Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

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

EC Number: -
CAS Number: 8018-01-7

CLH-O-0000001412-86-263/F

Adopted

15 March 2019

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON MANCOZEB (ISO); MANGANESE ETHYLENEBIS(DITHIOCARBAMATE) (POLYMERIC) COMPLEX WITH ZINC SALT

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: mancozeb (ISO); manganese ethylenebis(dithiocarbamate) (polymeric) complex with zinc salt
EC number: -
CAS number: 8018-01-7
Dossier submitter: United Kingdom

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Belgium		MemberState	1
Comment received				
BE CA would thank the UK CA for this interesting CLH dossier proposal. Mancozeb, through its main metabolite ethylenethiourea (ETU), has been demonstrated to specifically target thyroid, inducing histopathological and endocrine changes, eventually leading to thyroid neoplastic findings in rat, but also developmental alterations in pups. The ETU metabolite is known to inhibit the thyroid peroxidase, reducing the formation of thyroid hormone precursors. The disturbance of the hypothalamus-pituitary-thyroid axis trough circulating T4 decrease leads to THS levels increase, therefore inducing thyroid hypertrophy. Prolonged exposure might result in thyroid hyperplasia, adenomas or carcinomas. Observations of developmental malformations in rat due to mancozeb are also attributed to the formation of its main metabolite ETU, which is classified as a Repr. 1B developmental toxicant. Transient impairment of maternal thyroid hormone levels have been shown to affect neural brain organization and behavior. Therefore, although the three HH endpoints are discussed separately, BE CA would stress the various consequences induced by the same endocrine disruption mechanism and the need for specific criteria's to assess the endocrine system. BE CA also notes that ETU is not only the main metabolite of mancozeb, but also its main degradation product. In the CLH Report, ETU is reported as an impurity in a concentration range of maximum 0,09% and is therefore considered as not relevant for classification and labelling.				

In the CLH dossier, the reported purity of mancozeb is ≥ 85% (p.4). Considering the reported impurities (max 0,09% ETU) and additives (max 2,5% hexamethylene tetramine), please indicate the other compounds entering in the composition of the substance.

Because of the instability of mancozeb, BE CA express their concern regarding the non-relevance of ETU for classification. In particular, the DS indicated that the purity of the tested mancozeb ranged from 80% - 92,3%, suggesting that the ETU percentages might potentially be higher than 0,09%. An underestimation of the ETU concentration in mancozeb might affect its final classification. Therefore, BE CA is of the opinion that the purity of mancozeb is a crucial question.

Dossier Submitter's Response

Thank you for your comments. Please see later comments for responses regarding the effects in the thyroid and the classification for carcinogenicity and reproductive toxicity.

Information on the full composition of mancozeb is considered to be confidential and is already provided in the IUCLID. All impurities and additives have been taken into account and, with the exception of ETU and hexamethylene tetramine, are not considered to be relevant to the classification and labelling.

In the technical specification for the active substance, the maximum concentration of ETU permitted is <0.3%. However, analysis of the batches demonstrate that ETU is typically present at lower levels (i.e., <0.09%).

RAC's response

Thank you for your comments.

RAC is mandated to compare the available data against the current CLP criteria and use the current CLP guidance. As to the developmental toxicity, mechanistic studies with ETU (e.g., Emmerling 1978) indicate that the main ETU-induced malformations in the rat (hydrocephalus, meningocele, tail malformations) are not mediated by maternal thyroid disruption.

RAC confirms that according to the information in the RAR, the maximum concentration of ETU in the analysed batches was 0.09%.

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Germany		MemberState	2

Comment received

The German CA agrees with the proposal of classification for environmental hazards as Aquatic acute 1 (H400) and Aquatic chronic 1 (H410) and the acute/chronic M-factor of 10.
We do not agree with the proposal to remove the current classification for developmental toxicity – see specific comment.

Dossier Submitter's Response

Noted – thank you for your support regarding the environmental classification.
Please see later response regarding developmental toxicity.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2018	Netherlands		MemberState	3
Comment received				
The Dutch CA supports the proposal to retain the current harmonized classification for Aquatic Acute 1 (M=10) and to add classification as Aquatic Chronic 1 (M=10). The available acute and chronic toxicity data support the classification proposal. Regarding the chronic fish toxicity data, it is agreed that an EC10 is preferred above the NOEC when both can be derived from the same study. The critical point with regard to chronic classification is the degradation potential of mancozeb. We agree that mancozeb should not be regarded as rapidly degradable, as mineralization was shown to be limited, and while primary degradation was rapid, one of the transformation products (EBIS) has a harmonized classification as Aquatic Acute category 1 and Aquatic Chronic category 1.				
Dossier Submitter's Response				
Noted – thank you for your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	France		MemberState	4
Comment received				
§ 1.1 Table 1 (p 4): Dithane M45 is the name of one of the representative formulation of the RAR, it should not be proposed as another name for Mancozeb.				
Dossier Submitter's Response				
We agree with these comments and it should not have been included in the CLH report.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Belgium		MemberState	5
Comment received				
BE CA would thank the UK CA for this interesting CLH dossier proposal. In general mancozeb, through its main metabolite ethylenethiourea (ETU), has been demonstrated to specifically target thyroid, inducing histopathological and endocrine changes, eventually leading to thyroid neoplastic findings in rat, but also developmental alterations in pups. The ETU metabolite has been shown to inhibit the thyroid peroxidase, reducing the formation of thyroid hormone precursors. The disturbance of the hypothalamus-pituitary-thyroid axis trough circulating T4 decrease leads to THS levels increase, therefore inducing thyroid hypertrophy. Prolonged exposure might result in thyroid hyperplasia, adenomas or carcinomas. Observations of developmental malformations in rat due to mancozeb are also attributed to the formation of its main metabolite ETU, which is classified as a Repr. 1B developmental toxicant. Transient impairment of maternal thyroid hormone levels have been shown to affect neural brain organization and behavior.				

Therefore, although the three HH endpoints are discussed separately, BE CA would stress the various consequences induced by the same endocrine disruption mechanism and the urgent need for specific criteria's to assess the endocrine system in the CLP guidance.
Dossier Submitter's Response
We note your desire to have ED criteria in the CLP guidance; however as endocrine disruption is not a hazard class/endpoint in the CLP Regulation, we are unsure of its value.
RAC's response
Please see response to comment No. 1.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Belgium		MemberState	6
Comment received				
The carcinogenic potential of mancozeb has been investigated in various carcinogenicity, long-term toxicity and 2-generation reproduction toxicity studies in rats and mice. Results demonstrated that mancozeb specifically targets thyroid, leading therefore to thyroid neoplastic lesions in rat. Carcinogenic observations in rat include follicular cell adenomas and carcinomas from a dietary concentration of 750 ppm (30,9 – 40,2 mg/kg bw/day) in carcinogenicity studies (OECD 453, Anonymous 1990a ; Belpoggi et al, 2020). These findings are supported by further results in F0 and F1 adults in two reproductive toxicity two-generation studies, showing increased follicular adenomas in males from 68,9 mg/kg bw/day (Anonymous 1988 ; Anonymous 1992c). Finally, in a 12 week oral toxicity study, reporting include enlarged thyroid with proliferating epithelial cells, suggesting pre-neoplastic foci after a subchronic exposure. Other studies also consistently showed thyroid hyperplasia after repeated exposure to mancozeb in rat. In mice, no thyroid neoplastic findings are reported in oral carcinogenicity studies at doses up to 1000 ppm. However, repeated toxicity studies showed non-neoplastic thyroid hyperplasia in mice from 1000 ppm after only four weeks exposure. Thyroid follicular hyperplasia also appeared in dog from 34 mg/kg bw/day (convert to ppm) after 13 weeks oral exposure. Epidemiology studies did not demonstrated any relation between thyroid cancer and mancozeb exposure. However, the absence of epidemiology findings do not imply that the animal findings should be disregarded for classification. BE CA would also stress that although rare, thyroid follicular cell cancer remains relevant for human and cannot be excluded. Human studies also demonstrated that nodular thyroids are more likely to harbor incidental carcinoma (Smith et al., 2013). In particular, the Dossier Submitter concluded that carcinogenicity might appear only at "unrealistic doses in human", without further elaboration following this statement. BE CA would remind that the absence of relevance of a mode of action to human should not be based on a quantitative argumentation, but only qualitative. Thereupon, no indication has been found in the last version of the CLP guideline (July 2017) about the non-relevance to				

human of HTP axis disturbance-mediated thyroid tumour in rat. Only certain thyroid tumours in rodents mediated by UDP glucuronyltransferase induction are considered not to be relevant to human in the CLP Guideline. BE CA also note that the EU Specialised Experts Guideline on non-genotoxic thyroid carcinogens (1999) was considered in the CLP Guideline, as stated in the references.

Finally, the DS argued that ETU is not classified for carcinogenicity, although it has been shown to cause thyroid tumours in rats and mice. BE CA note that the actual harmonized entry of ETU has been translated from previous classification under Directive 67/548/EEC.

Considering the thyroid carcinogenic findings in rat associated with the coherence of the observations in mice and dogs (targeting specifically thyroid follicular cells in both) and the proposed mode of action (the ETU metabolite disturbing the HPT axis through the inhibition of the thyroid peroxidase), BE CA is of the opinion that the relevance of thyroid carcinogenicity for human cannot be excluded and that a Carc. 2 classification is warranted. We are also of the opinion that a STOT RE classification for thyroid is not sufficient to cover carcinogenic effects.

