeks)	- (12 to 104 weeks)	- (12 to 104 weeks)	- (104 weeks)	Anonymous (1990a)
1.0/1.3	- (26 to 104 weeks)	- (26 to 104 weeks)	- (26 to 104 weeks)	- (104 weeks)	Anonymous (1992a)
1.7/2.2	- (13 weeks)	- (13 weeks)	- (13 weeks)	NA [d]	Anonymous (1986b).

Dose (mg/kg bw/day)	Key Event 1 Decreased blood T4 levels	Key Event 2 Increased blood TSH levels	Key Event 3 Increased thyroid follicular cell hypertrophy and/or hyperplasia (determined as increased thyroid gland weight or histopathologically)	Key Event 4 Formation of thyroid tumours	Reference
1.7/2.1	- (13 weeks)	ND	- (13 weeks)	NA	Anonymous (1989)
1.7/2.4	ND	ND	- (25 weeks)	- (25 weeks)	Anonymous (1988b)
1.7	ND	ND	- (25 weeks)	- (25 weeks)	Anonymous (1992c)
2.3/3.1	- (12 to 104 weeks)	- (12 to 104 weeks)	- (12 to 104 weeks)	- (104 weeks)	Anonymous (1990a)
3.5/4.4	- (13 weeks)	- (13 weeks)	- (13 weeks)	NA	Anonymous (1986b).
3.9	- (4 days)	ND [c]	ND	ND	Flippin *et al* (2009)
4.0/5.1	- (26 to 104 weeks)	- (26 to 104 weeks)	- (26 to 104 weeks)	- (104 weeks)	Anonymous (1992a)
4.8/ 6.7	- (12 to 104 weeks)	- (12 to 104 weeks)	- (12 to 104 weeks)	- (104 weeks)	Anonymous (1990a)
7.8	- (4 days)	ND	ND	ND	Flippin *et al* (2009)
6.8/8.5	- (13 weeks)	ND	- (13 weeks)	NA	Anonymous (1989).
6.9/10.5	ND	ND	- (25 weeks)	- (25 weeks)	Anonymous (1988b)
7.4/9.2	- (13 weeks)	- (13 weeks)	- (13 weeks)	NA	Anonymous (1986b)
10	ND	ND	- (12 weeks)	NA	Szepvolgyi *et al* (1989)
10	ND	ND	- (25 weeks)	- (25 weeks)	Anonymous (1992c)
15.6	- (4 days)	ND	ND	NA	Flippin *et al* (2009)
15/18	+ [e] (13 weeks)	+ (13 weeks)	- (13 weeks)	NA	Anonymous (1986b)
16.8/ 20.8	+ (26 to 104 weeks)	- (26 to 104 weeks)	+ (26 to 104 weeks)	- (26 to 104 weeks)	Anonymous (1992a)
31.3	+ (4 days)	ND	ND	ND	Flippin *et al* (2009)
30.9/ 40.2	+ (12 to 104 weeks)	+ (12 to 104 weeks)	+ (52 & 104 weeks)	+ (104 weeks)	Anonymous (1990a)
27/ 34	+ (13 weeks)	ND	- (13 weeks)	NA	Anonymous (1989)
50	ND	ND	- (12 weeks)	NA	Szepvolgyi *et al* (1989)

Dose (mg/kg bw/day)	Key Event 1 Decreased blood T4 levels	Key Event 2 Increased blood TSH levels	Key Event 3 Increased thyroid follicular cell hypertrophy and/or hyperplasia (determined as increased thyroid gland weight or histopathologically)	Key Event 4 Formation of thyroid tumours	Reference
62.5	+ (4 days)	ND	ND	ND	Flippin *et al* (2009)
57/75	+ (13 weeks)	+ (13 weeks)	+ (13 weeks)	- (NA)	Anonymous (1986b)
68.9/114.2	ND	ND	+ (25 weeks)	+ (25 weeks)	Anonymous (1988b)
74	ND	ND	- (25 weeks)	+ (25 weeks)	Anonymous (1992c)
75	ND	ND	+ (6 weeks)	ND	Horing *et al* (1990).
75	ND	ND	+ (12 weeks)	NA	Szepvolgyi *et al* (1989)
113	ND	ND	+ (12 weeks)	NA	Szepvolgyi *et al* (1989)
125	+ (4 days)	ND	ND	ND	Flippin *et al* (2009)
150	ND	ND	+ (6 weeks)	ND	Horing *et al* (1990).
169	ND	ND	+ (12 weeks)	NA	Szepvolgyi *et al* (1989)
250	+ (4 days)	ND	ND	ND	Flippin *et al* (2009)
253	ND	ND	+ (12 weeks)	NA	Szepvolgyi *et al* (1989)
300	ND	ND	+ (6 weeks)	ND	Horing *et al* (1990).
379	ND	ND	+ (12 weeks)	NA	Szepvolgyi *et al* (1989)
500	+ (4 days)	ND	ND	ND	Flippin *et al* (2009)
600	ND	ND	+ (6 weeks)	ND	Horing *et al* (1990).
1000	+ (4 days)	ND	ND	ND	Flippin *et al* (2009)

[a] Double value indicates the dosage in males and females respectively, where they are different.
[b] - : negative response; [c] ND : Not determined. [d] NA : Not applicable. [e] + : positive response

Species: Mouse

Table A1-4: Concordance of dose-response and temporal relationships for the Key Events in studies in mice. Data are taken from the studies in Table A1-2.

Dose (mg/kg bw/day)	Key Event 1 Decreased blood T4 levels	Key Event 2 Increased blood TSH levels	Key Event 3 Increased thyroid follicular cell hypertrophy and/or hyperplasia (determined as increased thyroid gland weight or histopathologically)	Key Event 4 Formation of thyroid tumours	Reference
Ordered from low to high dosage	**Key events shown in order from earliest event to later (left to right). Results show the time that the event was observed. Quantitative changes in severity are not shown.**				
0.15	[a] ND	ND	- (4 weeks)	[b] NA	Anonymous (1985b)
1.5	ND	ND	- (4 weeks)	NA	Anonymous (1985b)
1.8/2.2[c]	ND	ND	- (13 weeks)	NA)	Anonymous (1985c)
4/5	- [d] (52 to 78 weeks)	- (52 to 78 weeks)	- (52 to 78 weeks)	- (52 to 78 weeks)	Anonymous (1991a)
15	ND	ND	- (4 weeks)	NA	Anonymous (1985b)
13/18	- (52 to 78 weeks)	- (52 to 78 weeks)	- (52 to 78 weeks)	- (52 to 78 weeks)	Anonymous (1991a)
18/22	ND	ND	- (13 weeks)	NA	Anonymous (1985c)
150	ND	ND	+ [e] (4 weeks)	NA	Anonymous (1985b)
131/180	+ (52 to 78 weeks)	- (52 to 78 weeks)	- (52 to 78 weeks)	- (52 to 78 weeks)	Anonymous (1991a)
180/220	ND	ND	+ (13 weeks)	NA	Anonymous (1985c)
1500	ND	ND	+ (4 weeks)	NA	Anonymous (1985b)
1800/2200	ND	ND	+ (13 weeks)	NA	Anonymous (1985c)

[a]- : negative response. [b] ND : Not determined. [c] NA : Not applicable. [d] Double value indicates the dosage in males and females respectively, where they are different; [e] + : positive response

Species: Dog

Table A1-5: Concordance of dose-response and temporal relationships for the Key Events in studies in dogs. Data are taken from the studies in Table A1-2.

Dose (mg/kg bw/day)	Key Event 1. Decreased blood T4 levels	Key Event 2 Increased blood TSH levels	Key Event 3 Increased thyroid follicular cell hypertrophy and/or hyperplasia (determined as increased thyroid gland weight or histopathologically)	Key Event 4 Formation of thyroid tumours	Reference
Ordered from low to high dosage (down).	**Key events shown in order from earliest event to later (left to right). Results show the time that the event was observed. Quantitative changes in severity are not shown.**				
0.3	- [a] (13 weeks)	ND [b]	- (13 weeks)	NA [c]	Anonymous (1986c)
1.8/1.9 [d]	- (52 weeks)	ND	- (52 weeks)	NA	Anonymous (1990c)
2.3	- (52 weeks)	ND	- (52 weeks)	NA	Anonymous (1991c)
3	- (13 weeks)	ND	- (13 weeks)	NA	Anonymous (1986c)
5.7	- (13 weeks)	ND	- (13 weeks)	NA	Anonymous (1987c)
7.6/7.8	- (52 weeks)	ND	- (52 weeks)	NA	Anonymous (1990c)
23	+ [e] (52 weeks)	ND	+ (52 weeks)	NA	Anonymous (1991c)
28/29	- (52 weeks)	ND	- (52 weeks)	NA	Anonymous (1990c)
29	+ (13 weeks)	ND	+ (13 weeks)	NA	Anonymous (1986c)
34	+ (13 weeks)	ND	- (13 weeks)	NA	Anonymous (1987c)
40	+ (52 weeks)	ND	+ (52 weeks)	NA	Anonymous (1991d)
50/56	+ (52 weeks)	ND	+ (52 weeks)	NA	Anonymous (1990c)
102/110	+ (13 weeks)	ND	+ (13 weeks)	NA	Anonymous (1986c)
113	+ (26 weeks)	ND	+ (26 weeks)	NA	Anonymous (1991c)
340/204	+ (13 weeks)	ND	+ (13 weeks)	NA	Anonymous (1987c)

[a] Double value indicates the dosage in males and females respectively, where they are different.
[b] - : negative e response; [c] + : positive response ND : Not determined. NA : Not applicable

In general, the dose response and temporal relationship among the Key Events fits the expected pattern for rats, mice and dogs. The temporality of events in subchronic studies proceeds in the expected order. T4 reduction is usually an early event, preceding histopathological changes and weight increases in the thyroid.

Thyroid follicular hypertrophy precedes hyperplasia. Tumours are only observed in rats, and in this species, the appearance of adenomas and carcinomas is always a late event. However, the limited frequency of observations means that in several studies, Key Events 1 to 3 cannot be distinguished temporally because at the time of the first observation all three events had occurred. In rats, the Key Event of T4 reduction occurs at doses below those that induce tumours therefore the dose response supports the MoA.

In rats, the dose at which mancozeb starts to affect the rat thyroid (T4 reduction and TSH increase) is approximately 15 mg/kg bw/day (Table A1-3). The studies of Anonymous (1986b), Anonymous (1992a) provide a lowest effect level (LOEL) at approximately this dose, in spite of being of different durations. The 4 day gavage study of Flippin *et al* (2009) and the 13 week study of Anonymous (1989) gives a slightly higher LOEL of ~30 mg/kg bw/day. The dose responses from the carcinogenicity studies indicate that tumours were observed at doses of approximately 30 mg/kg bw/day (Anonymous, 1990a). No thyroid tumours were noted with mancozeb in the study of Anonymous (1992a) where the highest dose levels only reached 16.8/20.8 mg/kg bw/day. This study was therefore conducted at dose levels below the LOEL for tumours. In the 2-generation studies of Anonymous (1988b) and Anonymous (1992c) parental rats received doses up to approximately 70 mg/kg bw/day for about 25 weeks and thyroid adenomas were seen at this dose but not at the lower doses (approximately 10 mg/kg bw/day and below). These studies therefore support the dose responses seen in the studies mentioned above.

In the mouse, the dose at which mancozeb starts to affect the thyroid is 150 mg/kg bw/day, approximately 10-fold higher than in the rat (Table A1-4). At this dose, T4 was reduced and histopathological changes were seen in the thyroid gland (Anonymous, 1985b; Anonymous, 1985c; Anonymous, 1991a). Mancozeb was not carcinogenic in mice. However the highest dose tested (MTD) was 131 to 180 mg/kg bw/day, the approximate LOEL in this species for thyroid effects. The MoA would predict that a higher dose would be necessary for tumour induction and therefore the absence of tumours also fits the hypothesis.

The sensitivity of the dog is between that of the rat and mouse with a LOEL of approximately 30 mg/kg bw/day for effects on the thyroid (Anonymous, 1986c; Anonymous, 1987c, 1991c, d; Anonymous, 1990c). T4 was reduced and thyroid follicular hyperplasia was reported. TSH levels were not determined in the dog (Table A1-5).

In conclusion, the data on dose response and temporality fit with the proposed MoA for mancozeb in all three species.

Consistency & Specificity – Biological Plausibility

Consistency & Specificity

The weight of evidence linking the Key Events with the toxicological response is consistent with the observations in many studies in rats, mice and dogs. The same pattern of response among Key Events has been seen in all studies. The incidence of severity (quantitative response) is also consistent; for example, in rats dosed mancozeb in a 4 day study at approximately 30 mg/kg bw/day, 100% of rats had reduced T4 (Key Event 1) (Flippin *et al*, 2009) whilst in a carcinogenicity study, not all rats develop tumours, the incidence of thyroid carcinomas in male and female rats was 23% and 6.6% (Anonymous, 1990a). Thyroid tumours in rats are preceded by hyperplasia that occurs at lower doses, after a shorter duration and at a higher incidence than seen for tumours. These observations also fit with the MoA, where, at similar doses, the incidence/severity of later key events would not be expected to be greater than that of earlier key events.

One study did not show consistent results was a carcinogenicity study on mancozeb in rats conducted by Belpoggi *et al* (2002), where thyroid tumours appear to have been found at doses lower than the LOEL of ~15 mg/kg bw/day described above for thyroid effects in rats. The authors indicate that tumours may have been increased at 0.5 mg/kg bw/day. However, the study had an unusual design where rats were dosed until their natural death and there are very few study details and no historical control data. This makes interpretation of the incidence data at the lower doses difficult. This study is therefore not considered to be reliable for the purposes of this MoA analysis.

Two studies have addressed reversibility. In the 13 week rat study of Anonymous (1989), a recovery phase of 4 weeks was included, after which changes (reduction of plasma T4) were reversed. A recovery phase of 6 weeks was also included in the 13 week dog study of Anonymous (1987c), after which effects (reduction of plasma T4, thyroid weight increases and histopathological changes) were reversed. Reversibility is consistent with the proposed MoA where hormonal changes may be reset by the normal feedback-control systems and non-neoplastic cellular changes may also be reversed. Irreversibility is associated with the later development of thyroid follicular carcinomas.

The evidence is also consistent with ETU being the active metabolite responsible for the effects on the thyroid hormonal system and the tumours in rats. ETU causes an identical spectrum of changes to mancozeb, in rats, mice and dogs, with the Key Events also being the same. The LOELs for ETU in these species are lower than those for mancozeb and ETU causes thyroid follicular tumours in both rats and mice (Table A1-6). These facts are entirely consistent with metabolism of mancozeb to ETU being a requirement for the thyroid effects of mancozeb.

