Committee for Risk Assessment

RAC

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

Adopted

15 March 2019

Annankatu 18, P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

15 March 2019

CLH-O-0000001412-86-263/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **mancozeb (ISO); manganese ethylenebis(dithiocarbamate) (polymeric) complex with zinc salt**

EC Number: **-**

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

The proposal was submitted by the **United Kingdom** and received by RAC on **29 December 2017.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

The United Kingdom has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **26 February 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **27 April 2018**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Michal Martínek**

Co-Rapporteur, appointed by RAC: **Helena Polakovičová**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **15 March 2019** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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	mancozeb (ISO); manganese ethylenebis(dithiocarbamate) (polymeric) complex with zinc salt	-	8018-01-7	Repr. 2 Skin Sens. 1 Aquatic Acute 1	H361d*** H317 H400	GHS08 GHS07 GHS09 Wng	H361d*** H317 H400		M=10	
Dossier submitters proposal	006-076-00-1	mancozeb (ISO); manganese ethylenebis(dithiocarbamate) (polymeric) complex with zinc salt	-	8018-01-7	**Retain** Skin Sens. 1 Aquatic Acute 1 **Add** STOT RE 2 Aquatic Chronic 1 **Remove** Repr. 2	**Retain** H317 H400 **Add** H373 (thyroid, nervous system) (oral) H410 **Remove** H361d***	**Retain** GHS08 GHS07 GHS09 Wng	**Retain** H317 H400 **Add** H373 (thyroid, nervous system) (oral) H410 **Remove** H361d***		**Retain** M=10 **Add** M=10	
RAC opinion	006-076-00-1	mancozeb (ISO); manganese ethylenebis(dithiocarbamate) (polymeric) complex with zinc salt	-	8018-01-7	**Retain** Skin Sens. 1 Aquatic Acute 1 **Add** STOT RE 2 Carc. 2 Aquatic Chronic 1 **Modify** Repr. 1B	**Retain** H317 H400 **Add** H373 (thyroid, nervous system) H351 H410 **Modify** H360D	**Retain** GHS08 GHS07 GHS09 Dgr	**Retain** H317 H400 **Add** H373 (thyroid, nervous system) H351 H410 **Modify** H360D		**Retain** M=10 **Add** M=10	
Resulting entry in Annex VI if adopted by RAC and agreed by Commission	006-076-00-1	mancozeb (ISO); manganese ethylenebis(dithiocarbamate) (polymeric) complex with zinc salt	-	8018-01-7	Carc. 2 Repr. 1B STOT RE 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H351 H360D H373 (thyroid, nervous system) H317 H400 H410	GHS08 GHS07 GHS09 Dgr	H351 H360D H373 (thyroid, nervous system) H317 H410		 M=10 M=10	

GROUNDS FOR ADOPTION OF THE OPINION

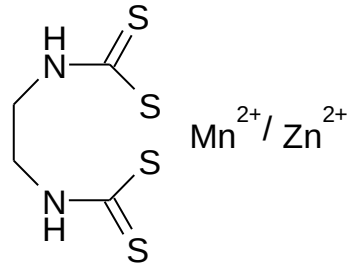
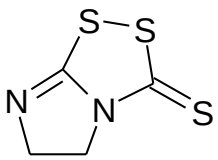
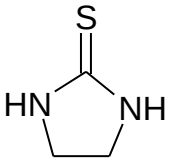
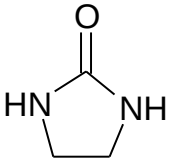
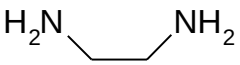
RAC general comment

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).

HUMAN HEALTH HAZARD EVALUATION

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

Skin sensitization studies		
Type of study; Reference	**Method**	**Observations**
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**.

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.

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) • Thyroid follicular cell hyperplasia (in all animals) ≤ 29 mg/kg bw/d: no thyroid-related findings	100 mg/kg bw/d
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) • ↑ thyroid weight (m absolute 1.4-fold, not stat. sign.) 2.3 mg/kg bw/d: no effects	25 mg/kg bw/d
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.

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.

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.