BE CA also regret that the dermal exposure to mancozeb is not addressed in this dossier. Squamous cell papillomas and keratoacanthomas have been reported after a 60 weeks topical application of 100 mg/kg bw/day on dorsal Swiss albino mouse skin, associated with a high rate of mortality (Shukla et al. 1990). BE CA would therefore appreciate any information available on dermal carcinogenicity studies during the assessment of mancozeb carcinogenicity

References :
Commission Group of Specialised Experts in the fields of carcinogenicity, mutagenicity and reprotoxicity: Non genotoxic thyroid carcinogens in the rodent. 1999
Smith JJ, Chen X, Schneider DF, Broome JT, Sippel RS, Chen H, Solórzano CC - Cancer after thyroidectomy: a multi-institutional experience with 1,523 patients. J Am Coll Surg. 2013 Apr;216(4):571-7;
Y.Shukla Y, M.Antony M, Kumar S, Mehrotra NK - Carcinogenic activity of a carbamate fungicide, mancozeb on mouse skin. Cancer Letters, Volume 53, Issues 2–3, September 1990, Pages 191-195

Dossier Submitter's Response

Thank you for your comments. The statement that "carcinogenicity might appear only at unrealistic doses in humans" has been fully justified in the CLH dossier. When quantitative differences between experimental animals and humans are large, they could have an impact on hazard classification, which should always represent a realistic hazard to human health. The EU Specialised Experts paper (1999) on non-genotoxic thyroid carcinogens, which is not specific to thyroid tumours mediated by UDPGT induction, is referenced in the CLP guidance.

ETU has no harmonised classification for carcinogenicity. The carcinogenicity criteria have not changed from Directive 67/548/EEC to Regulation 1272/2008. In addition, no new data relevant to the carcinogenicity of ETU are available.

We agree that STOT-RE classification for thyroid effects is not intended to cover carcinogenicity effects. However, the DS remains of the view that although

hypothyroidism induced by mancozeb is relevant to humans, the WoE supports the contention that thyroid cell proliferation and hyperplasia is unlikely to occur in humans.

We have requested the dermal study in mice (publication by Shukla et al., 1990). In this study, female Swiss albino mice were exposed to mancozeb at a dose of 100 mg/kg bw dissolved in 100 microliters DMSO 3 times per week. Development of tumours was observed after 31 weeks (217 days) of mancozeb application. A high rate of mortality was observed after 54 weeks (378 days) of mancozeb application due to its toxicity and the study was terminated after 60 weeks. On histological examination, these tumours were found mostly to be benign in nature, e.g., squamous cell papillomas and keratoacanthomas. Overall, mancozeb in DMSO was carcinogenic to mouse skin. This study, however, is not considered to be a reliable investigation of the potential of mancozeb to cause carcinogenicity to mouse skin because DMSO was used as the application vehicle. Mancozeb is highly unstable in DMSO. Therefore it is most likely that the squamous cell papillomas and keratoacanthomas (mostly benign in nature) may well 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.

RAC's response

Thank you for your comments. RAC agrees with your position regarding human relevance of mancozeb-induced thyroid tumours in the rat and that classification in Category 2 is warranted.

RAC has reviewed the mouse dermal carcinogenicity study by Shukla *et al.* (1990). This study reported a clear increase in benign skin tumours at a dose of mancozeb causing marked general toxicity and a dramatic reduction in survival leading to termination of the study after 60 weeks. In addition, the study by Tygai *et al.* (2011; cited as Shilpa *et al.* in the CLH report) 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. Although both studies indicate a potential for induction of local benign skin tumours at the doses tested, the 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.

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Spain		MemberState	7

Comment received

Carcinogenicity

Administration of mancozeb to rats for 2 years, resulted in an increased incidence of thyroid follicular tumours (carcinomas and adenomas) at the top dose of 750 ppm (30 to 40 mg/kg bw/d) (Anonymous, 1990). Thyroid tumours in rats were associated with thyroid toxicity (effects on thyroid hormones, thyroid hypertrophy and hyperplasia). Thyroid follicular adenomas were also seen in two rat multi-generation studies mainly in adult males of the F0 and F1 generations.

The mode of action (MoA) involves the disturbance of the HPT (hypothalamus-pituitary-thyroid) axis via the metabolite ETU. Mancozeb is metabolised to ETU in all mammals by

approximately 7% by weight. ETU inhibits the activity of the thyroid peroxidase (TPO), enzime involves in the synthesis of thyroid hormones. The decreased production of the thyroid hormones T4 (thyroxine) and T3 (triiodothyronine) results in turn in disturbance of the hypothlalamus-pituitary-thyroid axis (HPT) as the hormonal feedback control mechanisms attempt to adjust thyroid hormone concentrations to normal endogenous levels. Prolonged exposure can eventually result in thyroid follicular cell hyperplasia and development of tumours of the thyroid gland (adenomas and carcinomas).

There is qualitative evidence that mancozeb, via its metabolite ETU, could potentially cause hypothyroidism in humans as the operation of the HPT axis is qualitatively similar across mammalian species. However there is quantitative evidence that exposure of humans to ETU following mancozeb exposure is lower than rats because of differences in the metabolism of ETU. In vitro metabolism studies in several species show that the metabolism of ETU in hepatocytes increases in the following order: rats < mice < humans, with rabbits and dogs being similar to humans. There are also substantial quantitative dynamic differences in the physiology of the thyroid gland between rats and humans, that means that human thyroid is less responsive to thyroid disrupters than rats.

Thyroid tumours are a relatively common finding in rat long-term studies, whilst the only known human thyroid carcinogen is ionizing radiation. In addition, there is no clear evidence that hypothyroidism (goitre) in humans progresses to neoplasia and whilst thyroid hypertrophy has been observed in humans, thyroid hyperplasia is rare. Besides, the overall conclusion from the epidemiology and medical studies is that environmental or workplace exposure to mancozeb does not disrupt the thyroid hormonal system in humans and is not associated with thyroid tumours in humans.

In the ECB C&L guidance document on thyroid tumours (EC, 1999, ECBI49/99-Add1.Rev2) it is concluded that when a non-genotoxic substance produces a low/medium potency perturbation on the thyroid-pituitary axis the mechanism of action is not relevant for humans and do not need to be classified for carcinogenicity. Based on its T25 values (the chronic daily dose which will give 25% of the animals tumours at a specific site, after correction for spontaneous incidence, within the standard life-span of that species) mancozeb is a medium potency rat thyroid carcinogen with a clearly established non-genotoxic MoA and therefore does not need to be classified.

The thyroid tumours induced by mancozeb in rats would in theory occur in humans only at very high, unrealistic dose levels. Therefore, it is highly unlikely that mancozeb would cause thyroid hyperplasia and tumours in humans. This is consistent with the current harmonised classification of mancozeb. ETU, which causes thyroid tumours in rats and mice, is neither classified for carcinogenicity in its harmonised entry. The Spanish CA considers the available information does not provide enough evidence to support a classification of mancozeb for carcinogenicity.

Dossier Submitter's Response
Thank you for your support.
RAC's response
Thank you for your comments. Your support for the DS's position is noted.

However, RAC considers classification in Category 2 more appropriate for the following reasons:

- Thyroid follicular cell hyperplasia was seen upon administration of low doses of ETU in monkeys (Leber *et al.*, 1978), a species possessing thyroxine-binding globulin.
- The *in vitro* studies on interspecies differences in metabolism of ETU (Saghir *et al.*, 2005; Zhu, 2015) showed 2- to 5-fold faster metabolism in liver S9 or primary hepatocytes from humans than from rats. However, extrapolation to *in vivo* situation is not straightforward and the magnitude of the potential difference is not sufficient to disprove human relevance of the rat thyroid tumours.
- Although humans are quantitatively less sensitive than rats to the induction of malignant thyroid tumours from sustained stimulation of the thyroid by TSH, association of increased TSH with thyroid cancer was observed in some studies (see IARC, 1999; European Commission, 2017).
- The negative epidemiology studies on EBDC fungicides and the fact that the only currently known human thyroid carcinogen is ionizing radiation do not necessarily call into question the human relevance of the rodent thyroid tumours arising via non-genotoxic mechanisms. Non-genotoxic thyroid carcinogens have a threshold (for mancozeb around 20 mg/kg bw/d in the rat) that is very unlikely to have been reached in the epidemiology studies on EBDCs.
- The fact that the ECB C&L guidance document on thyroid tumours is referred to in the CLP guidance does not necessarily mean that the CLP guidance has fully endorsed the ECB document. In their decisions RAC takes into account all available information, with an emphasis on substance-specific data.

Reference

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

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	France		MemberState	8

Comment received

10.7.5 Comparison with the CLP criteria page 40

• In the absence of human data category 1A is not triggered.