Effects of ETU

The lowest dose at which ETU affects T4 in rats is 1-2 mg/kg bw/day compared to mancozeb where the LOEL is approximately 15 mg/kg bw/day. The most comprehensive study of the endocrine effects of ETU is the extended one generation study of Anonymous (2013). In this study some histopathological effects on the thyroid were described at 0.2 mg/kg bw/day but not all animals were affected and therefore 1-2 mg/kg bw/day is a clear LOEL. This is supported by older studies, although the doses at which effects are seen depends to some extent upon the duration of dosing. After administration for between 28 and 90 days, effects are seen at doses from 11 mg/kg bw/day (Kurttio *et al*, 1986); 12.5 mg/kg bw/day (Freudenthal *et al*, 1978); 15 mg/kg bw/day (Arnold *et al*, 1983), 7.5 mg/kg bw/day (O'Neil and Marshall, 1984); 14 mg/kg bw/d (Anonymous, 1986b) or 6 mg/kg bw/day (NTP, 1992). Dosing for up to 120 days resulted in a slightly lower effect level (5 mg/kg bw/day) (Graham and Hansen, 1972). A recovery period of up to 4 weeks was included in the study of Arnold *et al* (1983) following 7 weeks dosing of ETU. As demonstrated for mancozeb, the effects of ETU on the thyroid were reversible after this period. Effects at exceptionally low doses (0.05 mg/kg bw/day) are described by Nebbia and Fink-Gremmels (1996) after 5 days administration but the changes are small and inconsistent within the paper. As the data are also inconsistent with the data from the other 10 studies, the dose response in this paper is considered unreliable.

Long-term administration of ETU to rats in carcinogenicity studies caused increased incidences of thyroid follicular adenomas and carcinomas (Table A1-6). In an NTP (1992) study where ETU was administered in feed for 2 years, changes in T4 and TSH were seen at doses from 1.25 mg/kg bw/day. Further evidence of disturbance of the HTP axis occurred at higher doses, with histopathological changes and tumours appearing in the thyroid gland at doses from 3.8 mg/kg bw/day. This study included groups exposed as adults only and groups additionally exposed during the perinatal period. The frequency of tumours was increased by additional perinatal exposure to ETU.

Similar results were obtained in a proprietary 2-year carcinogenicity study on ETU. Thyroid follicular adenomas and carcinomas were increased in males but not females at 125 ppm (8.9 mg/kg bw/day) (Table A1-6). T4 was decreased and TSH was increased at this dose level (Anonymous, 1992e). An increased incidence of adenomas of the pituitary were also seen in male rats at 125 ppm in this study (50% incidence at 125 ppm compared with 26.6% incidence in controls). It is likely that these tumours are also a consequence of disruption of the HPT axis. In an earlier 2-year study on ETU, tumours were not noted but disturbance of the HTP axis was indicated by thyroid follicular hyperplasia at doses of 1.25 mg/kg bw/day and above (Graham *et al*, 1975).

In the mouse, the LOEL for effects of ETU on the thyroid is approximately 15 mg/kg bw/day (Anonymous, 1985b; Anonymous, 1985c; NTP, 1992) compared with about 150 mg/kg bw/day for mancozeb. Reliable short-term studies where T4 was measured have not been conducted in mice. Thyroid weight and histopathological findings were seen at doses from 15 mg/kg bw/day after 4 weeks administration (Anonymous, 1985b), histopathological findings were seen at doses from 17 mg/kg bw/day after 4 weeks administration whilst thyroid weight increased at 170 mg/kg bw/day after 3 months (Anonymous, 1985c). Histopathological findings were seen at doses from 19 mg/kg bw/day in both sexes, and thyroid follicular adenomas in males from 75 mg/kg bw/day following administration for 3 months (NTP, 1992).

In 2-year carcinogenicity studies in mice, ETU caused increased incidences of thyroid follicular adenomas and carcinomas, also seen in rats (Table A1-6). In an NTP (1992) study where ETU was administered in feed for 2 years, changes in T4 and TSH were seen at doses from 17 mg/kg bw/day. Further evidence of disturbance of the HTP axis occurred at higher doses, with histopathological changes and tumours appearing at doses from 50 mg/kg bw/day. This study also included groups exposed as adults only and groups additionally exposed during the perinatal period. The frequency of tumours after additional perinatal exposure to ETU was similar to adult alone. As in rats, an increased incidence of hyperplasia or adenomas of the pituitary was also seen, at 1000 ppm, in both sexes. It is likely that these tumours are also a consequence of disruption of the HPT axis.

In rats and mice therefore, the data are consistent with the proposed MoA where ETU is responsible for thyroid tumour induction, and thyroid tumours are preceded by hyperplasia occurring at lower doses and after shorter durations. Higher doses of ETU for longer durations result in further cellular changes that lead to tumours. The rat is also more sensitive than the mouse to the effects of ETU, the difference in lowest effect levels being about 10-fold. One reason for the difference in sensitivity for rats and mice to ETU is that ETU is more rapidly metabolised and excreted in mice compared in rats. Thus, internal exposure is lower in mice. Further evidence of the sensitivity of the rat HPT axis to the effects of ETU is illustrated by the observation of tumours in other (non-lifetime) study types. In the 2-generation reproductive study of Anonymous (1990d) thyroid adenomas were seen at the highest dose (8-19 mg/kg bw/day) in F0 and F1 generations. In the extended one-generation reproductive toxicity study of Anonymous (2013) a low incidence of thyroid adenomas occurred in the high dose group (10 mg/kg bw/day) in both generations.

The liver is a target organ of ETU in the mouse. Liver weight and MFO activity were increased at doses of 150 mg/kg bw/day after 4 weeks administration (Anonymous, 1985b) and at doses of 170 mg/kg bw/day after 3 months (Anonymous, 1985c). Similar changes were described in NTP (1992) following 3 months administration. Increased incidences of hepatocellular adenoma and carcinoma occurred after 2 years administration at doses of 50 or 150 mg/kg bw/day. However these changes have not been seen with mancozeb.

The dose response data for ETU in the dog is not very consistent. In two studies, the LOEL for effects on the thyroid in the dog ranged from 2 to 66 mg/kg bw/day (Anonymous, 1991f, 1992d). In a 3 month study, effects on T4, T3, thyroid weight and histopathology occurred at doses of 66 mg/kg bw/day but not below (Anonymous, 1991f). After 52 weeks of dosing, similar effects were seen at doses of 2 mg/kg bw/day and above (Anonymous, 1992d). In comparison, the LOEL for effects on the thyroid with mancozeb in dogs was approximately 30 mg/kg bw/day. These data also support the MoA.

Table A1-6: Thyroid follicular tumour incidences in rats and mice administered ETU

Reference	Dose ppm (mg/kg bw/day; males/females)	Tumour incidence (% tumour bearing animals)	
		Males	Females
Rat			
NTP (1992)	0	2AC[a]	6AC
	83 (3.8) [b]	26AC*	16AC*
	250 (12.5) [b]	74AC*	61AC*
Anonymous (1992)	0	3.3A, 0C	0A, 0C
	0.5 (0.04/0.05)	0A, 0C	0A, 0C
	2.5 (0.17/ 0.25	0A, 0C	0A, 0C
	5 (0.38/0.49)	0A, 0C	0A, 0C
	125 (8.9/13.6)	13.3A*, 6.6C*	0A, 0C
Mouse			
NTP (1992)	0	2AC	0AC
	330 (50) [b]	2AC	4AC*
	1000 (150) [b]	58AC*	76AC*

[a]A=adenomas, C=carcinomas, AC=combined incidence of adenomas and carcinomas.
[b]Exposure estimated according to OECD (2002).
*Statistically significant (as described in the study report).

Biological plausibility

The proposed MoA is consistent with data from other thyroid toxicants, acting via similar MoAs (TPO inhibition) or other MoAs that also have reduction of blood T4 levels as the first Key Event. Increased secretion of TSH then follows, and the progression of thyroid follicular cells to hypertrophy, hyperplasia and neoplasia (IARC, 1999, 2000; Hard, 1998; McClain 1995; Hurley, 1998). The proliferative response of thyroid follicular cells to TSH has also been shown by Bayer *et al* (1992). The biological changes observed with mancozeb are entirely consistent with the known biology of the thyroid hormonal system and the role of the hypothalamus and pituitary.

The tumour response in the rat caused by mancozeb is also typical of a rodent thyroid carcinogen in that the incidence of thyroid follicular cell tumours is greater in males compared to females. In all studies where tumours were observed, the incidence was markedly greater in males (Anonymous, 1990a; Anonymous, 1988b; Anonymous, 1992c). The same trend can also be seen with ETU in rats. Male rats have generally been found to be more sensitive than female rats, with respect to the proportion of chemicals inducing thyroid tumours (Hurley, 1998). This is supported by TSH levels being typically higher in male rats compared to females and therefore a greater potential for thyroid follicular cell stimulation to hypertrophy and hyperplasia.

Consistency across chemicals

The MoA for ETU is also consistent with that of chemical analogues. Other thioureas, such as the pharmaceuticals PTU (used to control hyperthyroidemia in humans) and TMTU are also inhibitors of TPO. MoA studies have been conducted with several structural analogues and all thioureas studied were inhibitors of TPO although some (e.g. PTU) were irreversible whilst others (e.g. ETU, tetramethylthiourea) were reversible (Doerge and Takazawa; 1990; Freyberger and Ahr, 2006). PTU produces the same Key Events as ETU/mancozeb in rats but PTU is a more potent chemical than ETU. Yamasaki *et al* (2002) dosed male rats with PTU by gavage in a 28 day repeat dose study (OECD TG 407) and a male pubertal assay (dosing from 23 to 53 days of age) at doses of 0.01 and 1 mg/kg bw/day. T4 was reduced at 1 mg/kg bw/day in the pubertal assay and at both 0.01 and 1 mg/kg bw/day in the 28 day repeat dose study.

Summary
The evidence for consistency, specificity and biological plausibility is summarised in Table A1-7. Almost all of the studies are consistent in their findings and interpretation, strongly supporting the proposed MoA. Alternative MoAs, such as genotoxicity, have also been considered, but discounted (see seciton 'Other Potential Modes of Action' below).

Table A1-7: Comparative weight of evidence for the MoA for mancozeb-induced rat thyroid tumours.

	Supporting evidence for Key Events	**Potentially inconsistent evidence**
Consistency & Specificity	- A consistent pattern of response for the Key Events has been seen in rats, mice and dogs administered mancozeb, but with quantitative differences in dose response. An identical pattern (but also with quantitative differences in dose response) is seen with ETU.	- None.
	- The pattern of observations across species/organs/test systems is consistent with what is expected based on the postulated MoA. Inhibition of thyroid hormones by ETU occurs in most species, consistent with the well-conserved nature of TPO. Rat is the most sensitive species to mancozeb, consistent with the biology of the rat HPT axis and less efficient metabolism of ETU compared to other species. Thyroid is the major target organ, consistent with TPO being the key molecular target of ETU. The pituitary gland may also be affected in rats, consistent with the action of ETU on the HTP axis in the most sensitive species.	- One study with ETU in rats had some inconsistencies (Nebbia and Fink-Gremmels, 1996).
	- In studies where mancozeb or ETU have been dosed to rats or dogs (Arnold *et al*, 1983; Anonymous, 1989; Virgo, 1987) reduction of blood T4 and histopathological changes in the thyroid gland are reversed following a recovery period after cessation of dosing.	- None.
	The dose–response is consistent with the MoA. T4 reduction occurs at doses below those that induce tumours. Doses of mancozeb causing thyroid disturbance and doses of mancozeb causing thyroid tumours are proportionately higher than those of ETU. The carcinogenicity study on mancozeb in rats that did not produce tumours (Anonymous, 1992a) was conducted at dose levels below those established to be the LOEL for tumours.	- One carcinogenicity study appeared to produce thyroid tumours at doses lower than those causing thyroid effects (Belpoggi *et al* (2002). However the study design is non-standard and results are not reliable.
	- The incidence of tumours (as % tumour bearing animals) is always lower than earlier effects. For example, all animals in an affected group will show thyroid follicular hyperplasia whilst only a proportion will go on to develop tumours.	- None.
	- ETU also induces thyroid tumours in both rats and mice, consistent with it being the active metabolite. However the dose at which ETU induces tumours is higher in mice than rats, consistent with rat being the more sensitive species.	- None.
	- Other structural analogues produce similar effects to ETU.	- None.

	Supporting evidence for Key Events	**Potentially inconsistent evidence**
Biological Plausibility	- The relationship between reduced blood T4 levels, increased TSH and thyroid follicular cell hypertrophy, hyperplasia and eventually tumours has been described for many thyroid perturbants (Hill *et al*, 1989; Hurley *et al*, 1998; McClain and Rice, 1999). The key initial event in most cases is reduced T4 and the MoA from this to thyroid tumours in rodents is well established. Evidence supporting the role of the pituitary and TSH is also well established.	- None.
	- The tumour incidence in rats, after administration of mancozeb or ETU, is higher in males than females. This is also consistent with other rat thyroid carcinogens and sex differences in TSH levels	- None.

Qualitative and Quantitative human concordance

In this section, concordance for each key event, between and within species will be assessed. This includes species differences in metabolism of mancozeb/ETU, possible species differences in the molecular target (TPO) and species concordance for the key biological events. Qualitative and quantitative human concordance analysis for the key events are summarised in Table A1-9.

The metabolism of mancozeb/ETU

In studies in laboratory animals, mancozeb is partially absorbed (about 50% absorption after oral administration) and rapidly excreted. ETU is the major metabolite. The bioconversion of mancozeb to ETU was 7% on a weight basis, similar to the bioconversion factor for other ethylenebisdithiocarbamates (EBDC). In monkeys, oral doses were very poorly absorbed (Emmerling, 1978). The spectrum of metabolites produced was similar in laboratory and farm animals indicating similar metabolism of mancozeb across species. The pharmacokinetics and metabolism of ETU have been studied in mice, rats, guinea pigs, cats, and monkeys. These studies have shown that ETU is rapidly excreted, primarily in the urine and more quickly in mice than in rats. Half-lives for elimination from maternal blood were 5.5 and 9.4 hours in mice and rats, respectively (Ruddick *et al*, 1977). Literature studies indicate that mancozeb and ETU are also rapidly absorbed and eliminated after oral administration in humans. The elimination half-life of ETU was estimated to be 19-23 hours in humans (Aprea *et al*, 1996; Lindh *et al*, 2008).