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	Negative ±S9 Toxicity not reported

Genotoxicity studies		
Type of study; Reference	**Method**	**Observations**
	Vehicle: water	
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 (only in the RAR, not in the CLH report)	OECD 471 GLP *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	Negative ±S9 Toxicity at 78 µg/plate (–S9) and 39 µg/plate (+S9)
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%)

Genotoxicity studies		
Type of study; Reference	**Method**	**Observations**
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	Negative No mortalities Clinical signs: transient slight piloerection, hunched posture, ptosis No convincing evidence of bone marrow toxicity

Genotoxicity studies		
Type of study; Reference	**Method**	**Observations**
	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	
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 2000 polychromatic erythrocytes per animal Vehicle: maize oil	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
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

Genotoxicity studies		
Type of study; Reference	**Method**	**Observations**
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.

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.

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	<u>Non-neoplastic findings</u> 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 <u>Neoplastic findings</u> 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	<u>Non-neoplastic findings</u> 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 <u>Neoplastic findings</u> None
Carcinogenicity, dietary Belpoggi *et al.* 2002	Non-guideline Non-GLP Animals treated for 2 years but observed until spontaneous death	<u>Non-neoplastic findings</u> ≤ 1000 ppm (≈ 50 mg/kg bw/d): none (thyroid function not investigated); survival not affected

	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	<u>Non-neoplastic findings</u> 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 <u>Neoplastic findings</u> None
18-month carcinogenicity, dietary Anon. 1992b	Doses: 0, 25, 100, 1000 ppm; equivalent to 0, 4.3, 17, 170 mg/kg bw/d 60/sex/dose	<u>Non-neoplastic findings</u> 1000 ppm (170 mg/kg bw/d): • Minor reductions in bw and bw gain

Available only as a summary from a WHO/FAO review		≤ 100 ppm (17 mg/kg bw/d): • No toxicologically significant treatment-related effects <u>Neoplastic findings</u> • 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.)

<u>Dietary carcinogenicity studies, Anon. (1991a) and Anon. (1992b)</u>

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.

<u>Dermal carcinogenicity study, Shukla et al. (1990)</u>

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. However, a number of factors increasing and decreasing the concern have to be taken into account for the classification (CLP, Annex I, Section 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.

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.

Assessment and comparison with the classification criteria

Developmental toxicity

Developmental studies evaluated in the initial DAR (2000)

The rat and rabbit prenatal developmental toxicity (PNDT) studies with mancozeb (or mancozeb and 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 Anon. 1980	EPA OPPTS 870.3700 Non-GLP 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	**Mancozeb** <u>Maternal toxicity</u> 512 mg/kg bw/d: • 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

Developmental toxicity studies with mancozeb (or mancozeb and ETU) evaluated in the initial DAR (2000)		
Type of study; Substance; Reference	**Method**	**Observations**
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	<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)

Developmental toxicity studies with mancozeb (or mancozeb and ETU) evaluated in the initial DAR (2000)		
Type of study; Substance; Reference	**Method**	**Observations**
	Dosing GD 7-19 20 females/group	• 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 <u>Developmental toxicity</u> 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	<u>Maternal toxicity</u> 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 <u>Developmental toxicity</u> 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.

<u>Relationship between maternal hypothyroidism and developmental neurotoxicity</u>

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-pregnant 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.

ENVIRONMENTAL HAZARD EVALUATION

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 in in the following table.

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%	*Lepomis macrochirus* Acute, semistatic, 96 h	Mortality	96-h LC_{50}: 0.083 mg a.s./L (mm)	Anonymous, 1987e

Test substance and guideline	Species, test conditions	Endpoint	Results	Reference
OECD TG 203, GLP			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	
Mancozeb Tech. Purity: > 90% OECD TG 203, GLP	*Oncorhynchus mykiss* Acute, semistatic, 96 h	Mortality	96-h LC_{50}: 0.088 mg a.s./L (mm) 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	Anonymous, 1997e
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

Test substance and guideline	Species, test conditions	Endpoint	Results	Reference
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."
References :
Kaars Sijpesteijn, A. (1984). Mode of action of some traditional fungicides. Pages 135-153 in: Mode of Action of Antifungal Agents. A. P. J. Trinci and J. F. Ryley, eds. Cambridge University Press, Cambridge.
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**.