• Follicular cell adenoma/adenocarcinoma in rats:

According to the CLP criteria, for experimental findings several factors should be taken into consideration to assess the strength of evidence and to conclude whether mancozeb triggers cat.1B, cat.2 or no classification

- While it is acknowledged that genotoxic mode of action can be excluded and the increased incidence of thyroid tumours is limited to one of the two species tested for exposure carcinogenicity (i.e.: rats), the reduced tumour latency (tumours observed in the 2-generation studies), the progression of lesions to malignancy (increased carcinoma in the 2-years studies), the consistency between sexes are lines of evidence to support classification.
- The postulated mode of action (inhibition of TPO) is considered sufficiently supported by

both specific data on mancozeb and ETU. However, contrary to DS opinion, the non-relevance of this mode of action due to quantitative differences between rat and human is not considered sufficiently substantiated. Indeed, several arguments put forwards are not agreed page 40-41: - the differences between rat and human regarding T4 clearance may be a sound reason in case of thyroid tumours resulting liver enzymatic induction which is not the MoA under consideration. - Differences in metabolism between rat and human are not substantiated by mancozeb-specific data. As regard ETU, metabolism of ETU in hepatocytes increases in the following order: rats < mice < humans, with rabbits and dogs being similar to humans (Daston, 1990; Saghir et al, 2005; Zhu, 2015). - Based on the dose levels at which decreased T4 (KE 1) is observed in the different tested species, mice are less sensible than rats while dogs seem as sensitive as rats. No life span exposure study in dog is available to investigate carcinogenicity. - DS considers that while thyroid tumours are a relatively common finding in rat long-term studies, these tumours are rare in human and not linked to hypothyroidism. As discussed in a EU workshop on thyroid disruption in 2017 –Final Report Framework Contract ENV.A.3/FRA/2014/0029, "thyroid cancers are the most rapidly rising type of all human malignancies in both women and men, with average annual increases of 6% year on year (Howlader et al. 2012, Kitahara and Sosa 2016). Low levels of TH lead to rises in TSH, and TSH in turn stimulates thyroid gland growth. Boelaert et al. (2006) were the first to describe that serum TSH is elevated in patients with malignant thyroid nodules when compared to subjects with benign thyroid tumours." - In AHS study, a significant association between use of the fungicide maneb/mancozeb and hypothyroidism in female spouses but not in workers. A recent published epidemiological study in Italian grapevine worker indicates a possible mild thyroid disrupting effect due to occupational exposure to mancozeb (Medda et al 2017) Based on the above listed consideration, the established mode of action may be relevant to human and classification for carcinogenicity seems warranted. Since one type of tumour in one species is observed Carc.cat2 could be appropriate.
Dossier Submitter's Response
Thank you for your comments. We agree that some uncertainties remain, but the balance of the evidence points towards no classification for carcinogenicity.
RAC's response
Thank you for your comments. RAC agrees with your position regarding human relevance of mancozeb-induced thyroid tumours in the rat and that classification in Category 2 is warranted.

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Belgium		MemberState	9
Comment received				
The carcinogenic potential of mancozeb has been investigated in various carcinogenicity, long-term toxicity and 2-generation reproduction toxicity studies in rats and mice. Results demonstrated that mancozeb specifically targets thyroid, leading therefore to thyroid neoplastic lesions in rat. Carcinogenic observations in rat include follicular cell adenomas and carcinomas from a				

dietary concentration of 750 ppm (30,9 – 40,2 mg/kg bw/day) in carcinogenicity studies (OECD 453, Anonymous 1990a ; Belpoggi et al, 2020). These findings are supported by further results in F0 and F1 adults in two reproductive toxicity two-generation studies, showing increased follicular adenomas in males from 68,9 mg/kg bw/day (Anonymous 1988 ; Anonymous 1992c). Finally, in a 12 week oral toxicity study, reporting include enlarged thyroid with proliferating epithelial cells, suggesting pre-neoplastic foci after a subchronic exposure. Other studies also consistently showed thyroid hyperplasia after repeated exposure to mancozeb in rat.

In mice, no thyroid neoplastic findings are reported in oral carcinogenicity studies at doses up to 1000 ppm. However, repeated toxicity studies showed non-neoplastic thyroid hyperplasia in mice from 1000 ppm after only four weeks exposure. Thyroid follicular hyperplasia also appeared in dog from 34 mg/kg bw/day (convert to ppm) after 13 weeks oral exposure.

Epidemiology studies did not demonstrated any relation between thyroid cancer and mancozeb exposure. However, the absence of epidemiology findings do not imply that the animal findings should be disregarded for classification. BE CA would also stress that although rare, thyroid follicular cell cancer remains relevant for human and cannot be excluded. Human studies also demonstrated that nodular thyroids are more likely to harbor incidental carcinoma (Smith et al., 2013).

In particular, the Dossier Submitter concluded that carcinogenicity might appear only at "unrealistic doses in human", without further elaboration following this statement. BE CA would remind that the absence of relevance of a mode of action to human should not be based on a quantitative argumentation, but only qualitative. Thereupon, no indication has been found in the last version of the CLP guideline (July 2017) about the non-relevance to human of HTP axis disturbance-mediated thyroid tumour in rat. Only certain thyroid tumours in rodents mediated by UDP glucuronyltransferase induction are considered not to be relevant to human in the CLP Guideline. BE CA also note that the EU Specialised Experts Guideline on non-genotoxic thyroid carcinogens (1999) was considered in the CLP Guideline, as stated in the references.

Finally, the DS argued that ETU is not classified for carcinogenicity, although it has been shown to cause thyroid tumours in rats and mice. BE CA note that the actual harmonized entry of ETU has been translated from previous classification under Directive 67/548/EEC.

Considering the thyroid carcinogenic findings in rat associated with the coherence of the observations in mice and dogs (targeting specifically thyroid follicular cells in both) and the proposed mode of action (the ETU metabolite disturbing the HPT axis through the inhibition of the thyroid peroxidase), BE CA is of the opinion that the relevance of thyroid carcinogenicity for human cannot be excluded and that a Carc. 2 classification might be warranted. We are also of the opinion that a STOT RE classification for thyroid is not sufficient to cover carcinogenic effects.

BE CA also regret that the dermal exposure to mancozeb is not addressed in this dossier. Squamous cell papillomas and keratoacanthomas have been reported after a 60 weeks topical application of 100 mg/kg bw/day on dorsal Swiss albino mouse skin, associated with a high rate of mortality (Shukla et al. 1990). BE CA would therefore appreciate any information available on dermal carcinogenicity studies during the assessment of

mancozeb carcinogenicity References : Commission Group of Specialised Experts in the fields of carcinogenicity, mutagenicity and reprotoxicity: Non genotoxic thyroid carcinogens in the rodent. 1999 Smith JJ, Chen X, Schneider DF, Broome JT, Sippel RS, Chen H, Solórzano CC - Cancer after thyroidectomy: a multi-institutional experience with 1,523 patients. J Am Coll Surg. 2013 Apr;216(4):571-7; Y.Shukla Y, M.Antony M, Kumar S, Mehrotra NK - Carcinogenic activity of a carbamate fungicide, mancozeb on mouse skin. Cancer Letters, Volume 53, Issues 2–3, September 1990, Pages 191-195
Dossier Submitter's Response
This is a repetition of comment no 6.
RAC's response
Please see response to comment no. 6.

Date	Country	Organisation	Type of Organisation	Comment number
26.04.2018	Denmark		MemberState	10
Comment received				
The case for none relevance for humans is not convincing. It has not been shown that the mode of action is not relevant for humans. The focus has been on the dose level. Carc 2 could be considered according to CLP criteria.				
Dossier Submitter's Response				
Thank you for your comments. Non-relevance to humans has been sufficiently demonstrated in the CLH report. The focus is on a realistic hazard to human health and large dynamic and kinetic quantitative differences in mancozeb-induced thyroid tumours between experimental animals and humans. Carc 2 is not justified.				
RAC's response				
Thank you for your comment. RAC agrees that human non-relevance of the thyroid tumours observed in the rat studies with mancozeb has not been convincingly demonstrated and Carc. 2 is justified.				

Date	Country	Organisation	Type of Organisation	Comment number
20.04.2018	Netherlands	EU Mancozeb Task Force	Company-Manufacturer	11
Comment received				
The EU MTF supports the position of the RMS that Mancozeb should not be classified for Carcinogenicity. A background document is provided in the file attached. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Mancozeb_ECHA Comments_EU MTF.zip				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Thank you for your comments. Please see response to comment no. 7.				

Date	Country	Organisation	Type of Organisation	Comment number
11.04.2018	United States		Individual	12
Comment received				
CLH report on Carcinogenicity (Section 10.7 to 10.7.6; pages 26-41). Agreed that the evidence is not sufficient for carcinogenicity classification. However, the carcinogenicity evidence is not conclusive. ECHA note – An attachment was submitted with the comment above. Refer to public attachment MANCOZEB WEIGHT OF EVIDENCE CARCINOGENICITY - QBECHA April 2018.pdf				
Dossier Submitter's Response				
Thank you for your comments. The DS remains of the view that the evidence is conclusive but insufficient for classification.				
RAC's response				
Thank you for your comments. Please see response to comment no. 7.				