The metabolism of ETU across species has been compared in two *in vitro* studies (table A1-8). Saghir *et al* (2005) compared rat, mouse and human using liver S9 as a source of metabolising enzymes. Zhu (2015) compared rat, mouse, dog, rabbit and human using primary hepatocytes as the source of metabolising enzymes. The results of the two studies were similar in that metabolism in the rat was lower than in humans. However, metabolism in mouse hepatocytes in Zhu (2015) was lower than in rat hepatocytes whilst the opposite occurred in Saghir *et al* (2005). The latter result concurs with *in vivo* comparisons of rat and mouse metabolism where metabolism is clearly greater in mice. Metabolism in dog and rabbit appears similar to humans. In these studies, metabolism in humans appears more efficient than rodents, indicating that ETU may be readily removed in humans. These studies were conducted with pools of human S9/hepatocytes and therefore no indication of polymorphisms in metabolism were obtained. Recent literature studies indicate that metabolism of ETU by flavin-dependent monooxygenase (FMO) may be subject to polymorphisms in humans (Krueger *et al*, 2005; Koukouritaki *et al*, 2007). However, the contribution of genetic variants of FMO towards the overall metabolism of ETU in humans is not known. Previous studies (reported in the DAR, 2000) have indicated that ETU is metabolised by both cytochrome P450 isoforms and FMO enzymes. Therefore the impact of genetic variants of FMO in the human population may be low.

The evidence that mancozeb is metabolised to ETU in experimental animals and humans is therefore high. The evidence for species differences in the metabolism of ETU is also high and there is plausible evidence that humans are efficient metabolisers of ETU.

Table A1-8: Metabolism of ETU in different species

Metabolism of ETU in liver S9 after 1 hour (% of total ETU added) (Saghir *et al*, 2005)					
ETU initial concentration	**Mouse (200)**	**Rat (105)**	**Human pool 1 (6)**	**Human pool 2 (7)**	**Human pool 3 (7)**
100 µM	4.98	2.11	10.76	12.81	11.72
Metabolism of ETU in hepatocytes after 3 hours (% of total ETU added) (Zhu, 2005)					
ETU initial concentration	**Mouse (59)**	**Rat (21)**	**Rabbit (6)**	**Dog (3)**	**Human (10)**
2 µM	ND	11.2	23.4	25.8	22.1
200 µM	10.5	12.2	13.3	22.5	17.0

ND: not detectable
Values in parentheses are the number of individuals in the pools (all females). Note that uninduced S9 was used in the Saghir study.

TPO inhibition in different species

TPO is a well-conserved enzyme and is the key enzyme responsible for thyroid hormone synthesis in many species. ETU has been shown to inhibit TPO in hog and rat TPO (Doerge and Takazawa, 1990; Freyberger and Ahr, 2006; Paul *et al*, 2014). Clear evidence of species differences are lacking because of use of different methods and substrates. Paul *et al* (2013) compared the inhibition of TPO in pigs with that of rats. Although ETU was not used in this paper, the comparison, showed that PTU was a more potent inhibitor of rat TPO than pig TPO. This result indicates that rat TPO may be more responsive to thiourea-type inhibitors than pig TPO.

The evidence that TPO is the molecular target in this MoA is overwhelming, however the evidence for species differences between humans and experimental animals is uncertain.

Species concordance for the Key (biological) Events

There is qualitative concordance between the biological Key Events 1-3 among experimental rats, mice and dogs. The studies in Tables A1-2 and A1-3 present overwhelming evidence that mancozeb caused decreased blood levels of T4, increased TSH blood levels and thyroid follicular hypertrophy/hyperplasia in rats, mice and dogs. There is also overwhelming evidence that these changes are caused by the metabolite, ETU. The quantitative differences in dose response among these species have been discussed previously. The final adverse effect (Key Event 4) of formation of thyroid follicular tumours only occurs in rats and therefore qualitative concordance among experimental species (rats and mice) does not occur. The likely reasons for these (species differences in metabolism) have been discussed previously.

The following discussion focusses on concordance between experimental animals and humans in the response to mancozeb. There are no direct human studies on mancozeb and the thyroid but there are some epidemiology and worker exposure studies. These are referred to in Table A1-9 and described separately below.

Qualitative concordance for Key Event 1 (T4 reduction) between experimental species and humans is plausible because the HPT axis operates in a qualitatively similar manner across species. In an experimental study with 2 phases, monkeys were administered ETU in the diet for 5 months (Leber *et al*, 1978). In Phase I, doses were 2-250 ppm (0.01-12.5 mg/kg bw/day) and in Phase II doses were 50-450 ppm (2.5-22.5 mg/kg bw/day). In Phase I, T4 was marginally decreased at 50 ppm (2.5 mg/kg bw/day) with other thyroid changes occurring at higher doses (e.g. weight increase at 12.5 mg/kg bw/day). In Phase II, T4 was decreased at doses greater than 150 ppm (7.5 mg/kg bw/day). These results indicate that monkeys respond to ETU but the reliability of the dose-response is not uncertain. The monkeys in Phase I suffered with tuberculosis and this phase had to be terminated early. The DAR (2000) questioned the reliability of the study as the NOAEL was derived from both phases, however the monkeys did respond to ETU.

There is no direct data on the thyroid from humans intentionally exposed to mancozeb or ETU, therefore there are no quantitative comparisons available. However, there are fundamental quantitative differences in the function and regulation of the HPT axis between rodents and humans and evidence that humans are less sensitive to thyroid disruption (see below). The more potent structural analogue, PTU, is used pharmaceutically to reduce T4 in human cases of hyperthyroidism. The dosage for humans is approximately

300 mg/day, equating to ~4 mg/kg bw/day for a 70 kg adult. In adult rats, T4 was reduced at PTU doses of 0.01 and 1 mg/kg bw/day in a 28 day study (Yamasaki *et al*, 2002). This indicates that rats are more sensitive than humans to PTU, although both species respond.

Qualitative concordance for Key Event 2 (TSH increase) between experimental species and humans is possible because the HPT axis operates in a qualitatively similar manner across species. In the monkey study described above, TSH was increased at doses of 7.5 mg/kg bw/day (Leber *et al*, 1978). As described for T4 reduction, there are no quantitative comparisons available but the fundamental quantitative differences in the function and regulation of the HPT axis means that humans are less sensitive to thyroid disruption than rats (see Table A1-9).

Qualitative concordance for Key Event 3 (increased thyroid follicular cell hypertrophy and/or hyperplasia) between experimental species and humans is possible because the HPT axis operates in a qualitatively similar manner across species. In the monkey study described above, thyroid follicular hyperplasia was observed. Iodine deficiency in humans leads to goitre. There are no quantitative comparisons available for humans but the fundamental quantitative differences in the function and regulation of the HPT axis means that humans are less sensitive to thyroid disruption than rats (see Table A1-9).

Qualitative concordance for the adverse effect Key Event 4 (Formation of thyroid follicular tumours) between rats and humans is unlikely to occur because humans are resistant to thyroid cancer. Exposure studies and epidemiology studies with mancozeb and ETU in humans have showed no increased incidence of thyroid tumours (see below). The fundamental quantitative differences in the function and regulation of the HPT axis means that humans are less sensitive to thyroid disruption than rats (see Table A1-9).

Table A1-9: Species concordance analysis of key events in the MoA for induction of thyroid tumours in rats by mancozeb

Key Event (name)	Qualitative Concordance		Quantitative Species Concordance	Confidence/ Uncertainty
	(Evidence in Experimental Species)	(Evidence in Humans)	(Experimental Species and Humans)	(Experimental Species and Humans)
Metabolism of mancozeb to ETU (activation) and metabolism of ETU (deactivation).	**Yes:** Metabolism of mancozeb to ETU (7% by weight) shown in all species studied (rats, mice, farm animals, monkeys). ETU is metabolised in rats, mice, dogs, guinea pigs, cats, and monkeys	**Yes**: Metabolism of mancozeb to ETU has been shown in humans. ETU is metabolised in humans.	**Plausible**: Metabolism of mancozeb to ETU is likely to be similar in experimental species and humans. Metabolism of ETU shows quantitative species differences. The rat shows lowest metabolism whilst human, mouse, dog and rabbit appear to be efficient metabolisers of ETU.	Overwhelming evidence from *in vivo* and *in vitro* studies for qualitative similarity. High evidence for quantitative species differences in experimental species and humans. The *in vitro* data on mouse in one study is not consistent with *in vivo* data but other *in vitro* is.
Molecular Initiating Event: **Inhibition of TPO by ETU**	**Yes**: Inhibition of TPO by ETU has been shown *in vitro* in rats and pigs. The down-stream consequences of TPO inhibition have been shown in rats, mice and dogs administered ETU.	**Likely**: Inhibition of human TPO by ETU has not been demonstrated but T4 reduction has been shown in monkeys administered ETU. TPO is a well-conserved enzyme.	**Not known**: Some species differences in TPO activity have been shown but no data on ETU and human TPO.	Overwhelming evidence for qualitative similarity and TPO being the molecular target in experimental animals. Uncertain evidence for quantitative species differences. Data for quantitative species differences is based on chemical analogues not ETU

Key Event (name)	Qualitative Concordance		Quantitative Species Concordance	Confidence/ Uncertainty
	(Evidence in Experimental Species)	(Evidence in Humans)	(Experimental Species and Humans)	(Experimental Species and Humans)
				itself.
Key Event 1: **Decreased blood T4 levels**	**Yes**: *In vivo* studies show that mancozeb reduces blood T4 levels in rats, mice and dogs. ETU has a similar qualitative effect.	**Plausible**: No direct data on mancozeb in humans. Exposure studies and epidemiology studies with mancozeb are negative. T4 was reduced in monkeys administered ETU. The ETU structural analogue, PTU, is used pharmaceutically to reduce T4 in human hyperthyroidism.	There are important differences between the physiology of the thyroid between rats (rodents) and humans. Humans may be quantitatively less sensitive than rats to chemicals reducing T4 levels. The biochemical response to TSH between rats (rodents) and humans is also quantitatively different. The rodent thyroid is far more dynamic than that of humans. The follicular cells appear to be in active synthesis compared to humans where they are quiescent. Humans have a greater reserve of thyroid hormone than rats, allowing for a bigger buffering capacity. T4 has a much shorter half-life in rats than in humans (12 hours compared to 5–9 days), and constitutive levels of TSH are about 25-fold higher in rats compared to humans. The shorter half-life of T4 is thought to be related to the absence of thyroid binding globulin (TBG) in adult rats, which leads to a higher turnover rate of thyroid hormones. In adult humans, thyroid hormones are tightly bound to TBG in blood. The consequence of these differences is that the thyroid of rodents is a much more active organ than that of humans. Thyroid tumours are a relatively common finding in rat	Overwhelming evidence for qualitative similarity among laboratory animals. Qualitative similarity with humans is plausible but no direct data on mancozeb. The reliability of the monkey study on ETU is uncertain. Strong evidence for quantitative differences in thyroid physiology with humans being less sensitive. The dose of PTU required to reduce T4 in humans is 4-40 fold higher than in rats.
Key Event 2: **Increased blood TSH levels**	**Yes:** In vivo studies show that mancozeb increases blood TSH levels in rats. Direct evidence in mice and dogs is lacking but the later events of increased thyroid follicular cell hypertrophy and/or hyperplasia indicate this occurs. ETU has a similar qualitative effect on TSH.	**Possible**: No direct data on mancozeb in humans. Exposure studies and epidemiology studies with mancozeb are negative. TSH is part of the thyroid hormonal system in humans. TSH was increased in monkeys administered ETU.		Overwhelming evidence for qualitative similarity among laboratory animals. Qualitative similarity with humans is plausible but no direct data on mancozeb. The reliability of the monkey study on ETU is uncertain. Strong evidence for quantitative differences in thyroid physiology with humans being less sensitive.
Key Event 3: **Increased thyroid follicular cell hypertrophy and/or hyperplasia (determined as increased thyroid gland weight or histopathologically)**	**Yes**: *In vivo* studies show that mancozeb increases thyroid follicular cell hypertrophy and/or hyperplasia in rats, mice and dogs. ETU has a similar qualitative effect.	**Uncertain:** No direct data on mancozeb in humans. Exposure studies and epidemiology studies with mancozeb are negative. Monkeys administered ETU showed increased thyroid follicular cell hyperplasia. Iodine deficiency in humans leads to goitre.		Overwhelming evidence for qualitative similarity among laboratory animals. Qualitative similarity with humans is plausible but no direct data on mancozeb. The reliability of the dose response of the monkey study on ETU is uncertain. Strong evidence for quantitative differences in thyroid physiology with humans being less sensitive.
Key Event 4: **Formation of thyroid**	**No**: Carcinogenicity studies show that mancozeb increases	**No**: Exposure studies and epidemiology studies with mancozeb		Strong evidence that mancozeb produced thyroid tumours in rats

Key Event (name)	Qualitative Concordance		Quantitative Species Concordance	Confidence/ Uncertainty
	(Evidence in Experimental Species)	(Evidence in Humans)	(Experimental Species and Humans)	(Experimental Species and Humans)
follicular tumours	thyroid follicular cell adenomas and carcinomas in rats. This did not occur in mice.	and ETU in humans have showed no increased incidence of thyroid tumours.	long-term studies, whilst the only known human thyroid carcinogen is ionizing radiation. Several analyses have been conducted to investigate the human relevance of rodent thyroid follicular tumours and have concluded that the relevance is low (Hill *et al*, 1998; Hurley, 1998; Hard, 1998).	but not mice. Strong evidence for quantitative differences in thyroid physiology with humans being less sensitive

Evidence for thyroid dysfunction or carcinogenicity in humans

The association between exposure to mancozeb and endocrine-related outcomes has been investigated both in medical studies and epidemiology studies of workers handling mancozeb and the general public who may be exposed to it. Many studies have not distinguished between EBDC pesticides generally and mancozeb. ETU in urine or blood has also been used as a biomarker of EBDC exposure.

In medical studies where data from workers in mancozeb/EBDC manufacturing plants were analysed to determine whether there was any evidence of thyroid dysfunction, no effects were found. De Fonso (1976) analysed mortality data from workers exposed to mancozeb in a manufacturing plant in the USA (1948-1975) and found that thyroid cancer in exposed workers was not increased. In another study, also in the USA, workers manufacturing mancozeb for many years were given thyroid function tests. Exposure to mancozeb was not associated with increased thyroid abnormalities (Anonymous, 1985a). A similar study in the Netherlands found that factory workers handling mancozeb had normal levels of thyroid hormones (Anonymous, 1990b). Ongoing monitoring of workers at mancozeb manufacturing plants have shown no evidence of thyroid dysfunction.

ETU is also a by-product in rubber manufacture. Two studies of workers at rubber manufacturing plants also monitored thyroid hormone levels (Smith, 1984) and thyroid cancer and foetal abnormalities (Smith, 1976). One group of workers (mixers) was reported to have lowered T4 levels, but not other groups. No increased incidence of thyroid cancer was found.

Epidemiology studies have also examined the possible association between mancozeb (EBDC) exposure and effects on the thyroid. The quality of these studies varies widely and several are contradictory. Details are provided in Dossier M-CA Section 5.9 (2015).