Additional references

ATSDR (2012) Toxicological profile for manganese. https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=102&tid=23 (accessed on 04/01/2019)

Chandra, M.; Riley, M.G.I.; Johnson, D.E. (1992) Spontaneous neoplasms in aged sprague-dawley rats. Archives of Toxicology 66:496-502

Chernoff, N., *et al.* (1979) Perinatal toxicity of maneb, ethylene thiourea and ethylenebisisothiocyanate sulfide in rodents. Journal of Toxicology and Environmental Health 5:821-834

Domico, L.M., et al. (2006) Acute neurotoxic effects of mancozeb and maneb in mesencephalic neuronal cultures are associated with mitochondrial dysfunction. Neurotoxicology 27:816-825

EU Commission Group of Specialised Experts in the fields of carcinogenicity, mutagenicity and reprotoxicity (1999) Non-genotoxic thyroid carcinogens in the rodent bioassay, ECBI/49/99 Add. 1 Rev. 2

European Commission (2017) Supporting the organisation of a workshop on thyroid disruption – Final report. Framework Contract ENV.A.3/FRA/2014/0029 on implementation of the Community strategy on Endocrine Disrupters. Written by Brunel University London and DTU National Food Institute Denmark

Gift, J.S., et al. (2013) Scientific considerations for evaluating cancer bioassays conducted by the Ramazzini Institute. Environmental Health Perspectives 121:1253-1263

Khera, K.S.; Tryphonas, L. (1977) Ethylenethiourea-induced hydrocephalus: pre-and postnatal pathogenesis in offspring from rats given a single oral dose during pregnancy. Toxicology and Applied Pharmacology 42:85-97

Lang, P. (1992) Spontaneous neoplastic lesions and selected non-neoplastic lesions in the Crl:CD®BR rat. Charles River Laboratories

Lu, M.-H.; Kennedy, G.L. (1986) Teratogenic evaluation of mancozeb in the rat following inhalation exposure. Toxicology and Applied Pharmacology 84:355-368

McLeod, D.S.A., et al. (2012) Thyrotropin and thyroid cancer diagnosis: a systematic review and dose-response meta-analysis. Journal of Clinical Endocrinology and Metabolism 97:2682-2692

McMartin, D.N., et al. (1992) Neoplasms and related proliferative lesions in control Sprague-Dawley rats from carcinogenicity studies : historical data and diagnostic considerations. Toxicologic Pathology 20(2):212-225

Medda, E., et al. (2017) Iodine nutritional status and thyroid effects of exposure to ethylenebisdithiocarbamates. Environmental Research 154:152-159

Moog, N.K., et al. (2017) Influence of maternal thyroid hormones during gestation on fetal brain development. Neuroscience 342:68-100

Nagane (2008) Bacteria reverse mutation test of Fortuna Technico (mancozeb technical) using Salmonella typhimurium. Report No. 7223. JAI Research Foundation, India.

National Toxicology Program (NTP) (2019) Historical controls. https://ntp.niehs.nih.gov/results/dbsearch/historical/index.html (accessed on 21/02/2019)

Ruddick, J.A.; Khera, K.S. (1975) Pattern of anomalies following single oral doses of ethylenethiourea to pregnant rats. Teratology 12:277-281

Saillenfait, A., et al. (1991) Difference in the developmental toxicity of ethylenethiourea and three N,N'-substituted thiourea derivatives in rats. Fundamental and Applied Toxicology 17:399-408

Shukla, Y., et al. (1990) Carcinogenic activity of a carbamate fungicide, mancozeb on mouse skin. Cancer Letters 53:191-195

Stula, E.F.; Krauss, W.C. (1977) Embryotoxicity in rats and rabbits from cutaneous application of amide-type solvents and substituted ureas. Toxicology and Applied Pharmacology 41:35-55

Teramoto, S.; Shingu, A.; Kaneda, M. (1978b) Effects of ethylenethiourea upon developing brain and limb buds in the rat. Congenital Anomalies 18:241-249

Tyagi, S., et al. (2011) Neoplastic alterations induced in mammalian skin following mancozeb exposure using in vivo and in vitro models. OMICS 15:155-167 (in the RAR cited as Shilpa et al.)

van Wendel de Joode, B., et al. (2016) Pesticide exposure and neurodevelopment in children aged 6-9 years from Talamanca, Costa Rica. Cortex 85:137-150

Zheng, J., et al. (2016) Quantitative assessment of preoperative serum thyrotropin level and thyroid cancer. Oncotarget 7:34918-34929

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).