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Greece		MemberState	13
Comment received				
It is proposed that the potential for mancozeb classification as Carc. 2 is revisited, based on the following observations in the Anonymous(1990) study: - The incidence of thyroid adenomas and carcinomas is statistically significantly increased in male rats at the dose of 31 mg/kg bw/d and in female rats (not significantly) at 40 mg/kg bw/d; - A causal relationship is established between mancozeb treatment and the increased incidence of thyroid tumours (both benign and malignant neoplasms); - A clear mode of action involving the disturbance of the HPT axis via the metabolite ETU has been identified for these tumours. No reference is made in the CLP on the T25 value. Further justification should be provided in the RAR with regard to current guidance. [See also Mancozeb RAR, Vol. 3, B.6.5, conclusion on carcinogenicity and classification, page 123-125]				
Dossier Submitter's Response				
Many thanks for your comments. The EU Specialised Experts paper (1999) on non-genotoxic thyroid carcinogens, including the use of the T25 concept, is referenced in the CLP guidance. Non-relevance to humans has been sufficiently demonstrated in the CLH report. The focus is on a realistic hazard to human health and large dynamic and kinetic quantitative differences in mancozeb-induced thyroid tumours between experimental animals and humans. Carc 2 is not justified.				
RAC's response				
Thank you for your comment. RAC agrees that mancozeb should be classified with Carc. 2.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	France		MemberState	14
Comment received				
No comment				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Belgium		MemberState	15
Comment received				
The developmental toxicity of mancozeb has been investigated through various developmental studies in rat and in rabbit. In rat, the two oldest developmental toxicity studies showed high maternal toxicity at the top doses (360-512 mg/kg bw/day), including treatment-related deaths (Anonymous 1980 ; Anonymous 1988c) after exposure during gestation days 6 to 15. This high maternal toxicity was associated with developmental toxicity such as incomplete ossification of fetuses, resorptions and malformations such as meningoencephalocele or dilated brain ventricle. A third rat oral developmental toxicity study carried in 1999b showed developmental toxicity in the same dose-range, including congestion of lungs, liver and kidney associated with dumbbell shaped thoracic centra after 225 and 500 mg/kg bw/day on gestation days 6-15. However, no maternal toxicity was reported at doses up to 500 mg/kg bw/day. BE CA is of the opinion that the validity of this study is a central question for a proper assessment of the developmental toxicity of mancozeb. No effects were reported in the two recent rat studies, at doses up to 160 mg/kg bw/day (Anonymous 2015d ; Jacobsen et al. 2012). In rabbit, abortion was associated with high maternal toxicity (severe body weight loss, even leading to death) in a dose-range 80-150 mg/kg bw/day (Anonymous 1987b ; Anonymous 1991b). We acknowledge that the generated ETU levels inducing fetotoxicity only appear at doses that are maternally toxic. However, due to the established developmental toxicity of ETU (classified as Repr. 1B H360D), main metabolite of mancozeb, and its reported mode of action on thyroid, a Repr. 2 classification should be maintained in order to prevent pregnant women exposure.				
Dossier Submitter's Response				
Thank you for your comments. As pointed out in the CLH report, the reliability of the 1999b study in Wistar rats is questionable as the developmental findings are inconsistent with those of the other available rat studies and the lack of maternal toxicity up to 500 mg/kg bw/d does not tally with the maternal effects seen in the other studies. The DS				

remains of the view that classification with R2 should be removed as the recent investigations in the developmental toxicity of mancozeb have demonstrated that teratogenic levels of ETU will only be generated at mancozeb doses (360-521 mg/kg bw/day) which cause excessive maternal toxicity. This is because only a small amount (approximately 7%) of mancozeb is converted to ETU in animals; in addition, the rate of this conversion is likely to be slow, such that systemic peaks of ETU are only generated at very high doses of mancozeb. The relevance to hazard identification and classification of such teratogenic effects seen only in the presence of excessive maternal toxicity (death/killing in extremis, paralysis, body weight and food consumption decreases, and suffering) is questionable.
RAC's response
Thank you for your comment. RAC agrees that classification for developmental toxicity should be retained. According to the CLP regulation, a dose with excessive maternal toxicity, normally not considered for classification, is a dose causing maternal mortality greater than 10% (CLP, Annex I, 3.7.2.4.4). This condition seems to be fulfilled for the top dose of 512 mg/kg bw/d in the study of Anon. (1980) where 1 out of 22 dams died, 2 were killed due to abortion, most animals showed clinical signs of marked toxicity, and food consumption was strongly reduced. However, this dose was already associated with a high incidence of severe malformations (e.g., 54% of foetuses with dilated brain ventricles occurring due to loss of brain tissue). 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 this 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. 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). Thus, the study of Anon. (1980) is considered to point towards Cat. 1B rather than Cat. 2. As to the recent study by Anon. (2015d), RAC notes that maternal toxicity at the top dose of 160 mg/kg bw/d was rather limited and obviously a higher dose could have been tested. Due to the way the top dose was selected, the study of Anon. (2015d) does not address the concerns raised by the Anon. (1980) study. In addition, RAC notes that a single oral dose of 30 mg/kg bw ETU to pregnant rats on GD 15 can induce very severe hydrocephalus in some of the pups (Khera and Tryphonas, 1977 – see the RAR). This corresponds to approx. 430–860 mg/kg bw mancozeb (using a conversion factor of 3.5%–7%, the derivation of which is described in the RAC opinion), which is a dose range not associated with significant general toxicity in the rat. This again points towards Category 1B. As to the study by Anon. (1999b), RAC agrees with the DS that its reliability is questionable as the lack of maternal toxicity at 500 mg/kg bw/d (no reduction in food consumption or body weight gain, no clinical signs) is not in line with the findings of other studies (Anon. 1980, Anon. 1988c, Anon. 2015d). Thus, RAC is of the view that mancozeb meets the criteria for classification in Category 1B.

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Spain		MemberState	16
Comment received				

Developmental toxicity

Malformations (mainly of head and neck) were seen in the rat at severely maternally toxic dose levels (Anonymous,1980). Mancozeb was classified as Reprotox category 2 (H361d) as a result of this study. More recent investigations of the developmental toxicity of mancozeb and ETU in the rat (Anonymous, 2015d & a) have demonstrated that the foetal malformations observed by Anonymous (1980) were attributable to the production of a teratogenic dose of ETU and that this was only produced at mancozeb exposures which caused excessive maternal toxicity (death/killing in extremis, paralysis, body weight and food consumption decreases and suffering). ETU is an established developmental toxicant (harmonised classification with Repr Cat 1B; H360D) which causes malformations (mainly of head and neck) in the rat in the absence of maternal toxicity. When mancozeb was administered to rats at a dose (160 mg/kg bw/d) that was maternally toxic (decreases in body weight and food consumption) but did not caused excessive maternal toxicity (Anonymous, 2015d), insufficient ETU was generated to produce teratogenicity as ETU-driven teratogenic effects.

When the harmonized classification of mancozeb was considered in 2003-2006 by the Technical Committee on Classification an Labelling (TC c&L), the commission Working Group of Specialised Experts (SE) in the field of reproductive toxicity advised to address the concern about thyroid effects observed with mancozeb and brain development and to obtain information about the comparative kinetics and metabolism in man. Recent investigations of the developmental neurotoxicity of mancozeb consisting in two NNT studies in rat (Anonymous, 2018b,c; Axelstad et al, 2011) and an extended one generation reproduction toxicity study on ETU in rats (Anonumous, 2013) have demonstrated that neither mancozeb nor ETU cause neurological damage in offspring, at doses with thyroid hormones are affected in dams. Additional investigations of the developmental toxicity of mancozeb in the rat form the open literature did not identify effects on neurological endpoints, sexual behaviour, post-natal development and puberty onset. Besides, the in vitro metabolism of ETU in hepatocytes increases in the following order. Rats < mice <humans, with rabbits and dogs being similar to humas (Saghir et al, 2005; Zhu, 2015).

The dossier submitter is of the view that these recent investigations have demonstrated that teratogenic levels of ETU will only be generated at mancozeb doses which cause excessive maternal toxicity. This is because only a small amount (approximately 7%) of mancozeb is converted to ETU in animals; in addition, the rate of this conversion is likely to be slow, such that systemic peaks of ETU are only generated at very high doses of mancozeb. It was also argued by the applicant that, althought ETU is teratogenic, several lines of evidence indicate that this not related to its MoA in the thyroid. In the dossier submitter view, the weight of evidence indicates that the risk of developmental toxicity after exposure of humans to mancozeb is very low.

On overall, we are aware that the relevance to man is lower and probably the levels of ETU produced by mancozeb would not reach the threshold for teratogenic effects. However, they are very serious malformations and the explanation for no classification is risk rather than hazard based. In the opinion of the Spanish CA, a possible relationship to treatment cannot be discounted and therefore it can´t be ruled out their relevance to

humans. Therefore, we do not agree to the proposed removal of the classification Repro. Cat2. The Spanish CA is of the opinion that classification with category 2 (H361d) for toxicity to the developmental is the most appropriate in this case.
Dossier Submitter's Response
Thank you for your comments. We agree that the malformations are treatment-related and relevant to humans. However, as these occur only in the presence of severe maternal toxicity, including death, they are not relevant for classification. The proposal to remove R2 is not based on risk but on the presence of severe maternal toxicity.
RAC's response
Thank you for your comment. RAC agrees that classification for developmental toxicity should be retained but considers mancozeb to meet the criteria for classification in Category 1B. Please see RAC's response to comment No. 15.