Steenland *et al* (1997) found low levels of ETU in the urine of workers using EBDC fungicides. T4 levels were similar amongst the exposed workers and a non-exposed group whilst TSH adjusted for age appeared slightly elevated. Age was a confounding factor in this study and in fact TSH in all groups was within normal levels. The authors stated that the results were of "borderline significance". Correlation between blood ETU and thyroid disorder was studied in a group of workers in the Philippines (Panganiban *et al*, 2004). There was no difference in thyroid function between directly exposed, indirectly exposed and non-exposed groups. The authors claimed that the exposed workers had a higher incidence of solitary thyroid nodules but the analyses performed in this paper are so poor that the associations and conclusions are considered unreliable. Goldner *et al* (2010) analysed the association between thyroid disease and pesticide use in two papers. The first (2010) investigated female spouses in the Agricultural Health Study in the USA (1993-1997). The second (2013) investigated male applicators in the same Agricultural Health Study. In the first study, the authors claimed a

role of maneb/mancozeb in the development of thyroid disease whilst in the second study maneb/ mancozeb was not associated with thyroid effects. Both studies had significant limitations, such as an inability to distinguish between prevalence and incidence of disease and to determine whether exposure preceded disease onset. The results should therefore be interpreted cautiously. A study by Nordby *et al* (2005) investigated the association between mancozeb exposure and thyroid cancer in farmers' families. The study had significant limitations including a lack of adjustment for confounding factors and very crude measures of exposure. The authors concluded that there was no association between mancozeb exposure and thyroid cancer.

The overall conclusion from these epidemiology and medical studies is that environmental or workplace exposure to mancozeb does not disrupt the thyroid hormonal system in humans. Monitoring data shows that exposure levels are generally well within current reference doses and these are protective of human health.

Summary

Having considered all the evidence, the Human Relevance Framework then addresses the following three questions regarding the human relevance of mancozeb-induced thyroid tumours.

- **Is the weight of evidence sufficient to establish the hypothesised mode of action in experimental species?**

Yes, there is clear evidence that mancozeb causes thyroid cancer in rats via metabolism to ETU, inhibition of TPO, reduction of thyroid hormone synthesis and consequent disruption of thyroid homeostasis.

- **Can human relevance of the specific mode of action be reasonably excluded on the basis of fundamental qualitative differences in key events between experimental species and humans?**

No. TPO is a well-conserved key enzyme involved in the synthesis of thyroid hormones. Drugs such as PTU and methimazole are TPO inhibitors that operate via this MoA to treat hyperthyroidemia in humans. The HPT axis also operates in a similar qualitative way in experimental species and humans, although there are fundamental differences in the physiology of the thyroid system indicating humans are less sensitive than rats to thyroid disrupters.

- **Can human relevance of the specific mode of action be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?**

Yes. The *in vivo* and *in vitro* metabolism data support the rat being the most sensitive species to mancozeb because metabolism of ETU is least efficient in this species. Differences in dose response in rats, mice and dogs administered mancozeb or ETU show that effect levels are lowest in rats and highest in mice, supporting this argument. Thyroid tumours do not develop in mice exposed to mancozeb because at MTD, insufficient ETU is generated to cause tumours.

In addition to kinetic differences there are dynamic differences between rats and humans that indicate that humans are less sensitive to thyroid disruption than rats. Differences in the physiology of the thyroid, a longer half-life for T4, the presence of thyroid binding globulin in blood in humans means that human thyroid has a much greater buffering capacity than the rat thyroid. Many studies in humans have also demonstrated that thyroid cancer is very rare, even in patients with Graves disease or goitre. A study of environmental and heritable causes of cancer in Sweden showed that the environment did not play a principal causative role in thyroid cancer in humans (Czene *et al*, 2002). However, it is a commonly observed finding after chemical exposure in rats.

Other potential Modes of Action

Other possible MoAs for tumour induction, such as genotoxicity and other non-genotoxic thyroidal modes of action have been excluded. Mancozeb and ETU are not genotoxic and there is no evidence that other thyroidal modes of action contribute to the carcinogenicity of mancozeb (EU, 2009; M-CA Dossier Section 5, 2015).

Genotoxicity

Mancozeb, ETU and EBDCs as a group of chemicals have been extensively tested for genotoxicity in various bacterial, fungal, and mammalian cell in *in vitro* test systems; in assays for structural chromosomal damage in somatic cells *in vivo*; and for heritable effects in Drosophila gene mutation assays. The only studies in which

positive genotoxic effects have been observed with mancozeb have significant deficiencies, or are flawed in various ways. Overall, neither mancozeb nor ETU is mutagenic when properly tested in higher organism test systems in the two major endpoints used to assess genotoxicity, gene mutation and chromosomal damage. Similarly, ancillary tests have not provided significant evidence of genotoxic damage. The overall body of toxicological data coming from a number of *in vitro* and *in vivo* assays indicates that mancozeb and ETU are not genotoxic (EU, 2009; M-CA Dossier Section 5, 2015).

Alternative non-genotoxic thyroidal modes of action

A common mode of action for chemicals causing thyroid follicular tumours in rats is enhanced metabolism of thyroid hormones by liver enzyme induction leading to reduced T4 levels and disruption of the HPT axis (Dellarco *et al*, 2006; Zoeller, 2007; Miller *et al*, 2009). Chemicals acting via this mechanism induce hepatic xenobiotic metabolising enzymes including T4-uridine diphosphate glucuronosyl transferase (UDPGT), the enzyme responsible for conjugation of thyroid hormones prior to excretion. The potential for a contributory effect of this to the main MoA of TPO inhibition has been examined. However, in several studies in rats, mancozeb appeared to have little effect on the liver. Flippin *et al* (2009) reported that mancozeb did not induce the activity of mixed function oxidase (MFO) enzymes or UDPGT. Szepvolgyi *et al* (1989) and Anonymous (1986b) reported that MFO activity was decreased, whilst Anonymous (1989), Anonymous (1992a), and Anonymous (1990a) reported that the liver was unaffected. This MoA therefore does not appear to have a contributory role for mancozeb.

Another potential contributory MoA is inhibition of iodothyronine deiodinase. Iodothyronine deiodinase is an enzyme present in peripheral tissues that is responsible for the deiodination of T4 to T3, which is a more active ligand than T4 for the thyroid hormone receptor. Inhibition of iodothyronine deiodinase has been demonstrated to be a contributory MoA for PTU. ETU however, did not inhibit this enzyme, demonstrating it is not a contributory MoA for ETU (Freyberger and Ahr, 2006; O'Neil and Marshall, 1984).

Other MoAs, such as interaction with the thyroid hormone receptor have also been excluded (Hass *et al*, 2012; Kjeldsen *et al*, 2013).

Uncertainties/Inconsistencies and Identification of Data Gaps

Inconsistencies and uncertainties have been identified in the previous discussions. However, they are few in number compared to the data supporting the arguments presented.

Classification of mancozeb and carcinogenic potency considerations

In addition to the factors discussed above, the possible classification of mancozeb for carcinogenicity should consider carcinogenic potency using the T25 concept (the chronic daily dose which will give 25% of the animals tumours at a specific site, after correction for spontaneous incidence, within the standard life-span of that species). Carcinogens of high potency have a T25 value of: ≤ 1 mg/kg bw/day; those of medium potency a T25 value of 1 to ≤ 100 mg/kg bw/day, and those of low potency a T25 value of > 100 mg/kg bw/day. The T25 value for mancozeb as a rat thyroid carcinogen was calculated from the study of Anonymous (1990a) using the method in EC (1999) (see Section 10.7.5 of the main document). The T25 value for mancozeb and rat thyroid adenomas was 23.6 mg/kg bw/day whilst that for mancozeb and rat thyroid carcinomas was 33.5 mg/kg bw/day. In both cases this places mancozeb into the medium potency carcinogen group. According to a TCC&L paper on thyroid tumours (ECBI49/99-Add1.Rev2) non-genotoxic carcinogenic substances producing thyroid tumours in rodents with low or medium potency by a clearly established perturbation of the thyroid hormone axis, in general, do not need to be classified. Mancozeb is a medium potency rat thyroid carcinogen with a clearly established non-genotoxic MoA and therefore does not need to be classified.

Conclusions in relation to problem formulation

There is sufficient evidence to establish the MoA for thyroid disruption caused by mancozeb-induced thyroid tumours in rats. Mancozeb is a medium potency (T25 value of 1 to ≤ 100 mg/kg bw/day) rat thyroid carcinogen. There is qualitative evidence that mancozeb, via its metabolite ETU, could potentially cause hypothyroidism in humans as the operation of the HPT axis is qualitatively similar across mammalian species. However there is quantitative evidence that exposure of humans to ETU following mancozeb exposure is lower than rats because of differences in the metabolism of ETU. There are also substantial quantitative dynamic

differences in the physiology of the thyroid gland between rats and humans that mean that the human thyroid is less responsive to thyroid disrupters than rats. Thyroid cancer in humans is a rare event whilst thyroid cancer in rats is common. Epidemiology and medical studies also show no increased incidence of hypothyroidism or thyroid cancer in exposed individuals. The question formulated "**Are thyroid tumours in rats caused by mancozeb, relevant for humans who may be exposed to mancozeb?"** can be answered by concluding that the significant quantitative kinetic and dynamic factors means that relevance is low and the risk to humans is negligible. The clearly established non-genotoxic MoA, the medium potency and low risk to humans means that mancozeb should not be classified as a carcinogen.

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ANNEX II: EVALUATION OF DEVELOPMENTAL TOXICITY, POSSIBLE MODES OF ACTION AND RELEVANCE TO HUMANS

Introduction and objectives

In this annex the developmental toxicity studies on mancozeb are discussed in more detail with respect to toxicokinetic factors controlling levels of its metabolite ETU; possible modes of action (MoA) and the relevance of these for humans. ETU is teratogenic in rats but not in rabbits and therefore this raises questions about human relevance; for example, are humans more similar to rats or to rabbits; how much exposure to mancozeb has to occur to reach the lowest adverse effect level (LOAEL) for ETU in sensitive species? ETU inhibits the formation of thyroid hormones but there is evidence that ETU-induced teratogenicity is not caused by thyroid hormone modulation. The objective of this annex is to provide further detail and discussion in order to help resolve these issues.

Mancozeb and developmental toxicity in non-human, mammalian species

Regulatory background

Mancozeb has been the subject of several regulatory guideline developmental toxicity studies. In 1980, a rat developmental toxicity reported by Anonymous, investigated a dose level of 512 mg/kg bw/day when given to pregnant rats on gestation days 6-15. This dose level of mancozeb was in excess of the maximum tolerated dose inducing maternal death, severe weight loss, embryo-foetal death and foetal malformations. The malformations were attributed to ETU and not to maternal toxicity because of the consistency of the observations with the ETU positive control group (50 mg/kg bw/day) in the same study. The malformations were also consistent with the earlier studies of ETU reported by Khera (1973)[#]. On the basis of the work of Khera (1973) the no observed effect level of ETU for malformation in the rat was indicated to be 5 mg/kg bw/day. A more recent study (Saillenfait, 1991) indicated that the no observed effect level for malformation in the rat was 15 mg/kg bw/day.

[#]N.B. Although the data reported by Khera in 1973 is relevant in the context of foetal malformation, it should be noted that the studies predate GLP, predate formal test guidelines and the study designs are not consistent with current OECD/EPA test guidelines. For these reasons and also the lack of reported detail, the publication has not been summarised in this document.

In 1993, a Specialised Expert Group reviewed the data from the following studies on the prenatal developmental toxicity of mancozeb in rats and rabbits

- Larsson, 1976 (rat – non-guideline publication, not summarised in this document nor included in the DAR)
- Anonymous, 1980 (rat)
- Anonymous, 1987b (rabbit).

The Specialised Expert Group acknowledged that the foetal malformations present in rats were "…attributed to the formation of ETU", a metabolite of all EBDC fungicides, but as "… ETU levels produced by the parent compound would not reach the threshold for teratogenic effects" classification was not justified.

The Draft Assessment Report of mancozeb was prepared by Italy (2000). The review of developmental toxicity was based on the following studies:

- Anonymous, 1980 (rat)
- Anonymous, 1988c (rat)
- Anonymous, 1999b (rat)
- Anonymous, 1987b (rabbit)
- Anonymous, 1991b (rabbit).

In Addendum 1 to the DAR (Addendum 1 Draft Assessment Report and Proposed Decision of Italy prepared in the context of the possible inclusion of mancozeb in Annex I of Council Directive 91/414/EEC (2002)) it was concluded that "mancozeb is not a teratogenic agent".

"This evaluation of the mancozeb developmental toxicology database clearly demonstrates that:

the maternal NOEL reported in the Anonymous rat development study is not consistent with the toxicity profile of mancozeb and on the basis of the questionable validity, this study should not be incorporated into the developmental hazard evaluation of mancozeb

- *developmental toxicity associated with mancozeb in the rat is only observed at maternally toxic dosages*
- *the highest reported NOEL for maternal toxicity is 55 mg/kg bw/day for rabbits and 60 mg/kg bw/day for rats (discounting the Anonymous findings)*
- *the highest reported NOEL for embryotoxicity/teratogenicity is 100 mg/kg bw/day for rabbits and 128 mg/kg bw/day for rats*

As stated in EC Council Directive 67/548/ECC, the classification of a substance as a possible risk of harm to the unborn child (R63) is based on the following criteria:

- *results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of developmental toxicity in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but which are not a secondary non-specific consequence of the other toxic effects, but where the evidence is insufficient place the substance in Category 2 (R61 May cause harm to the unborn child).*

Findings from the valid, well-conducted developmental studies showed no evidence to cause a suspicion of developmental toxicity in the absence of marked maternal toxicity. Thus, the criteria for R63 are not met.

On the basis of the current classification criteria and the results of the developmental toxicity database evaluation, mancozeb should not be classified as a developmental toxicant."

Mancozeb was reconsidered for health effects at the Classification and Labelling Working Group Meeting in January 2003. The question of classification for developmental effects (i.e., R63) based on foetal malformations such as cleft palate was discussed briefly and deferred to the next meeting (ECBI/52/03). Industry's position against the need for R63 was presented in ECBI/89/02 Add 11. In the January 2003 Follow-up Period, Greece submitted a position paper (ECBI/89/02 Add. 3) proposing R63 based on teratogenic and foetotoxic potential in rats from the studies of Anonymous (1980) and Anonymous (1988c). At the Working Group meeting in September 2003 the decision was again deferred to allow the meeting to revisit the opinion of the Specialised Experts, who had decided against the need for R63 in 1993. In 2005, the Specialised Experts were asked to evaluate any new studies since the 1993 meeting and to consider whether this additional data would change the conclusion they had reached. It was recognised that a few new studies had been reported and that these supported the recommendation of the Specialised Experts 1993 meeting as no developmental effects were observed as also stated in the 91/414/EEC (DAR) monograph. The Specialised Expert Group was referred to the guidance produced by an expert group on parental toxicity (ECBI/30/04) on how to evaluate maternal toxicity. This guidance clarified how to evaluate developmental effects observed in the presence of maternal toxicity. It was pointed out that a key issue was the highest dose tested because it caused unacceptable maternal toxicity (death, paralysis, suffering, total litter loss). As such toxicity contravened Annex V test guideline requirements, the highest dose (512 mg/kg bw/day) was considered irrelevant for decision-making purposes. Nevertheless, the developmental toxicity studies with mancozeb were considered to provide clear evidence of the mechanistic link to ETU and were therefore relevant to the assessment of classification. The consensus opinion of the Specialised Expert Group was that classification of mancozeb for developmental toxicity was warranted. There was an equal distribution of opinion towards classification for Repr. Cat. 2, R 61 and Repr. Cat. 3, R 63. The Specialised Expert Group advised the evaluation of developmental neurotoxicity (OECD TG 426) for ethylenebis(dithiocarbamates) (EBDC) to address the concern about thyroid effects and brain development (ECBI/58/05).

At the Technical Committee meeting on classification and labelling (2006) the decision was taken that mancozeb should be classified as a Category 3 developmental toxin and given the R63 risk phrase – "Possible risk of harm to the unborn child".

Response to the regulatory evaluation of mancozeb

The issue of developmental toxicity in the rat and the concerns of the Specialised Expert Group (2006) have been readdressed by the EU Mancozeb Task Force.

Developmental neurotoxicity

The concern for developmental neurotoxicity (DNT) was addressed by conducting a guideline and GLP compliant developmental neurotoxicity study on mancozeb in rats using the dietary route of exposure. The preliminary to this study confirmed that mancozeb (and ETU) was present in maternal and foetal blood and in milk (Anonymous, 2008b). In the main study, no evidence of developmental neurotoxicity was observed up to the highest dose tested (30 mg/kg bw/day) (Anonymous, 2008c). A lack of behavioural effects in offspring of female rats exposed (by oral gavage) to mancozeb during pregnancy and lactation was also reported in a literature study (Axelstad *et al*, 2011). In this study, rats were exposed to mancozeb at doses up to 150 mg/kg bw/day from gestation day 7 until lactation day 14. Despite significant toxicity in dams characterised by body weight loss and hind limb paralysis, and maternal hypothyroxinemia (resulting in reduction of the highest dose to 100 mg/kg bw/day) no effects were seen in offspring behaviour (spatial learning in a radial arm maze, motor activity measurements and acoustic startle response). In addition, an extended one generation reproduction toxicity study has been conducted on ETU using the dietary route of exposure (Anonymous, 2013). There were no effects on neurobehavioural endpoints or neuropathology at doses up to 10 mg/kg bw/day. A small decrease in brain weight and "size" was noted but was considered to be a consequence of effects on body weight, reflecting reduced growth during late lactation, juvenile and early adult periods and not evidence of specific developmental neurotoxicity.

The detailed results of the following studies to investigate DNT (conducted since the 2006 decision) are provided in the main document (Section 10.8).

- Mancozeb: A dietary exposure and dose range finding developmental neurotoxicity study in rats (Anonymous, 2008b) (reported in Addendum 4 to DAR 2000).
- Mancozeb: An oral (dietary) developmental neurotoxicity study in rats (Anonymous, 2008c) (reported in Addendum 4 to DAR 2000).
- ETU: An F1 Extended One Generation Reproductive Toxicity in Crl:CD(SD) rats (Anonymous, 2013) (required by US EPA).

Developmental toxicity (morphological)

The strategy adopted to address the concerns for potential developmental toxicity and morphological effects in rats was to conduct a GLP compliant developmental toxicity study to current OECD test guidelines with dose levels selected to meet test guideline and animal welfare requirements. To better understand the quantitative relationship between mancozeb and ETU production, initial studies were undertaken where mancozeb and ETU levels in maternal and foetal blood samples were analysed as part of dose range finding studies (Anonymous, 2015a & b). To investigate the dose-response of ETU and foetal malformation, a developmental toxicity study of ETU to current OECD test guidelines was undertaken (Anonymous, 2015d). This study also included a satellite group so that blood samples could be taken. ETU concentrations in maternal and foetal blood samples were then determined in order to establish blood levels of ETU at teratogenic and non-teratogenic doses of ETU. In addition, new studies on ETU in the rabbit have been conducted under REACh, legislation.

The detailed results of the following additional studies to investigate developmental toxicity (conducted since the 2006 decision) are provided in the main document (Section 10.8).

- Mancozeb: A 14-day oral (gavage) tolerability study in non-pregnant Sprague Dawley rats (Anonymous, 2015b).
- Mancozeb: A preliminary oral (gavage) study in pregnant Sprague Dawley rats (Anonymous, 2015c).
- Mancozeb: An oral (gavage) prenatal developmental toxicity study in Sprague Dawley rats (Anonymous, 2015d)

- ETU: A 14-day tolerability study in non-pregnant rabbits (Anonymous, 2010d) (required under REACh, legislation).
- ETU: Pilot developmental toxicity study in rabbits (Anonymous, 2010b) (required under REACh, legislation).
- ETU: Developmental toxicity study in rabbits (Anonymous, 2010c) (required under REACh, legislation).
- ETU: An oral (gavage) prenatal developmental toxicity study in Sprague Dawley rats (Anonymous, 2015d).

The key findings in context with the toxicokinetics are presented below.

The quantitative relationship between mancozeb and ETU production and its relevance for developmental toxicity

Early studies established that in rats, mancozeb is rapidly absorbed, metabolised and eliminated, following oral administration. Approximately 50% of mancozeb is absorbed and conversion of mancozeb to ETU is approximately 7% (by weight). ETU has an elimination half-life of 4-8 hours (Anonymous, 1986f; Anonymous, 1986g). The dietary exposure and dose range finding developmental neurotoxicity study in rats (Anonymous, 2008b) demonstrated that maternal dosing with mancozeb results in both maternal and foetal exposure. Neonatal pups are exposed to mancozeb secreted in milk. The toxicokinetics of ETU have also been studied in rats. When administered over a wide range of dose levels, absorption and elimination are rapid. ETU is predominantly eliminated (unchanged) in urine. Maternal dosing with ETU results in foetal exposure to ETU (Kato *et al*, 1976; Ruddick *et al*, 1977; Anonymous, 1987f).

While the early studies provide valuable information regarding the fate of mancozeb and ETU in rats, they rely largely on measurements made using non-specific endpoints i.e. radioactivity or a common moiety method measuring carbon disulphide derived from a range of sulphur containing metabolites and endogenous substances. In the new studies where the toxicokinetics of ETU and mancozeb have been determined as part of the investigation of the developmental toxicity of both substances in rats, a sensitive, specific LC-MS-MS analytical method for the determination of mancozeb and ETU in rat plasma was developed and validated. Use of this method ensured that only the analytes of interest were measured.

The key study details are as follows:

ETU: An oral (gavage) prenatal developmental toxicity study in Sprague Dawley rats (Anonymous, 2015d)

ETU was administered orally by gavage to groups of 24 bred female Crl:CD(SD) rats once daily from GD 6 through GD 19. Dosage levels were 0, 2.5, 5, 15 and 30 mg/kg bw/day. Clinical observations, body weights, and food consumption were recorded at appropriate intervals. On gestation day 20, a laparohysterectomy was performed and the uteri, placentae, and ovaries were examined. Maternal blood samples for toxicokinetic evaluation were collected from 4 confirmed pregnant females/group and foetal blood samples were collected from their litters on gestation day 20. All foetuses were weighed, sexed, and examined for external, visceral, and skeletal malformations and developmental variations.

Based on the absence of adverse maternal effects, a dosage level of 30 mg/kg bw/day was considered to be the NOAEL for maternal toxicity. Developmental effects were noted at 30 mg/kg bw/day, as evidenced by lower mean foetal weights and foetal malformations and developmental variations. Developmental effects were also noted at 15 mg/kg bw/day, but were limited to hydrocephaly in 2 litters. No test substance-related effects were noted at 2.5 and 5 mg/kg bw/day. Based on these results, a dosage level of 5 mg/kg bw/day was considered to be the NOAEL for embryo/foetal development, with a LOAEL of 15 mg/kg bw/day.

Blood concentrations of ETU on gestation day 20 were similar in maternal and foetal rats. ETU blood concentrations at 15 and 30 mg/kg bw/day were 1280 and 2940 ng/mL, respectively, in maternal rats and 1170 and 2790 ng/mL, respectively, in foetuses. Plasma concentrations of ETU in both maternal and foetal plasma

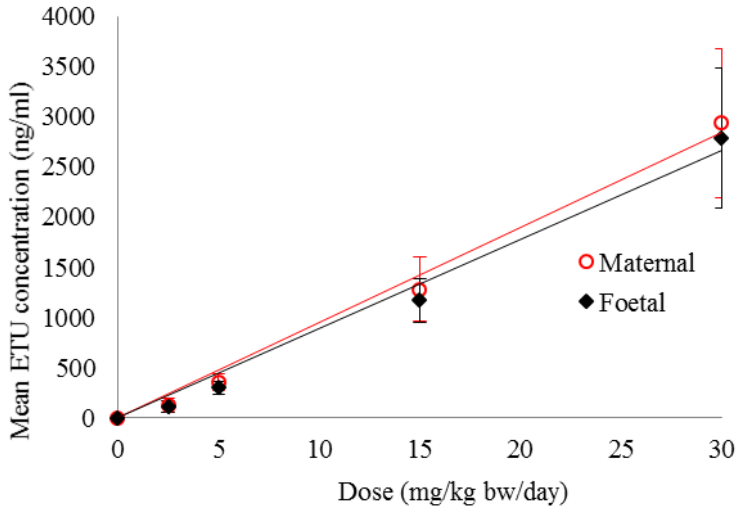
increased approximately linearly with maternal dose; foetal exposure was similar to that of the dams with maternal: foetal concentration ratios of 1.1 to 1.2 (Table A2-1, Figure A2-1).

Table A2-1: Mean plasma concentrations of ETU in maternal and foetal plasma on GD 20 after oral (gavage) administration of ETU

Dose (mg ETU/kg/day)	Maternal concentration (ng/mL)		Foetal concentration (ng/mL)		Maternal/foetal ratio
	Mean*	SD	Mean*	SD	
0	<LOQ		<LOQ		
2.5	124*[3]	68.7	113*[3]	59.5	1.1
5	355	88.1	299	66.9	1.2
15	1280	318	1170	221	1.1
30	2940	742	2790	694	1.1

* - N=4 except where designated as *[N]. <LOQ - concentration below the limit of quantitation (20 ng/mL).

Figure A2-1: Dose response for ETU in maternal and foetal plasma on GD 20 after oral administration of ETU



<u>Mancozeb: A 14-day oral (gavage) tolerability study in non pregnant Sprague Dawley rats (Anonymous, 2015b)</u>

Mancozeb was orally administered to non pregnant rats at doses of 60, 120, 180, 240, and 300 mg/kg bw/day for 14 days. Rat plasma concentrations for mancozeb ranged from BLQ < (10.0) to 213 ng/mL and for ETU ranged from BLQ < (10.0) to 1950 ng/mL, 6 hours after the final dose administration. Sporadic body weight losses occurred throughout the treatment period at dosage levels ≥180 mg/kg bw/day. The sporadic body weight losses, which occurred in the absence of reduced food consumption, produced lower (8.3%, 6.0%, and 9.8%) mean body weights at 180, 240, and 300 mg/kg bw/day, respectively, on study day 14 compared to the control group value. There were no effects on survival and macroscopic findings at any dosage level. Dosage levels of 60 and 120 mg/kg bw/day were considered to be well tolerated, based on the lack of toxicologically significant effects. Based on these results, dosage levels of 80, 120, and 160 mg/kg bw/day were selected for a preliminary oral (gavage) study of mancozeb in pregnant Crl:CD(SD) rats.

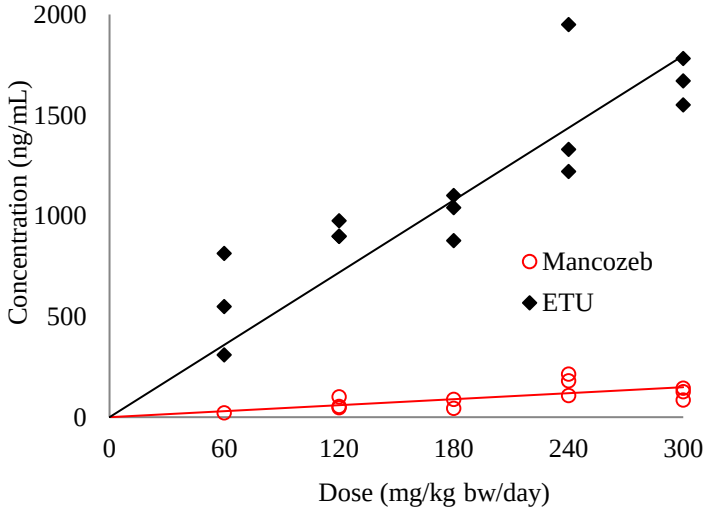
Although group sizes were small and concentrations variable, trend lines through the individual values show a clear dose response for exposure to ETU following administration of mancozeb. Mancozeb concentrations were significantly lower than those for ETU and represent the residue of rapid metabolism of mancozeb to ETU (Table A2-2, Figure A2-2).

Table A2-2: Plasma concentrations of mancozeb and ETU approximately 6 hours after the final dose of mancozeb

Dose (mg mancozeb/kg bw/day)	Mancozeb concentration range (ng/mL)	ETU concentration range (ng/mL)
0	<LOQ	<LOQ
60	<LOQ - 20.9	309 - 813
120	46.5 - 100	897 - 975
180	<LOQ - 87.6	876 - 1100
240	107 - 203	1220 - 1950
300	85.3 - 144	1550 - 1780

<LOQ - concentration below the limit of quantitation (20 ng/mL).

Figure A2-2: Dose response for mancozeb and ETU in plasma at approximately 6 hours after the final dose of mancozeb



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<u>Mancozeb: A preliminary oral (gavage) study in pregnant Sprague Dawley rats (Anonymous, 2015c)</u>

Mancozeb was administered orally by gavage to groups of 23 bred female Crl:CD(SD) rats once daily from GD 6 through to GD 19. Dosage levels were 0, 80, 120, and 160 mg/kg bw/day. Clinical observations, body weights, and food consumption were recorded at appropriate intervals. Blood samples for toxicokinetic analysis were collected from 3 maternal rats/group at 0 (pre-dose), 2, 4, 6, 12, and 24 hours after dose administration on gestation day 19; foetal blood samples were collected from each viable foetus immediately following maternal blood collection. Plasma samples collected from all maternal animals on gestation day 19 at the 2-, 4-, and 6-hour post-dose time points were also analysed for thyroxine (T_4) levels. All maternal animals utilized for toxicokinetic blood collection at the 0 (pre-dose), 2-, 4-, 6-, and 12-hour time points were euthanized following blood collection on gestation day 19, and pregnancy status was determined for each female. On gestation day 20, a laparohysterectomy was performed on each female not utilized for toxicokinetic

blood collection, as well as the females selected for the 24 hour toxicokinetic blood collection. The uteri, placentae, and ovaries were examined. The foetuses were weighed, sexed, and examined for external malformations and developmental variations.