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Germany		MemberState	17
Comment received				
The proposal to remove the current classification for developmental toxicity is not supported. In contrast, Repr 2, H361d, should be kept. There were severe malformations in rat fetuses at high doses (but still well below the limit dose of 1000 mg/kg bw/day). Most likely, they were due to exposure to the teratogenic metabolite ETU. The arguments put forward to support the removal of the existing classification are mainly based on quantitative considerations, i.e., the assumption that the ETU levels would not be sufficiently high to cause malformations. However, this approach is rather part of risk (but not of hazard) assessment and would also apply to many other developmental toxicants for which human exposure will never be of concern but which have been classified and labelled anyway. Occurrence of fetal malformations only at high doses (and in the presence of maternal toxicity even though there is no clear-cut mechanistic relation) is reflected by assigning category 2 (in contrast to ETU which is a Cat. 1B teratogen). Detailed considerations: The current harmonised classification of mancozeb as Repr. 2, H361d, was based on the study of Anonymus (1980) and the therein found effects like malformations of rat foetuses. The found malformations affected mostly head and neck of the foetuses. It is assumed that the metabolite ethylenethiourea (ETU) of mancozeb causes these effects on the foetal development. ETU disrupts the synthesis of thyroid hormones by inhibition of the thyroid peroxidase. Since thyroid hormones are essential for brain development in mammals an inhibition of the synthesis of these hormones and therefor a hormone deficiency has a significant influence in the foetal development. ETU is classified as Repr. 1B, H360D. Now three new prenatal development toxicity studies on rats with mancozeb are available (Anonymus 2015b, c & d) which should substantiate the removal of the current classification of mancozeb as Repr. 2, H361d. This proposal is not supported. From our point of view, the new studies and their results are not sufficient to relieve mancozeb from the suspicion to be reprotoxic. In contrast to the study of Anonymus (1980) no effects to the foetal development of rats were found in the studies Anonymus (2015 c & d). The used doses in the studies Anonymus (2015c & d) were propably chosen too low to cause effects on the foetal development. With regard to the dose range finding study (Anonymus, 2015b) doses up to 240-300 mg/kg bw/d mancozeb could be possible without causing severe maternal				

toxicity in the animals. Having results from this dose range would be very interesting and helpful to interpret the effects found in the Anonymus (1980) study.
However, in the study Anonymus (2015c) a decrease of the thyroxin levels was found in the foetuses at doses of 120 and 160 mg/kg bw/d which would correspond to the supposed mode of action for ETU by inhibition of the thyroid hormone synthesis. Since thyroid hormones are crucial for foetal development it is possible that effects were found at higher doses than 160 mg/kg bw/d.
For this reason we focus on malformations found in the study of Anonymus (1980), even if they occurred during severe maternal toxicity at the highest dose. In our opinion, mancozeb cannot be relieved from the suspicion to be toxic for the foetal development. Therefore, a removal of the harmonized classification of mancozeb as Repr. 2, H361d, is not supported.

Dossier Submitter's Response

Thank you for your comments. Please note that it has been demonstrated that there is no relationship between the reduced thyroid hormone levels induced by mancozeb and the malformations seen in the foetus. We agree that the malformations are treatment-related and relevant to humans. However, as these occur only in the presence of severe maternal toxicity, including death, they are not relevant for classification. The proposal to remove R2 is not based on risk but on the presence of severe maternal toxicity.

RAC's response

Thank you for your comment. RAC agrees that classification for developmental toxicity should be retained but considers mancozeb to meet the criteria for classification in Category 1B. Please see RAC's response to comment No. 15.

RAC agrees with the DS that a relationship between the ETU-induced brain malformations and reduced thyroid hormone levels has not been demonstrated.

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	France		MemberState	18

Comment received

10.8.9 Comparison with the CLP criteria page 65

• Teratogenic effects observed in rats in the presence of high maternal toxicity:
- The evidence suggested that the malformations seen in the rat with mancozeb were due to its main metabolite ETU (classified Repr. Cat2 H360D).
- It is also established that ETU is a main metabolite in human (used as biomarker).
- No specific data on conversion of mancozeb to ETU in human is available.

• DNT: it is acknowledged that marked maternal T4 reductions did not lead to any behavioural effects or any other investigated neurotoxic endpoints in the offspring. However, uncertainties on these negative results should be pointed out including:
- pups were not exposed directly by gavage in any of the DNT study and much brain development occurs postnatally in rats,
- limited milk transfer leads to low exposure level which could explain the absence of neurotoxic effects
- Neurobehavioral tests (learning and memory tests) implemented in standard DNT studies may not be sensitive enough to pick-up cognitive development.

Based on the above listed considerations, classification of mancozeb for developmental

toxicity is warranted and Repr. Cat2 H361d should not be removed.
Dossier Submitter's Response
Thank you for your comments. The DS remains of the view that classification with R2 should be removed as the recent investigations in the developmental toxicity of mancozeb have demonstrated that teratogenic levels of ETU will only be generated at mancozeb doses (360-521 mg/kg bw/day) which cause excessive maternal toxicity. This is because only a small amount (approximately 7%) of mancozeb is converted to ETU in animals; in addition, the rate of this conversion is likely to be slow, such that systemic peaks of ETU are only generated at very high doses of mancozeb. The relevance to hazard identification and classification of such teratogenic effects seen only in the presence of excessive maternal toxicity (death/killing in extremis, paralysis, body weight and food consumption decreases, and suffering) is questionable. In addition, no developmental neurotoxicity was seen in the available DNT study despite effects on the thyroid. The limitations of the DNT study highlighted by FR are well known; however, this is a generic testing issue.
RAC's response
Thank you for your comment. RAC agrees that classification for developmental toxicity should be retained but considers mancozeb to meet the criteria for classification in Category 1B. Please see RAC's response to comment No. 15.

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Sweden		MemberState	19
Comment received				
The dossier submitter (DS) presents new studies on developmental toxicity to support a review and revision of the current Annex VI entry for mancozeb. Based on information contained in the CLH-report, the Swedish Chemicals Agency does not agree with the DS's proposal to remove the harmonised classification of mancozeb as Repr. 2, H361d. DEVELOPMENTAL TOXICITY Included in the CLH-report are five developmental toxicity studies (3 in rat and 2 in rabbit) that were described in the original DAR (2000) under Directive 91/414. The 3 rat studies were also the basis for the current Repr.2, H361d harmonised classification of mancozeb. ETU, the main metabolite of mancozeb, has a harmonized classification as Repr. 1B, H360D. The CLH-report also includes a more recent developmental toxicity study (OECD TG 414) in SD rats (Anonymous, 2015d), which was negative for adverse effects on offspring but showed mild maternal toxicity (i.e. reduced food consumption with corresponding lower maternal body weight gain of 14% from GD 6-19). The DS proposes to disregard the findings in the older developmental toxicity studies since the doses at which adverse compound-related developmental effects in the offspring were observed also induced various degrees of maternal toxicity. The developmental effects observed were significant abnormalities and malformations (agnathia, cleft palate, meningoencephalocele), soft tissue effects (dilated ventricles, compressed spinal cord, lung emphysema, kidney congestion and dumbell shaped thoracic centre), and skeletal tissue effects (incomplete ossification of the skull, clavicle, scapula). While the maternal toxicity ranged from no toxicity in Wistar rats to clinical signs of lethargy, scruffy coat, diarrhoea, soft faeces, bloody vaginal discharge, hunched, dehydrated, lower body weight gain, reduced food consumption and those killed in extremis (3/22 dams) in SD rats. We do not agree with the DS's conclusion for the following reasons;				

• According to the CLP-regulation and guidance document, developmental effects, which occur 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 and that there is a causal relationship. As stated in the CLH-report by the DS, the Commission Working Group of Specialised Experts in the field of reproductive toxicity state that the developmental toxicity of mancozeb is not a secondary non-specific consequence of maternal toxicity. This is because ETU, the metabolite of mancozeb suggestive of causing the developmental effects of mancozeb-exposure, does not cause maternal toxicity at developmentally toxic dose levels. The DS has not provided an explanation to why various degrees of maternal toxicity is observed in the developmental toxicity studies in rats and why maternal toxicity is not observed in the study using Wistar rats (Anonymous, 1999b).
• The new OECD TG 414 developmental toxicity study (Anonymous, 2015d) only confirms that mild maternal toxicity (i.e. reduced food consumption with corresponding lower maternal body weight gain of 14% from GD 6-19) may arise at dose levels (top dose 160 mg/kg bw/day) slightly lower than those levels seen for developmental effects in the older toxicity studies (LOAELs 225 - 512 mg/kg bw in rat; Anonymous, 1980, 1988c, 1999b).
• Also, the non-guideline studies from the open literature (Jacobsen et. al., 2012, Overgaard et. al., 2013) provided in the CLH-report by the DS, used a top dose of mancozeb of 25 mg/kg bw/day (alone or in a mixture with other chemicals) at which no developmental effects were observed. This again shows that developmental effects occur at higher dose levels.

According to the CLP regulation, independent of maternal toxicity classification shall be considered where there is a significant toxic effect in the offspring (e.g. irreversible effects including structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies). This is the case for mancozeb. As stated by the Commission Working Group of Specialised Experts, mancozeb causes a high frequency of a specific pattern of major malformations that are similar to the malformations caused by the metabolite ETU (classified as a Repr. 1B, H360D). Discounting developmental effects that are observed at maternally toxic doses can only be done when a causal relationship is established. In our opinion, the DS has not clearly demonstrated such a link in order to be able to remove the current harmonized classification Repr 2, H361d.

DEVELOPMENTAL NEUROTOXICITY
Included in the CLH-report is also a new non-guideline DNT study (Anonymous, 2008c) conducted to address concerns about a potential relationship between thyroid effects and brain development. The study did not find neurodevelopmental effects in the offspring. However, the dose range chosen for the DNT study (0, 5, 15 and 30 mg/kg/day) is questionable.

From the OECD TG 426 dose range finding study (Anonymous, 2008b), the two highest doses (30 and 60 mg/kg/day) resulted in maternal toxicity i.e. lower mean body weights, body weight gains and food consumption, higher mean serum concentrations of TSH, lower mean serum concentrations of total T4 and higher incidence of thyroid gland follicular cell hypertrophy. The offspring in the 30 and 60 mg/kg/d groups had decreased body weights and body weight gains. However, in the OECD TG 426 it is stated that if the test substance has been shown to be developmentally toxic, the highest dose level should be the maximum dose that will not induce excessive offspring toxicity (or in utero or neonatal death or malformations). Based on the results from the older developmental

toxicity studies, NOAELs and LOAELs occur between 60 – 160 mg/kg bw/day and 225 – 512 mg/kg bw in rat, respectively (Anonymous, 1980, 1988c, 1999b), suggesting that a dose of 30 mg/kg bw/day would result in effects far from excessive offspring toxicity as stated by OECD TG.

This point of view is also shared by the European Commission in a report [1], where the authors state that doses above 30 mg/kg bw in the repeated dose toxicity studies were needed to induce T4 reduction after 4 days of exposure whereas doses of 15 – 75 mg/kg bw/day have been shown to cause adverse effects on the thyroid after 90 days of exposure. This indicates that the dams in the DNT study were exposed to mancozeb doses that were in the low range of those previously shown to affect the thyroid hormone system after a relatively short term exposure (GD6-PND 20= 35 days).

Also included in the CLH-report is a non-guideline DNT study from the open literature (Alexstad et. al., 2011). The study found maternal toxicity such as severe weight loss and hind limb paralysis at dose levels of 150 mg/kg bw, which is not consistent with that observed in the guideline developmental toxicity studies where pregnant rats were seen to tolerate 160 mg/kg bw/d (Anonymous, 2015d) with only a transient effect on body weight.

From these studies, it is in our opinion not possible to draw conclusions on developmental neurotoxicity from the exposure to mancozeb.

References

1. Supporting the organisation of a workshop on thyroid disruption. Framework Contract ENV.A.3/FRA/2014/0029 on implementation of the Community strategy on Endocrine Disrupters. Written by Brunel University London, Institute of Environment, Health and Societies National Food Institute, Technical University of Denmark. September– 2017.

Dossier Submitter's Response

Thank you for your comments. As pointed out in the CLH report, the reliability of the 1999b study in Wistar rats is questionable as the developmental findings at 225 and 500 mg/kg bw/d are inconsistent with those of the other available rat studies and the lack of maternal toxicity up to 500 mg/kg bw/d does not tally with the maternal effects seen in the other studies. We agree that the malformations seen with mancozeb in SD rats are treatment-related and relevant to humans, and we also agree that it has not been unequivocally demonstrated that they are secondary to maternal toxicity. However, as these occur only in the presence of **severe** maternal toxicity, including death, they are not relevant for classification.

In relation to developmental neurotoxicity, no effects were seen in the 2008 DNT study up to doses (30 mg/kg bw/d) causing maternal toxicity (including effects on thyroid weight and histopathology) and producing measurable levels of ETU in pup plasma and milk. Therefore, in our view, dose selection was adequate for this study. In addition, no developmental neurotoxicity was seen in another study (Alexstad et al., 2011) up to a much higher dose of 150 mg/kg bw/d.

RAC's response

Thank you for your comments. RAC agrees that classification for developmental toxicity should be retained but considers mancozeb to meet the criteria for classification in Category 1B. Please see RAC's response to comment No. 15.

RAC's confidence in the results of the study by Anon. (1999b) is low because the lack of maternal toxicity at 500 mg/kg bw/d (no reduction in food consumption or body weight

gain, no clinical signs) is not in line with the findings of other studies (Anon. 1980, Anon. 1988c, Anon. 2015d).

It should be noted that a considerable degree of interanimal and study-to-study variability in the threshold for hind limb paralysis and severe toxicity is seen in repeat dose toxicity studies with mancozeb (see the STOT RE section of the RAC opinion), so the low threshold for maternal toxicity in the study by Axelstad *et al.* (2011) is not implausible.

RAC agrees that the top dose in the DNT study of Anon. (2008c) was inappropriately low. A recent extended one-generation reproductive toxicity study with ETU (Anon., 2013) did not show any neurotoxic effects up to levels causing maternal T4 reduction by 70%. Unfortunately, effects on learning and memory were not investigated in the latter study. It should also be noted that the standard animal studies may not be sufficiently sensitive to detect subtle effects on cognitive development. Thus, even an absence of an effect on learning in a standard DNT study does not completely exclude an effect in humans at equivalent doses.

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Belgium		MemberState	20
Comment received				

Developmental toxicity
The developmental toxicity of mancozeb has been investigated through various developmental studies in rat and in rabbit.

In rat, the two oldest developmental toxicity studies showed high maternal toxicity at the top doses (360-512 mg/kg bw/day), including treatment-related deaths (Anonymous 1980 ; Anonymous 1988c) after exposure during gestation days 6 to 15. This high maternal toxicity was associated with developmental toxicity such as incomplete ossification of fetuses, resorptions and malformations such as meningoencephalocele or dilated brain ventricle.

A third rat oral developmental toxicity study carried in 1999b showed developmental toxicity in the same dose-range, including congestion of lungs, liver and kidney associated with dumbbell shaped thoracic centra after 225 and 500 mg/kg bw/day on gestation days 6-15. However, no maternal toxicity was reported at doses up to 500 mg/kg bw/day. BE CA is of the opinion that the validity of this study is a central question for a proper assessment of the developmental toxicity of mancozeb.

No effects were reported in the two recent rat studies, at doses up to 160 mg/kg bw/day (Anonymous 2015d ; Jacobsen et al. 2012).

In rabbit, abortion was associated with high maternal toxicity (severe body weight loss, even leading to death) in a dose-range 80-150 mg/kg bw/day (Anonymous 1987b ; Anonymous 1991b).

We acknowledge that the generated ETU levels inducing fetotoxicity only appear at doses that are maternally toxic. However, due to the established developmental toxicity of ETU (classified as Repr. 1B H360D), main metabolite of mancozeb, and its reported mode of action on thyroid, a Repr. 2 classification should still be considered in order to prevent pregnant women exposure.

Dossier Submitter's Response
This is a repeat of comment 15.
RAC's response
Please see the response to comment No. 15.

Date	Country	Organisation	Type of Organisation	Comment number
26.04.2018	Denmark		MemberState	21
Comment received				
Do not agree that the harmonised classification can be removed based on the arguments given in the RAR and CLH report. There are some inconsistencies in the argumentation. First, In the range finding study from 2015 rat plasma concentrations of mancozeb was detected up to 213 ng/mL and plasma concentrations of ETU up to 1950 ng/mL, 6 hours after the final dose administration. This means that even though only approximately 7 % of mancozeb by weight is converted to ETU, the internal dose of ETU (at least for some time) exceeds those of Mancozeb almost 10 fold. In the ETU developmental study from 2015 hydrocephaly was observed in the 15 mg/kg bw/d group. Since hydrocephalus is a very rarely seen malformation in control animals, there is no doubt that finding 7 offspring (even though it is only 2 % of all offspring) with this malformation in the ETU study was the beginning of the dose response curve for this malformation. The incidence increased in the 30 mg/kg bw/d group to 84% of all offspring. At the 15 mg/kg bw/d group the internal ETU concentrations were 1170 ng/ml in foetuses and 1280 ng/ml in dams. In the animals receiving a mancozeb dose of 160 mg/kg bw/d (prenatal developmental tox study with mancozeb 2015) an internal maximum ETU concentration of approximately 1040 ng/ml (1950 ng/ml / 300mg/kg bw/d * 160mg/kg bw/d) could be expected. This means that the internal concentration probably was not high enough to reveal the effects from ETU but that the dose should not be raised much for the risk of these effects. We therefore are of the opinion that it has not been sufficiently convincingly shown that only very high mancozeb doses (360-512 mg/kg bw/d), which induce excessive maternal toxicity, will cause the teratogenic effects. Secondly, another argument in the RAR/CLH report was related to the DNT study with mancozeb up to 30 mg/kg bw/d. The study was performed due to concerns for the disturbance of the thyroid hormone system and the potential effects on the development of the nervoussystem. However, the highest dose tested did not cause significant changes in either thyroid weight nor histopathology, and only caused a very limited decrease in maternal BW gain. We therefore find it is not possible to conclude that mancozeb cannot cause any adverse effects on the developing nervous system.				
Dossier Submitter's Response				
Thank you for your comments. The DS remains of the view that classification with R2 should be removed as the recent investigations in the developmental toxicity of mancozeb have demonstrated that teratogenic levels of ETU will only be generated at mancozeb doses (360-521 mg/kg bw/day) which cause excessive maternal toxicity. This is because only a small amount (approximately 7%) of mancozeb is converted to ETU in animals; in addition, the rate of this conversion is likely to be slow, such that systemic peaks of ETU are only generated at very high doses of mancozeb. The relevance to hazard identification and classification of such teratogenic effects seen only in the presence of excessive maternal toxicity (death/killing in extremis, paralysis, body weight and food consumption decreases, and suffering) is questionable.				

In relation to developmental neurotoxicity, no effects were seen in the 2008 DNT study up to doses (30 mg/kg bw/d) causing maternal toxicity (including effects on thyroid weight and histopathology) and producing measurable levels of ETU in pup plasma and milk. In addition, no developmental neurotoxicity was seen in another study (Alexstad et al., 2011) up to a much higher dose of 150 mg/kg bw/d, causing marked decreases in thyroid hormones.
RAC's response
Thank you for your comment. RAC agrees that classification for developmental toxicity should be retained but considers mancozeb to meet the criteria for classification in Category 1B. Please see RAC's response to comment No. 15. As to the toxicokinetic calculations based on studies of Anon. (2015a) and Anon. (2015c), please note that plasma ETU concentrations obtained at different timepoints after the last dose (e.g., 24 h vs 6 h) are not directly comparable. Therefore, an adjustment has been made (CLH report p. 170-171) and the result is that 15 mg/kg bw/d ETU is expected to produce the same peak plasma levels of ETU as approximately 430 mg/kg bw/d mancozeb under the conditions of these studies. RAC agrees that the top dose in the DNT study of Anon. (2008c) was inappropriately low. There is also a negative DNT study by Axelstad *et al.* (2011) that tested up to levels causing severe maternal toxicity. A recent extended one-generation reproductive toxicity study with ETU (Anon., 2013) did not show any neurotoxic effects up to levels causing maternal T4 reduction by 70%, although effects on learning and memory were not investigated in this study. However, it should be noted that the standard animal studies may not be sufficiently sensitive to detect subtle effects on cognitive development. Thus, even an absence of an effect on learning in a standard DNT study does not completely exclude an effect in humans at equivalent doses.