Maternal toxicity was observed at a dosage level of 160 mg/kg bw/day as evidenced by lower mean body weight gains and corresponding reduced mean food consumption compared to the control group, which resulted in a slightly lower mean body weight on gestation day 20. There were no test substance-related effects on maternal survival, clinical condition, or macroscopic findings, or on intrauterine growth, survival, and external foetal morphology at any dosage level. Based on these results, dosage levels of 10, 40, and 160 mg/kg bw/day were selected for a definitive prenatal development toxicity study of mancozeb administered orally, by gavage, to pregnant Crl:CD(SD) rats.

The results for mancozeb from the 24 hour toxicokinetic blood collection are shown in Table A2-3:

Table A2-3: Mean plasma concentrations (ng/mL) of mancozeb in maternal and foetal plasma on GD19 after oral (gavage) administration of mancozeb

Dose	80 mg mancozeb/kg bw/day		120 mg mancozeb/kg bw/day		160 mg mancozeb/kg bw/day	
Time after the final dose (hours)	**Mean***	**SD**	**Mean***	**SD**	**Mean***	**SD**
Maternal plasma						
0	<LOQ	NA	<LOQ	NA	<LOQ	NA
2	61.0	72.0	97.9*[2]	NA	119	93.2
4	74.2	35.4	81.6*[2]	NA	96.1	35.6
6	26.4	24.1	<LOQ*[2]	NA	42.0	10.9
12	<LOQ	NA	<LOQ*[2]	NA	<LOQ	NA
24	<LOQ	NA	<LOQ	NA	<LOQ	NA
Foetal plasma						
0	<LOQ	NA	<LOQ	NA	<LOQ	NA
2	25.9*[1]	44.8	<LOQ	NA	<LOQ	NA
4	<LOQ	NA	<LOQ	NA	<LOQ	NA
6	<LOQ	NA	<LOQ	NA	<LOQ	NA
12	<LOQ	NA	<LOQ	NA	<LOQ	NA
24	<LOQ	NA	<LOQ	NA	<LOQ	NA

* - N=3 except where designated as *[N]. <LOQ - concentration below the limit of quantitation (20 ng/mL). NA - Not applicable.

As observed in the previous study, concentrations of mancozeb in both maternal and foetal samples were low and variable. Peak mean concentrations in maternal plasma were observed between 2 and 4 hours after dosing indicating rapid absorption; concentrations had declined to <LOQ (20 ng/mL) by 12 hours after dosing. Concentrations of mancozeb in foetal plasma were generally <LOQ.

Concentrations of ETU in both maternal and foetal plasma attained peak values by 6 hours after dosing with mancozeb indicating that once absorbed, mancozeb is rapidly metabolised to ETU (Table A2-4, Figure A2-3). Maternal and foetal plasma concentration versus time curves for ETU were very similar; comparison of the dose responses for ETU in maternal and foetal plasma based on $AUC_{(0-24)}$ and C_{max} values demonstrated that exposure increased approximately in proportion with dose and that there was little difference in exposure between the dams and foetus (Figure A2-4).

Table A2-4: Mean plasma concentrations (ng/mL) and toxicokinetic parameters for ETU in maternal and foetal plasma on GD 19 after oral (gavage) administration of mancozeb

Dose	**80 mg mancozeb/kg bw/day**		**120 mg mancozeb/kg bw/day**		**160 mg mancozeb/kg bw/day**	
Time after the final dose (hours)	**Mean***	**SD**	**Mean***	**SD**	**Mean***	**SD**
Maternal plasma						
0	147	57.9	272	97.3	214	83.3
2	686	120	1030	201	1100	101
4	761	88.8	1020	102	1400	126
6	973	56.1	1470	245	1720	156
12	867	183	801	88.6	1160	332
24	315	37.0	356	101	548	146
C_{max}(ng/mL)	973		1470		1720	
T_{max} (h)	6		6		6	
$AUC_{(0-24)}$ (ng.h/mL)	16600		19600		25800	
Dose	**80 mg mancozeb/kg bw/day**		**120 mg mancozeb/kg bw/day**		**160 mg mancozeb/kg bw/day**	
Time after the final dose (hours)	**Mean***	**SD**	**Mean***	**SD**	**Mean***	**SD**
Foetal plasma						
0	139	90.6	266	108	163	69.2
2	613	90.6	938	154	963	45.1
4	685	104	980	157	1250	162
6	883	20.8	1290	304	1470	57.5
12	806	204	776	41.4	1190	289
24	304	41.2	337	101	459	131
C_{max}(ng/mL)	883		1290		1470	
T_{max} (h)	6		6		6	
$AUC_{(0-24)}$ (ng.h/mL)	15300		18300		23900	

* - N=3.

Figure A2-3: Mean plasma concentrations versus time curves for ETU in maternal and foetal plasma on GD 19 after oral administration of mancozeb

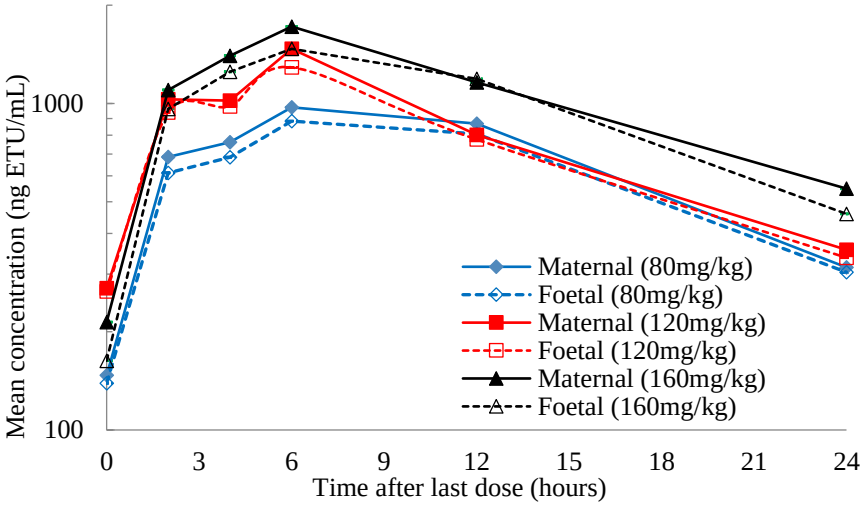
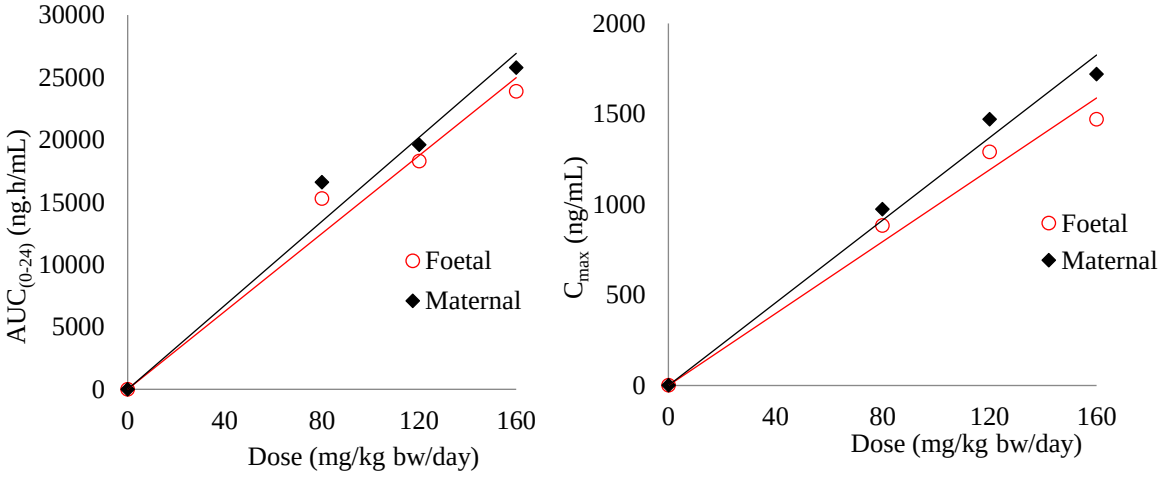


Figure A2-4: Dose response (AUC and C_{max}) for ETU in maternal and foetal plasma on GD 19 after oral administration of mancozeb



<u>Mancozeb: An oral (gavage) prenatal developmental toxicity study in Sprague Dawley rats (Anonymous, 2015d)</u>

Mancozeb was administered orally by gavage to groups of 25 bred female Crl:CD(SD) rats once daily from GD 6 through to GD 19. Dosage levels were 0, 10, 40, and 160 mg/kg bw/day. Clinical observations, body weights, and food consumption were recorded at appropriate intervals. On gestation day 20, a laparohysterectomy was performed and the uteri, placentae, and ovaries examined. The foetuses were weighed, sexed, and examined for external, visceral, and skeletal malformations and developmental variations.

Based on test substance-related effects on mean body weight gain and food consumption at 160 mg/kg bw/day, a dosage level of 40 mg/kg bw/day was considered to be the no observed adverse effect level (NOAEL) for maternal toxicity. No test substance related effects on intrauterine growth and survival or foetal morphology were noted at any dosage level. Therefore, a dosage level of 160 mg/kg bw/day, the highest dosage level tested, was considered to be the NOAEL for embryo/foetal development when mancozeb was administered orally by gavage to bred Crl:CD(SD) rats. There was no evidence of teratogenicity due to mancozeb in this study.

Measurements made in the previous studies provided the basis for an estimation of the dose levels of mancozeb necessary to produce peak concentrations of ETU equivalent to those likely to have been achieved following administration of ETU to pregnant rats at the NOEL (5 mg ETU/kg), LOAEL (15 mg ETU/kg) and effect (30 mg ETU/kg) doses.

Plasma concentration versus time data for ETU following administration of mancozeb was available over the range of doses from 80 to 160 mg mancozeb/kg; this was used to calculate the rate constants (k) for the decline in ETU concentrations at each dose levels, the mean, minimum and maximum values were determined.

Dose (mg mancozeb/kg bw/day)	**80**	**120**	**160**
k for ETU (h^{-1})	0.065684	0.077107	0.063419

Mean k value	0.068737
Minimum k value	0.063419
Maximum k value	0.077107

Measured maternal plasma concentrations of ETU at 24 hours after the final dose of ETU to pregnant rats were used along with the above values for k to estimate the maximum ETU concentrations at 6 hours after dosing (T_{max}) using the relationship:

$$C_{6h} = C_{24h}.e^{k.18}$$

Dose (mg ETU/kg bw/day)		**2.5**	**5**	**15**	**30**
C_{24h} (ng ETU/mL)	measured	124	355	1280	2940
C_{6h} (ng ETU/mL) mean	predicted	427	1223	4411	10131
C_{6h} (ng ETU/mL) minimum		388	1112	4008	9207
C_{6h} (ng ETU/mL) maximum		497	1422	5128	11779

Linear regression lines were fitted through the C_{6h} versus dose data and the parameters used to calculate the mean dose of mancozeb required to give concentrations of ETU equal to those at the NOEL (5 mg ETU/kg/day), LOAEL (15 mg ETU/kg/day) and effect (30 mg ETU/kg/day); an estimate of the uncertainty of the value was made by estimating the maximum and minimum values.

	NOEL (5mg ETU/kg bw/day)	**LOAEL (15 mg ETU/kg bw/day)**	**Effect (30 mg ETU/kg bw/day)**
Mean dose (mg mancozeb/kg/day)	143	429	859
Range of doses (mg mancozeb/kg/day)	130 - 166	390 - 499	780 - 998

These values indicate that doses of mancozeb up to those that caused maternal toxicity (160 mg/kg bw/day) in the oral (gavage) prenatal developmental toxicity study of mancozeb in Sprague Dawley rats (Anonymous, 2015d) result in peak plasma concentrations of ETU lower than those seen following administration of ETU at the NOAEL (5 mg/kg), LOAEL (15 mg/kg) and effect level (30 mg/kg) for developmental toxicity.

Discussion

The potential of mancozeb to induce reproductive and developmental toxicity has been thoroughly investigated in regulatory compliant studies.

Reproductive toxicity studies

These comprise:

- A two generation oral (dietary) reproduction toxicity study in rats (Anonymous, 1988b).
- A two generation oral (dietary) reproduction toxicity study in rats (Anonymous, 1992c).
- An oral (dietary) developmental neurotoxicity study in rats (Anonymous, 2008c)

The results of the two reproduction studies are in agreement and confirm that no reproductive or developmental toxicity due to mancozeb was observed at the highest dose level tested, which induced systemic toxicity. These studies clearly demonstrate that mancozeb does not induce reproductive toxicity and therefore requires no classification under CLP.

In addition, the main metabolite of mancozeb, ETU, has been thoroughly evaluated in a recent, regulatory compliant, extended one generation reproductive toxicity study (OECD TG 443) (Anonymous, 2013). The results of this evaluation confirmed that there was no evidence of ETU-related reproductive toxicity or of effects on the estrogen- or androgen-related endocrine pathways at any dose of ETU. No effects on neurobehavioral endpoints were detected at any dose of ETU, despite exposures during critical windows of development.

Neither mancozeb nor ETU induce reproductive toxicity.

Developmental toxicity studies

The main metabolite of mancozeb, ETU is a known teratogenic agent in rats. The relevant, regulatory compliant studies comprise:

- Mancozeb: oral (gavage) teratology study in the rat (Anonymous, 1988c)
- Mancozeb: oral (gavage) prenatal developmental toxicity study in the rat (Anonymous, 2015d)
- Mancozeb: oral (gavage) developmental toxicity study in rabbits (Anonymous, 1987b)
- Mancozeb: oral (gavage) teratogenicity study in the rabbit (Anonymous, 1991b)
- ETU: oral (gavage) prenatal developmental toxicity study in the rat (Anonymous, 2015a)
- ETU: oral (gavage) developmental toxicity study in rabbits (Anonymous, 2010c)

With respect to the evaluations of mancozeb in the rat, it is recognised that the teratogenicity study of Anonymous (1980) has been the focus of regulatory debate and that the results should not be ignored. However, they should be considered in context. It is recognised that this study (Anonymous, 1980) pre-dates GLP, the maternal data are inappropriately presented and most importantly, the highest dose tested exceeded the maximum tolerated dose by causing unacceptable maternal toxicity (death, paralysis, suffering, total litter loss). As the severe toxicity contravened Annex V test guideline requirements, the highest dose (512 mg/kg bw/day) should be regarded as irrelevant for decision-making purposes of the potential of mancozeb to induce developmental toxicity. Nevertheless, the results of this and other studies (Anonymous, 1988c; Anonymous, 2015d & a) demonstrate that the foetal malformations observed by Anonymous (1980) were totally attributable to the production of a teratogenic dose of ETU.

The prenatal developmental toxicity of ETU in the rat (Anonymous, 2015a) clearly identifies 5 mg/kg bw/day as a non-teratogenic dose. This result is entirely consistent with the results of studies published in the literature. Anonymous (2015a) also identified 15 mg/kg bw/day as a LOEL, causing a minimal teratogenic response, whilst 30 mg/kg bw/day was well within the teratogenic dose range. The mean doses of mancozeb calculated to produce blood levels of ETU equivalent to dosing ETU at 5, 15 and 30 mg/kg bw/day were 143 (range 130-166), 429 (range 390-499) and 859 (range 780-998) mg/kg bw/day respectively. Consequently, when mancozeb is administered to rats at a dose that is maternally toxic but does not exceed the maximum tolerated dose (160 mg/kg bw/day), insufficient ETU is generated to produce teratogenicity. Therefore, administration of mancozeb during organogenesis, at doses of 360 mg/kg bw/day (Anonymous, 1988c), 160 mg/kg bw/day

(Anonymous,, 2015c) or 128 mg/kg bw/day (Anonymous, 1980) did not induce foetal malformations nor generate a teratogenic dose of ETU. It should be noted that 360 mg/kg bw/day (Anonymous, 1988c) was not in excess of, but was close to, a maximum tolerated dose; slight paralysis of the hind limbs was induced in 5/25 females with one severely affected animal killed in extremis.

The highest dose of 160 mg mancozeb/kg bw/day was selected as an appropriate dose level for administration to the pregnant rat in the definitive, GLP, test guideline compliant, prenatal developmental toxicity study (Anonymous, 2015d). The dose, based on maternal toxicity, was within the range calculated to produce non-teratogenic levels of ETU. This dose of mancozeb did not impair foetal development in the rat. Therefore, 160 mg/kg bw/day, was the NOEL of mancozeb for embryo/foetal development in the rat.

The prenatal developmental toxicity of mancozeb and of ETU have also been evaluated in the rabbit. No teratogenicity was observed for either substance even in the presence of severe maternal toxicity. Mancozeb at ≥ 80 mg/kg bw/day was in excess of the maximum dose that could be tolerated by the pregnant dams (Anonymous,, 1987b and Anonymous, 1991b). The NOEL of mancozeb for maternal toxicity was 55 mg/kg bw/day (Anonymous, 1991b). The NOEL of mancozeb for developmental toxicity was 100 mg/kg bw/day (Anonymous, 1991).

For ETU, maternal toxicity (reduced body weight gain) was observed at 15 and 50 mg/kg bw/day (Anonymous, 2010c). Mean foetal weight was reduced at 15 and 50 mg/kg bw/day but the 3% difference from control at 15 mg/kg bw/day was within the laboratories historical control range and therefore considered not to be an adverse effect. There were no ETU-related increases in the incidences of foetal malformations or variations at any dose level. The results demonstrate that ETU is not a developmental or teratogenic agent in the rabbit.

The results of three prenatal developmental toxicity studies confirm that mancozeb is not a developmental toxicant in the rabbit and that ETU is not teratogenic in this species.

In conclusion, in well conducted, GLP, test guideline compliant studies, mancozeb has been shown to have no adverse effect on sexual function and fertility in the rat. Similarly, no adverse effects on development of the offspring of rats and of rabbits and no effects on the neurological development of rat offspring were seen. No classification of mancozeb for reproductive toxicity is therefore warranted.

ETU-mediated developmental toxicity: Possible modes of action

A role for thyroid hormones?

Thyroid hormones are essential for normal development and the maintenance of physiological functions in mammals. In young or foetal mammals, they also are essential for normal brain development and skeletal development. Cretinism, where affected individuals have severely stunted physical and mental growth, has long been known to be associated with severely reduced levels of thyroid hormones (Cranefield, 1962; Boyages and Halpern, 1993; Haddow *et al*, 1999).

Neurological effects in offspring exposed in utero have been described for several thyroid hormone perturbing chemicals including polychlorinated biphenyls (PCBs) (Crofton *et al*, 2000) and the pharmaceuticals, propylthiouracil (PTU) and methimazole (Axelstad *et al*, 2008; Brosvic *et al*, 2002; Shibutani *et al*, 2009; Mallela *et al*, 2014). In the case of PCBs, effects have been described in some human populations exposed via spillages as well as in experimental animal studies (WHO, 2012). However, no such human cases have been described for PTU and methimazole, which are administered to treat hyperthyroidemia. The difference may partly be attributable to different MoAs, or PCBs being sequestered in tissues and only slowly excreted compared to PTU and methimazole, and/or blood levels of these being controlled during treatment. Nevertheless, this example illustrates important differences in human risk for chemicals that have a similar key event of lowered plasma T4.

Potential for developmental neurotoxicity

The two DNT studies conducted on mancozeb in rats (Anonymous, 2008b, c; Axelstad *et al*, 2011) provide valuable information on whether the effects on thyroid hormones result in neurological changes in offspring. Both studies were conducted at dose levels of mancozeb that were above the lowest effect level (LOEL) of 15

mg/kg bw/day, derived in Annex 1, for effects on thyroid hormones. The main study of Anonymous used a maximum dose of 30 mg/kg bw/day (dietary administration) whilst Axelstad used a maximum of 150 mg/kg bw/day (gavage administration). Both studies demonstrated that T4 was reduced in dams at these doses. In pups, Axelstad showed that T4 was unchanged at PND 17 and 24 whilst this was not determined in the study of Anonymous 2008b. In both studies mancozeb had no effect on neurological parameters in offspring including learning, memory and startle response. Brain morphometry and histopathology were also unaffected.

In the extended one generation reproduction toxicity study on ETU in rats (Anonymous, 2013), the maximum dose was 10 mg/kg bw/day (dietary administration) and was therefore conducted at doses of ETU that were above the derived LOEL for effects on thyroid hormones (1-2 mg/kg) (see Annex 1). Effects on T4 were seen in both dams and pups at 2 and 10 mg/kg bw/day. As with mancozeb, there were no effects on neurological parameters in offspring including learning, memory and brain histopathology. These results for ETU contrast with neurological effects seen in rats dosed (via oral gavage) to PTU at 0.8 mg/kg bw/day in a developmental neurotoxicity study (Axelstad *et al*, 2008).

These studies demonstrate that neither mancozeb nor ETU cause neurological damage in offspring, at doses where thyroid hormones are affected in dams. They indicate that at current exposure levels, babies and children exposed to mancozeb are at very low risk for neurotoxicological effects. To date, reviews of epidemiology literature have not indicated that EBDCs may cause neurodevelopmental problems in humans (Ntzani *et al*, 2013; WHO 2012).

Potential for developmental toxicity (morphological)

ETU is developmentally toxic/teratogenic in the rat, causing malformations associated with neural tube development such as hydrocephaly and meningocele. Clear effects are seen at doses of 30 mg/kg bw/day and above with a NOAEL of 5 mg/kg bw/day (Anonymous, 2015d). Other species appear to be more resistant to the teratogenicity of ETU, with mice being largely unaffected at doses up to 800 mg/kg bw/day (Teramoto *et al*, 1978). Rabbits were also largely unaffected at doses up to 80 mg/kg bw/day (Khera, 1973) and no teratogenicity was observed in a recent guideline study of ETU in rabbits (Anonymous, 2010c). Hamsters appear to occupy a middle ground with malformations occurring at doses of 270 mg/kg bw/day. The species difference in response to ETU is due (at least) in part to greater metabolism of ETU in non- (or less) responsive species. This has been reviewed by Khera (1987) and Daston (1997).

Khera (1987) indicated that neuroblasts in the developing nervous system of the rat are the specific target of ETU. Several studies have investigated whether modulation of thyroid hormones are involved in the developmental toxicity of ETU. Initial studies indicated that they may be involved whilst later studies did not support this. The relevant studies are summarised in Table A2-5. The following is taken from the DAR on mancozeb (2000) and is still relevant: "In studies of the mechanism of action, combined treatment of immature rats with thyroxine (T3/T4) and ETU was found to reduce the incidences of hydrocephalus induced by 20 or 40 mg/kg daily doses of ETU (Emmerling, 1978). However, in a second study with ETU administered orally at a 40 mg/kg bw/day dose to hypothyroid (thyroparathyroidectomised), euthyroid (otherwise untreated), or thyroxine-supplemented rats, foetal malformations were unrelated to the alteration in thyroid function in the maternal rat (Lu and Staples, 1978). Combined treatment with ETU and the metabolic inhibitors, SKF-125 or methimazole, increased the incidence and severity of foetal malformations compared to those in groups given ETU only, while combined treatments with the enzyme inducers, phenobarbital or methylcholanthrene failed to influence the teratogenic outcome, thereby indicating that ETU per se may be responsible for the malformations (Khera and Iverson, 1981)."

Support for a direct effect of ETU on the foetus, rather than via thyroid hormones is provided by *in vitro* studies (Table A2-6). In the study of Stannistreet *et al* (1990), the effect of two thyroid hormone antagonists (ETU and methimazole) on rat embryos were compared *in vitro*. In this study, both ETU and methimazole caused abnormalities at concentrations of 0.5 mM and above but the pattern of changes was different. The authors concluded that ETU and methimazole had a direct effect on embryos rather than via changes in thyroid hormones. Further evidence that ETU has a direct effect on the developing embryo rather than via thyroid hormones is provided by experiments with rat foetal cell cultures (Khera, 1987) and whole embryo cultures (Daston *et al*, 1987). In the studies of Daston (1987, 1997), rat whole embryo cultures exposed directly to ETU developed the same type of malformations as those seen *in vivo*. These studies also lent further support

to the species sensitivity to ETU being at least partly due to ability to metabolise ETU. Hepatic S9 prepared from rat (the species most sensitive to ETU) liver was ineffective at reducing the incidence of malformations *in vitro* whereas addition of S9 prepared from mouse (the species least sensitive to ETU) liver reduced the incidence of malformations. These results are consistent with a direct effect that is ablated when ETU is removed by metabolism.

Khera (1987) concluded that ETU-induced foetal malformations were unrelated to maternal thyroid function. Whilst the exact MoA is not known, recent studies in rats where ETU has been used to model anorectal malformations indicate that it has a direct effect on genes involved in development (Mi *et al*, 2014; Wang *et al*, 2011).

Table A2-5: Studies investigating the effect of thyroid hormones on ETU-induced developmental toxicity in the rat

Study design	Findings relevant for developmental toxicity	Comments	Reference
Pregnant rats (up to 16/group) were administered ETU, T3/T4 or sodium iodide from GD 7-20, by gavage in water, in the following combinations: T3: 20 µg/kg + T4: 100µg/kg NaI 333 µg/kg ETU 20 mg/kg ETU 20 mg/kg + NaI ETU 20 mg /kg + T3/T4 ETU 40 mg/kg ETU 40 mg/kg + NaI ETU 40 mg/kg + T3/T4	The teratogenic response to ETU was reduced for some malformations (micrognathia, cleft palate, syndactyl, ablepharia) when T3/T4 was co-administered, but not for malformations such as hydrocephaly, meningocele, short and kinky tail. Sodium iodide co-administration did not provide any protective effect.	The results indicate the teratogenic effect of ETU may in part be secondary to the thyroid toxicity of ETU.	Emmerling (1978)
Rats (6-12 per group) were rendered hypothyroid by removal thyroids and parathyroids (TPTX). Pregnant TPTX and euthyroid (sham operated) rats were administered ETU (40 mg/kg bw/day) and /or T4 from GD 7-15, by gavage in water.	84-100% of the foetuses in all litters from the groups given ETU were malformed regardless of the thyroid status of the dams. 10% of the foetuses of TPTX dams that were not given ETU were malformed. No malformations were noted among the foetuses of the other groups not given ETU. Malformations, after ETU was given to euthyroid rats, were mostly of CNS, rib, and tail changes, often in the presence of clubbed foot. TPTX rats given ETU also had a high incidence of oedema, micrognathia, cleft palate and micromelia. These latter malformations were not seen in TPTX rats not given ETU. There was a high incidence of delayed ossification of the skull of foetuses from all groups given ETU and in	Results indicate that foetal malformations were unrelated to the alteration in thyroid function in the maternal rat. However, hypothyroidism altered the spectrum of malformations in response to ETU. Thyroidectomy also caused increased malformations but these were thought to be the results of maternal stress.	Lu and Staples, (1978)

	TPTX rats (related to body weight).		
Pregnant rats (5 per group) were administered ETU by gavage in water, at 0, or 60 mg/kg bw/day, on GD 13; in combination with the metabolic inhibitor SKF-525A (SKF) and the antithyroid drug methimazole (MMI). Further groups were pretreated with the MFO inducers phenobarbital (PB) or methylcholanthrene (MC) before ETU (60 mg/kg bw/day on GD 13).	MMI and SKF, alone or in combination were not teratogenic. MMI+SKF+ETU, or MMI + ETU, or SKF+ETU increased the incidence and severity of malformations compared to the group given only ETU. Pretreatment with PB or MC had no effect on the incidence of ETU-induced malformations.	Results indicate that ETU *per se* is responsible for malformations. Inhibition of ETU metabolism increases rate of malformations by inhibiting removal of ETU. SKF is an inhibitor of P450. MMI and ETU are thought to be metabolized via P450 and FMN therefore MMI may be a competitive inhibitor of P450 in the presence of ETU. The lack of effect of PB/MC may have been due to insufficient doses, inability to reduce the foetal levels of ETU or the P450 isoforms induced by PB/MC may not be involved in ETU metabolism.	Khera and Iverson (1981)

Table A2-6: Studies investigating the direct effect of ETU on developmental toxicity in the rat

Study design	Findings relevant for developmental toxicity	Comments	Reference
Rat foetal neuronal and non-neuronal cells were grown in the presence of ETU	ETU at concentrations greater than 0.5mM caused necrosis of neuronal cells and a reduction in the formation of neurites and fascicles. Non-neuronal cells were unaffected.	Study indicates a direct effect on neuronal cells	Khera (1987)
Rat embryos at GD 9.5 were incubated for 48 h with ETU or methimazole to determine possible direct effects on rat embryos of the two thyroid antagonists.	Both ETU and methimazole inhibited development of the embryos. At 0.5mM and above, ETU caused abnormalities. Most commonly of the neural tube. Methimazole also caused abnormalities at 0.5mM and above but the spectrum of changes was different from ETU. The concentration at which methimazole disturbed rat embryogenesis was higher than that which is reached in treated hyperthyroid patients.	Both of the thyroid antagonists affected development, but with different effects. The authors concluded that ETU has a direct effect on the rat embryo rather than through thyroid hormone changes.	Stanisstreet *et al* (1990)
Rat embryos at GD 10 were incubated for 48 h with ETU to determine possible morphological changes.	A dose-related inhibition of crown-rump length, protein & DNA content, somite number and increased abnormalities were seen from 0.04mM ETU. Abnormalities included hydrocephalus, decreased mandibular size, decreased telencephalon size, abnormal dorsiflexion and sub-ectodermal blisters. Osmolarity of the exocoelomic fluid was lowered.	The abnormalities seen here in vitro are seen at the same sites as those seen in rats after in vivo exposure to ETU. The authors suggested that ETU alters osmotic balance in the embryo and this may contribute towards the formation of defects.	Daston *et al* (1987)

Comparison of the dose responses for the teratogenicity of ETU and the effects on T4 shows an order-of-magnitude difference also implying that different MoAs may be responsible. The NOAEL for the ETU teratogenicity is 5 mg/kg bw/day, with the LOEL being about 15 mg/kg bw/day (Anonymous, 2015d) whilst the NOAEL and LOEL for effects on T4 were 0.2 and 2 mg/kg bw/day respectively (Anonymous, 2013).