Date	Country	Organisation	Type of Organisation	Comment number
20.04.2018	Netherlands	EU Mancozeb Task Force	Company-Manufacturer	22
Comment received				
The EU MTF supports the position of the RMS that Mancozeb should not be classified for Reproductive toxicity. A background document is provided in the file attached. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Mancozeb_ECHA Comments_EU MTF.zip				
Dossier Submitter's Response				
Thank you.				
RAC's response				
According to the CLP regulation, a dose with excessive maternal toxicity, normally not considered for classification, is a dose causing maternal mortality greater than 10% (CLP, Annex I, 3.7.2.4.4). This condition seems to be fulfilled for the top dose of 512 mg/kg bw/d in the study of Anon. (1980) where 1 out of 22 dams died, 2 were killed due to abortion, most animals showed clinical signs of marked toxicity, and food consumption was strongly reduced. However, this dose was already associated with a high incidence of severe malformations (e.g., 54% of foetuses with dilated brain ventricles occurring due to loss of brain tissue). 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 this 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. 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). Thus, the study of Anon. (1980) is considered to point towards classification in Category 1B.

As to the recent study by Anon. (2015d), RAC notes that maternal toxicity at the top dose of 160 mg/kg bw/d was rather limited and obviously a higher dose could have been tested. Due to the way the top dose was selected, the study of Anon. (2015d) does not address the concerns raised by the Anon. (1980) study.

In addition, RAC notes that a single oral dose of 30 mg/kg bw ETU to pregnant rats on GD 15 can induce very severe hydrocephalus in some of the pups (Khera and Tryphonas, 1977 – see the RAR). This corresponds to approx. 430–860 mg/kg bw mancozeb (using a conversion factor of 3.5%–7%, the derivation of which is described in the RAC opinion), which is a dose range not associated with significant general toxicity in the rat. This again points towards Category 1B.

As to the interspecies differences in metabolism of ETU, RAC notes the results of the two *in vitro* studies (Saghir *et al.* 2012; Zhu 2015) indicating a more rapid metabolism in humans compared to rats. However, translation of these findings into quantitative differences *in vivo* is not straightforward, and a substantial difference would be needed to alleviate the concern about the severe malformations induced by ETU in the rat.

Therefore, RAC is of the view that removing the current classification of mancozeb with Repr. 2 is not justified and that mancozeb meets the criteria for classification with Repr. 1B.

Date	Country	Organisation	Type of Organisation	Comment number
17.04.2018	United Kingdom	Tesh Consultants International (TCI)	Individual	23

Comment received

Comments refer to section 10.8 of CLH report
Tesh Consultants International (TCI) have been working in the field of developmental and reproductive toxicity for more than 50 years. We have prepared these comments as independent consultants for UPL Europe Ltd.

TCI have reviewed the reproductive toxicity data presented in the Mancozeb CLH Report: Proposal for Classification and Labelling, dated December 2017 and concur with the opinion of the UK Rapporteur that, taking into consideration the recent studies performed with mancozeb and its main metabolite, ethylene thiourea (ETU), a proven teratogen with a classification of Repro 1B, (Anonymous 2015 a, b, c and d), mancozeb per se does not warrant classification as a reproductive toxicant, viz.:

1. The experimental results from these studies indicate that the teratogenic effects in the rat, seen only at a dose level of mancozeb that resulted in severe maternal toxicity (512 mg/kg bwt/day), were likely to be due to the generation of peak plasma levels of ETU which were found to be within the teratogenic range for ETU. At a lower dose of mancozeb (360 mg/kg/day), which also resulted in marked maternal toxicity but did not give rise to foetal malformations, peak plasma levels of ETU were below those recorded at the lowest adverse effect level for ETU teratogenicity. At 160 mg/kg bwt/day, where

maternal toxicity was recorded but where there were no adverse developmental effects, peak plasma levels of ETU were similar to those recorded at the no-effect level for ETU. Maternal toxicity is, therefore, a much more sensitive endpoint than developmental toxicity.
2. A developmental neurotoxicity study in the rat with mancozeb revealed no indications of neurotoxicity in the offspring, despite reductions in the maternal T4 levels during neural tube development and maturation both pre- and post-natally.
3. Comparative toxicokinetic and metabolism studies with mancozeb and ETU in vivo and in vitro have demonstrated similar pathways in experimental animals, farm animals and humans. The rate of metabolism of ETU by human, rabbit and dog hepatocytes in vitro was more rapid than by rodent hepatocytes, possibly indicative of a more rapid clearance of ETU in the former species.

TCI's conclusions are based upon the following considerations:

Mancozeb, [manganese ethylenebis(dithiocarbamate) (polymeric) complex with zinc salt], is a fungicidal agent with a multi-site mode of action. The developmental toxicity of mancozeb was first investigated in rats in 1980 (Report: Anonymous 1980), and it was found that an oral dose level of 512 mg/kg body weight (bwt)/day gave rise to severe maternal toxicity, 3/22 females were killed in extremis, and 6/19 surviving females had total litter death at term. Foetal findings at this dose level included markedly reduced foetal weight, incomplete ossification and malformations including neural tube defects (meningocoele, exencephaly, dilated brain ventricles) cleft palate and kinky/short tail. At a lower dose level of 128 mg/kg bwt/day moderate maternal toxicity was recorded but there were no adverse foetal effects and at 32 mg/kg bwt/day or below there were no adverse maternal or foetal effects. The major metabolite of mancozeb, ethylene thiourea (ETU), is a proven teratogen, which has been classified as Repro 1B (H360D), and this was used as a positive control in this study. At a high dose level of 50 mg/kg/day similar foetal malformations were seen to those at the high dose level of mancozeb but there was no maternal toxicity.
A second developmental toxicity study in the rat was performed in 1988 (Report: Anonymous 1988c) and the the high oral dose level of mancozeb of 360 mg/kg bwt/day resulted in marked maternal toxicity, One female was killed in extremis after showing marked loss of body weight, hind limb paralysis and a general loss of condition. Foetal findings, however, were limited to minimal signs of reduced ossification – no foetal abnormalities were recorded. At 60mg/kg bwt/day and below there were no adverse maternal or foetal effects.

A third developmental toxicity study in rats was performed in 1999 (Report: Anonymous 1999b), using oral dose levels of 500, 225 and 100 mg/kg/day. In this study, no adverse maternal effects or foetal abnormalities were recorded at any dose level. These results did not conform to those seen in the previous two studies, but no explanation was found for the discrepancies and the results of this study were discounted for regulatory assessment.

Two developmental toxicity studies were performed in rabbits during the same time period. In the first study (Anonymous 1987b), the high oral dose level of 80 mg/kg bwt/day gave rise to severe maternal toxicity in 2/20 females such that they were killed in a moribund condition; 5 females aborted having exhibited moderate maternal toxicity but the remaining females, did not show any adverse clinical signs. No maternal toxicity was recorded at 30 mg/kg/day or below and no foetal abnormalities were seen at any dose level. In the second study (Anonymous 1991b) the high oral dose level was increased to100 mg/kg bwt day. There was evidence of moderate maternal toxicity but no deaths occurred. 5/16 females aborted compared with 2/13 control females and post-

implantation loss was marginally increased. At 55 mg/kg bwt/day and below no maternal toxicity was recorded and no foetal abnormalities were observed at any dose level.

In 1993 the Commission Working Group on the Classification and Labelling of Dangerous Substances reviewed the reproductive toxicity data and decided not to classify mancozeb as a reproductive toxin, on the grounds that it was likely that the foetal abnormalities recorded in the 1980 rat study were due to the main metabolite ETU and not to mancozeb per se, and that the effects were only seen at severely maternally toxic doses. Between 2003 and 2006 the data were re-reviewed in depth by the Technical Committee on Classification and Labelling (TC C&L) and, following much discussion, mancozeb was re-classified as Repro 3, R63. In 2009, this classification was translated to Repro 2; H361d under CLP.

In 2005, the TC C&L working group of specialist experts in the field of reproductive toxicology highlighted three areas of concern, viz:

1. There is uncertainty about the dose-response relationship for maternal toxicity and developmental toxicity between 100 and 500 mg/kg/day in rat developmental studies and it is not known, therefore, if maternal or developmental toxicity is the more sensitive effect.
2. The critical short- and long-term general toxicological target of mancozeb relates to inhibition of thyroid hormone synthesis. The effect is mediated by the inhibition of thyroid peroxidase by ETU. Thyroid hormone is crucial for brain development in mammals. Recent studies have suggested that transient impairment of maternal thyroid hormone levels in the rat and in man may affect neural brain organization and behaviour. Therefore, there is a concern that mancozeb and other ethylene bisdithiocarbamates may cause developmental neurotoxicity, which would argue for a Cat.2 classification.
3. There is a species difference in kinetics and metabolism of mancozeb including the formation of ETU as shown between rats and mice, which may partially explain the higher susceptibility of rats to ETU developmental toxicity. There is no information on the comparative kinetics and metabolism in man.