Mancozeb itself is not teratogenic when dosed at non-maternally toxic doses (up to 160 mg/kg bw/day, Anonymous, 2015d). The overall LOEL for effects of mancozeb on the rat thyroid was estimated to be 15 mg/kg bw/day (see analysis in Annex 1), indicating that thyroid hormones were likely to be reduced at the high dose of 160 mg/kg bw/day in the developmental toxicity study of Anonymous (2015d). Plasma T4 was measured in blood samples from the toxicokinetic phase of the preliminary developmental toxicity study on mancozeb (Anonymous, 2015c). Measurements were made at 2, 4, and 6 hours. At 4 hours, T4 levels in the 160 and 120 mg/kg bw/day groups were 23-40% lower than control values. However, there was no consistent dose response and changes were not statistically significant. Sample sizes were small (n=3) as the study was not designed to investigate this and did not have sufficient statistical power to draw a clear conclusion. However, results indicate that there may have been a weak effect on thyroid hormones at doses where there was no effect on development.

The large difference between doses of mancozeb where effects occur on thyroid hormones and the doses that can be reached in developmental toxicity studies before maternal toxicity occurs provides support for the absence of a link between thyroid hormones and developmental toxicity.

Comparison of ETU with other thyroid hormone antagonists shows that potent thyroid antagonists such as PTU and methimazole, unlike ETU, cause effects on the brain in experimental animal species but there is no indication that thyroid antagonists commonly cause teratogenicity. Shibutani *et al* (2009) observed impaired brain development with PTU and methimazole but not external malformations. Mallela *et al* (2014) treated rats and mice with PTU or methimazole in a developmental toxicity study, but did not observe gross external malformations or histopathological malformations. Axelstad *et al* (2008) also observed effects on behaviour and memory in a developmental neurotoxicity study on PTU, but no external or visceral malformations. The absence of morphological developmental effects at doses where developmental neurotoxicity is observed indicates that the reduction in thyroid hormones does not lead to commonly lead to external/visceral malformations.

In conclusion, ETU does not cause developmental neurotoxicity in rats, at doses where thyroid hormones are reduced. Although ETU is teratogenic in rats, similar effects are not seen in rabbits or mice. Several lines of evidence indicate that the morphological effects on rat foetuses are caused by a direct MoA and not via thyroid hormone reduction.

Human relevance

The effects of mancozeb/ETU in experimental animals and humans also depends upon absorption, distribution, metabolism and excretion in those species. Effective removal of ETU via metabolism and excretion means that internal exposure to ETU is reduced. The bioconversion of mancozeb to ETU is 7% on a weight basis, similar to the bioconversion factor for other ethylenebisdithiocarbamates (EBDC). In monkeys, oral doses were very poorly absorbed (Emmerling, 1978). The spectrum of metabolites produced was similar in laboratory and farm animals indicating similar metabolism of mancozeb across species. The pharmacokinetics and metabolism of ETU have been studied in mice, rats, guinea pigs, cats, and monkeys. These studies have shown that ETU is rapidly excreted, primarily in the urine and more quickly in mice than in rats. Half-lives for elimination from maternal blood were 5.5 and 9.4 hours in mice and rats, respectively (Ruddick *et al*, 1977). Literature studies indicate that mancozeb and ETU are also rapidly absorbed and eliminated after oral administration in humans. The elimination half-life of ETU was estimated to be 19-23 hours in humans (Aprea *et al*, 1996; Lindh *et al*, 2008).

The metabolism of ETU across species has been compared in two *in vitro* studies (the studies are discussed in more detail in Annex 1). Saghir *et al* (2005) compared rat, mouse and human using liver S9 as a source of metabolising enzymes. Zhu (2015) compared rat, mouse, dog, rabbit and human using primary hepatocytes as the source of metabolising enzymes. The results of the two studies were similar in that metabolism in the rat was lower than in humans. However, metabolism in mouse hepatocytes in Zhu (2015) was lower than in rat hepatocytes whilst the opposite occurred in Saghir *et al* (2005). The latter result concurs with *in vivo* comparisons of rat and mouse metabolism where metabolism is clearly greater in mice. Metabolism in dog and rabbit appears similar to humans. The *in vitro* metabolism studies show that the metabolism of ETU in hepatocytes increases in the following order: rats < mice < humans, with rabbits and dogs being similar to humans (Saghir *et al*, 2005; Zhu, 2015).

Metabolism of ETU is likely to play a major role in the sensitivity of different species to the developmental toxicity of ETU. Metabolism in humans appears more efficient than rodents, indicating that ETU may be readily removed in humans. These results indicate that humans are more similar to insensitive species than sensitive species (rats).

An analysis of the margin of exposure for mancozeb and developmental toxicity shows that the NOAEL for embryo/foetal development is 160 mg/kg bw/day (the highest dosage level tested in Anonymous, 2015d) compared with current reference values of: AOEL 0.035 mg/kg bw/day; ADI 0.05 mg/kg bw/day and ARfD 0.6 mg/kg bw/day (Review Report for mancozeb: SANCO/4058/2001-rev. 4.4 July 2009) demonstrating a large margin for all values. Epidemiology and medical studies have not shown that exposure to mancozeb is associated with developmental effects (See main document Sections 10.7-10.10).

Conclusions

Mancozeb and ETU do not cause developmental neurotoxicity in offspring when administered to pregnant rats. Mancozeb does not cause structural developmental abnormalities when administered to pregnant rats or rabbits. Although ETU is teratogenic, several lines of evidence indicate that this is not related to its MoA in the thyroid. Epidemiology studies also show that in humans, mancozeb does not cause developmental toxicity. The weight of evidence therefore indicates that the risk of developmental toxicity after exposure of humans to mancozeb is very low.

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ANNEX III: SUPPLEMENTARY DATA ON THE DEGRADANTS OF MANCOZEB

Aquatic bioaccumulation of the degradant ETU

The log Kow for the major degradant of mancozeb, ETU, has been estimated by QSAR analysis. The values calculated are in the negative for pH 5.5, 7, and 9, therefore the same conclusions can be drawn for ETU as were drawn for mancozeb.

Table A3-1: Summary of relevant information on aquatic bioaccumulation

Method	Test Substance	Results	Remarks	Reference
Predicted value (ACD Labs version 12.01) Non-GLP	Ethylenethiourea (ETU)	Log Kow = -0.66 (pH 5.5) Log Kow = -0.66 (pH 7.0) Log Kow = -0.66 (pH 9.0)	Acceptable. ETU is included in the residue definition, therefore these data are required.	Anonymous (2014) Study 2014/1321435

Table A3-2: Toxicity endpoints for the degradants of mancozeb

Guideline and GLP	Substance (purity in % w/w)	Species	Endpoint Effect	Exposure		Results		Reference	Submitted for original approval (O) or renewal (N)
				Design	Duration	Endpoint	Toxicity (mg a.s./L)		
Fish Acute Toxicity									
similar to OECD 203 GLP	ETU (purity: 100%)	*Oncorhynchus mykiss*	Mortality	Static	96h	LC_{50}	> 490	Anonymous, 1982a	O
OECD 203 GLP	ETU (purity 99.9%)	*Oncorhynchus mykiss*	Mortality	Static	96h	LC_{50}	> 500	Anonymous, 2001	O
OECD 203 GLP	EU (purity 90.8%)	*Oncorhynchus mykiss*	Mortality	Static	96h	LC_{50}	> 122	Anonymous, 2001a	O
Fish/Aquatic vertebrate Chronic Toxicity									
Similar to OECD 231	ETU (purity 99.9%)	*Xenopus laevis*	Development. Amphibian Metamorphosis	Semi-static	28d	NOEC	10	Anonymous, 2002	O
Invertebrate Acute Toxicity									
OECD 202 GLP	ETU (purity 97.7%)	*Daphnia magna*	Immobilisation	Static	48h	EC_{50}	49	Hutchinson & Spare, 1982b	O

Guideline and GLP	Substance (purity in % w/w)	Species	Endpoint Effect	Exposure		Results		Reference	Submitted for original approval (O) or renewal (N)
				Design	Duration	Endpoint	Toxicity (mg a.s./L)		
OECD 202 GLP	ETU (purity 97.7%)	*Americamysis bahia*	Immobilisation	Static	48h	EC_{50}	11	Soucy, 2008	N
OECD 202 GLP	ETU (purity 99.6%)	*Daphnia magna*	Immobilisation	Static	48h	EC_{50}	21.6	Hisgen, 2000	O
OECD 202 GLP	EU (purity 90.8%)	*Daphnia magna*	Immobilisation	Static	48h	EC_{50}	> 985	Palmer et al., 2001b	O
Invertebrate Chronic Toxicity									
Similar to OECD 211	ETU (purity 96.2%)	*Daphnia magna*	Reproduction	Flow-through	21d	NOEC	2.0	Graves, 1995	O
Algal Toxicity									
OECD 201 GLP	ETU (purity 99.6%)	*Pseudokirchneriella subcapitata**	Growth rate	Static	72h	E_rC_{50}	93.8	Reuschen-bach, 2000	O
OECD 201 GLP	EU (purity 90.8%)	*Selenastrum capricornutum**	Growth rate	Static	72h	E_rC_{50}	> 119	Palmer, 2001c	O
Theoretical degradant endpoints based on QSAR calculations									
Performed by the applicant using the software ECOSAR V 1.1 (U.S.EPA)	EBIS	Fish	Mortality	N/A	N/A	LC_{50}	0.183	N/A	N
		Daphnia	Immobilisation			EC_{50}	0.210		
		Algae	Growth rate			E_rC_{50}	0.102		

*Currently known as *Raphidocelis subcapitata*

Reply from the Applicant on the classification of relevant metabolites in order to determine the 'rapid degradability' classification of mancozeb

Harmonized classification is available for all relevant degradants of mancozeb (with exclusion of glycolic acid, which can be self-classified as non-toxic based on the relevant aquatic toxicity data for this metabolite) and has therefore to be adopted for classification purposes within the EU.

The metabolite EBIS is legally classified within the EU with H400 (Aquatic acute 1) and H410 (Aquatic Chronic 1) according to Annex VI of (EC) No 1272/2008 (CLP). Regarding the other relevant metabolites of mancozeb, no environmental hazard classification is given according to Annex VI of (EC) No 1272/2008 (CLP) (Table 1).

Table 1: **Acute and chronic classification of the relevant degradants of mancozeb**

Degradant (CAS No.)	Study type	Findings [mg/L]	DT_{50} (or DT_{90}/3.322 where behaviour is biphasic)	**Triggered classification and labelling**	Reference
EBIS (33813-20-6)	--	--	0.5 – 10.9 days[1]	**Aquatic acute hazard cat. 1 (H400)**	legal classification of EBIS in Annex VI of (EC) No 1272/2008 (CLP)
	--	--		**Aquatic chronic hazard cat. 1 (H410)**	
EDA (1,2-Ethanediamine) (107-15-3)	--	--	Not calculable	**No aquatic acute hazard cat.**	legal classification of EDA in Annex VI of (EC) No 1272/2008 (CLP)
	--	--		**No aquatic chronic hazard cat.**	
Ethanolamine (2-Aminoethanol) (141-43-5)	--	--	20.2 days	**No aquatic acute hazard cat.**	legal classification of ethanolamine in Annex VI of (EC) No 1272/2008 (CLP)
	--	--		**No aquatic chronic hazard cat.**	
Glycolic acid (79-14-1)	48-h EC_{50}	44.0	2060 days	**No aquatic acute hazard cat.**	Japanese Environment Agency (NR), Acute Immobilization Test of Glycolic acid to Daphnia magna Report Nr. 1998-生22
	21-d NOEC	4.38		**No aquatic chronic hazard cat.**	Japanese Environment Agency (NR), Reproduction Inhibition Test of Glycolic acid to Daphnia magna Report No: 1998-生23
Ethylene glycol (107-21-1)	--	--	11.9 days[2]	**No aquatic acute hazard cat.**	legal classification of ethylene glycol in Annex VI of (EC) No 1272/2008 (CLP)
	--	--		**No aquatic chronic hazard cat.**	
Oxalic acid (possibly M1) (144-62-7)	--	--	37.8 days[3]	**No aquatic acute hazard cat.**	legal classification of oxalic acid in Annex VI of (EC) No 1272/2008 (CLP)
	--	--		**No aquatic chronic hazard cat.**	

[1] = Decline calculated on total amount in water + sediment phase
[2] = DT50 calculated tentatively on 2 sample points
[3] = Study had two incubations; maximum occurred in one incubation at study end, but in the other incubation the substance declined before end to allow tentative DT50 calculation (three sample points from study end)

Note that EU and ETU were not further considered as it was already confirmed by CRD that you agree with our conclusion and no further data/evaluation is required:

„In conclusion, neither EU or ETU fulfil the criteria for classification for 'hazardous to the aquatic environment'."

In conclusion, only the metabolite EBIS must be considered as environmentally hazardous in the evaluation of ready biodegradability of the parent compound. Regarding its environmental behaviour, EBIS is rapidly formed from mancozeb and maximum half-lives of < 1 day are reported for the water phase before the

metabolite will be converted to the non-toxic ETU. Longer half-lives, as given in the table above, are related to the total water-sediment system. For the compartment water EBIS can therefore be classified as rapidly degradable too.

Moreover, looking at the known mode of action of EBDCs like mancozeb, this is explained in the literature by the transient formation of early intermediate products like EBIS (often named DIDT, forming other intermediates like ethylene diisothiocyanate) and their unspecific reaction with cell constituents such as thiol-containing enzymes (Ludwig & Thorn, 1960; Kaars Sijpesteijn, 1984).

Hence, as EBDCs like mancozeb act via their early intermediate products, the classification of mancozeb into Aquatic Acute Hazard Cat. 1 (H400) and Aquatic Chronic Hazard Cat. 1 (H410) already considers the aquatic toxicity of EBIS (which has the same classification). Therefore, the consideration of the classification of both molecules (mancozeb and EBIS) within the evaluation of the rapid degradability of mancozeb would overestimate the risk for the environment.

In conclusion, apart from EBIS the aquatic metabolites of mancozeb are not hazardous to aquatic environment. The classification of EBIS is already covered by the classification of mancozeb and therefore mancozeb can be assessed as rapidly degradable.

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