Since 2005, further investigations have been carried out to investigate these areas of concern, with the aim of clarifying the situation. The results of these investigations are summarized in the following sections:

1. The relationship between maternal toxicity and developmental toxicity

Commencing in 2015, studies were undertaken with the aim of clarifying the relationship between dose levels of mancozeb, maternal toxicity, systemic exposure to the metabolite ETU and developmental toxicity

Firstly, a dose-range-finding study in non-pregnant female rats (Anonymous 2015b) was undertaken to determine suitable dose levels for use in subsequent developmental toxicity studies and to determine plasma levels of mancozeb and ETU following oral administration of 60, 120, 180, 240 and 300 mg/kg bwt/day once daily for 13 consecutive days. Dose–related slight body weight loss was recorded at 180, 240 and 300 mg/kg bwt/day but there were no effects upon food consumption at any dose level. Analysis of plasma samples demonstrated a clear dose-response between administered mancozeb and plasma levels of ETU. Plasma levels of ETU were consistently higher than those of parent compound, due to rapid metabolism. Oral dose levels of mancozeb of 80, 120 and 160 mg/kg bwt day administered from
GD 6-GD 19 were selected for use in a preliminary developmental toxicity study.

In the preliminary study (Anonymous 2015c) with mancozeb, no adverse maternal clinical signs were recorded at 80,120 or 160 mg/kg bwt/day. At 160 mg/kg bwt/day mean maternal weight gain and food consumption were reduced but embryo-foetal survival and mean foetal weight were unaffected and there were no treatment-related external foetal malformations or variations at any dose level. Pharmacokinetic investigations at 0, 2, 4, 6, 12 and 24 hours after dosing on GD 19 revealed approximately dose-related exposure to mancozeb and ETU in both dams and foetuses with foetal/dam ratios of 0.92. Peak plasma levels of mancozeb were recorded at 2-4 hours post dosing, whilst peak levels of ETU were recorded at 6 hrs post-dosing, indicating rapid metabolism. Exposure to ETU, in terms of AUClast was between x55 and x71 higher than that to mancozeb.

A definitive developmental toxicity study (Anonymous 2015d) was then performed in rats, using oral dose levels of 10, 40 or 160 mg/kg bwt/day once daily from GD 6-GD19. No clinical signs were observed at any dose level but reduced body weight gain and lower food consumption was recorded at 160 mg/kg bwt/day. Embryo-foetal survival and growth and foetal morphology were unaffected by maternal treatment at 160 mg/kg bwt/day.
It has been shown conclusively, therefore, that mancozeb, administered throughout organogenesis at dose levels up to 160 mg/kg bwt/day, a dose level at which maternal toxicity was recorded, did not give rise to any adverse effects upon litter parameters or upon embryo-foetal development. A clear no-adverse-effect level for embryofoetal development was established as 160 mg/kg bwt/day. At all dose levels investigated, dams and foetuses were exposed to both mancozeb and ETU.

Concurrent with these studies, a developmental toxicity study was conducted with ETU in rats (Anonymous 2015a). ETU was administered by the oral route from
GD 6-GD 19 at dose levels of 2.5, 5, 15 and 30 mg/kg bwt day. Analyses of plasma levels of ETU in dams and foetuses on GD 20, i.e. 24 hrs after the last dose, were performed as part of this investigation.
No maternal toxicity was recorded at any dose level, but mean foetal weight was reduced at 30 mg/kg/day and the majority of foetuses had malformations, viz. hydrocephaly, meningocoele, rib and vertebral column and tail abnormalities and malrotated limbs. At 15 mg/kg bwt day, 7 foetuses from 2 litters had hydrocephaly but there were no other foetal morphological changes that were considered to be related to maternal treatment with ETU. At 2.5 and 5.0 mg/kg bwt/day there were no adverse effects upon maternal performance or foetal development. Plasma levels of ETU on GD 20 (24 hours after the last dose) demonstrated a linear dose-relationship. It was concluded that 5 mg/kg bwt/day was a clear no-effect level (NOEL), 15 mg/kg bwt/day was the lowest-adverse-effect level LOAEL) and 30 mg/kg bwt/day was clearly teratogenic.

From the plasma concentration versus time data obtained from the pharmacokinetic investigations conducted in the preliminary mancozeb study (Anonymous 2015c) it was possible to calculate the rate constant (k) for the decline in ETU concentrations at each of the three dose levels. The following values were obtained:
80 mg/kg bwt/day: k = 0.065684, 120 mg/kg bwt/day: k = 0.077101,
160 mg/kg bwt/day: k = 0.063419. Mean value: k = 0.068737

In order to calculate the plasma levels for ETU at the time that peak concentrations would have been achieved (6 hrs post-dosing) from the C24hr values obtained in the ETU developmental toxicity study (Anonymous 2015a) the values of k were then applied to the following formula:
$C6\text{h} = C24\text{h}.ek.18$

The calculated C6hr values are shown in text table 1:

Text Table 1: Calculated plasma concentrations of ETU at 6 hrs. post-dosing

Dose ETU mg/kg bwt/day 2.5 5.0 15.0 30.0
C24hr (ng ETU/ml) measured 124 355 1280 2940
C6hr (ng ETU/ml) mean calculated 427 1223 4411 10131
C6hr (ng ETU/ml) minimum calculated 388 1112 4008 9207
C6hr (ng ETU/ml) maximum calculated 497 1422 5128 11779

Linear regression analysis of the data from the preliminary developmental toxicity study with mancozeb (Anonymous 2015c) for C6hr for ETU versus the dose of mancozeb, enabled the calculation of the mean dose of mancozeb required to give peak concentrations of ETU equal to those at the NOEL, the LOAEL and the clearly teratogenic dose level, and also the maximum and minimum values, see Text Table 2:

Text Table 2: Calculated doses of mancozeb to achieve plasma C6hr of ETU

Dose ETU mg/kg bwt/day		2.5	5.0	15.0	30.0
C24hr (ng ETU/ml)	measured	124	355	1280	2940
C6hr (ng ETU/ml) mean	calculated	427	1223	4411	10131
C6hr (ng ETU/ml) minimum	calculated	388	1112	4008	9207
C6hr (ng ETU/ml) maximum	calculated	497	1422	5128	11779

It has been clearly demonstrated, therefore, that at a dose level of mancozeb of 160 mg/kg bwt/day, which was maternally toxic, peak plasma levels of ETU approximated to that calculated for the NOEL for ETU and were thus insufficient to exert a teratogenic response. Hence the absence of any developmental toxicity at 160 mg/kg bwt/day of mancozeb.
Similarly, at a dose level of 360 mg/kg/day mancozeb, at which marked maternal toxicity was recorded (Anonymous 1988c), peak plasma levels of ETU were slightly below the minimum calculated level at the LOAEL for ETU. Hence the finding of slight developmental delay but no malformations at 360 mg/kg bwt/day of mancozeb.
At a dose level of mancozeb of 512 mg/kg bwt/day, however, which was severely maternally toxic (Anonymous 1980), peak levels of ETU were within the calculated teratogenic range for ETU and foetal malformations were recorded.

On the basis of these data, the uncertainty expressed by the TC C&L as to whether, for mancozeb, maternal or developmental toxicity was the more sensitive effect has been addressed. It is apparent that maternal toxicity can be clearly separated from developmental toxicity, with maternal toxicity occurring at very much lower dose levels than developmental toxicity. It has also been demonstrated that developmental toxicity is a consequence of exposure of embryos to the main metabolite ETU rather than to mancozeb per se, since adverse developmental effects were only seen at a dose level that generated plasma levels of ETU within the teratogenic range. TCI consider, therefore, that mancozeb per se does not warrant classification as a developmental toxin.

2. Developmental neurotoxicity

In 2008 a dose-range-finding developmental neurotoxicity (DNT) study (Anonymous

2008b) was performed in rats in which mancozeb was administered continuously in the diet at target dose levels of 5, 30 and 60 mg/kg bwt/day to mated females from GD 6 until termination on post-natal day (PND) 21. There were no treatment-related clinical signs at any dose level but during the gestation period, body weight performance and food consumption showed dose-related reductions at 30 and 60 mg/kg/day. At these dose levels an increased incidence of thyroid gland hypertrophy was recorded on PND 21, together with a significant reduction in serum T4. Increased relative thyroid gland weights were noted in some females at 60 mg/kg bwt/day, in conjunction with follicular cell hypertrophy. Mean gestation length and parturition were unaffected by treatment. Offspring in the 30 and 60 mg/kg bwt/day groups had decreased body weights and body weight gains but there were no treatment-related effects at 5 mg/kg bwt/day. Plasma and milk analyses in the high dose animals (60 mg/kg bwt/day) showed that the foetuses were exposed to residues of both mancozeb and ETU on GD 20, as were the pups during the lactation period. The ETU residue levels increased with increasing doses of mancozeb.

Based upon the findings in the preliminary study, a definitive developmental neurotoxicity (DNT) study (Anonymous 2008c) was performed in accordance with OECD Guideline 426. Target dose levels of 0, 5, 15 and 30 mg/kg bwt/day were administered via the diet to mated female rats from GD 6 until termination on PND 21-PND 28.
At the high dose level of 30 mg/kg bwt/day, reduced maternal body weight gain was recorded following onset of treatment, statistically significant from GD 6-GD 12. When maternal body weight gain was corrected for litter size/litter weight, the difference from controls between GD 6-GD 20 was -9.4%. For the dams, mean absolute and relative weights of the thyroid gland were increased by 7.5% and 9.1% respectively and this corresponded with an increased incidence of thyroid follicular cell hypertrophy.
There were no test substance-related effects at any dose level on any of the F1 litter parameters including survival, clinical signs, functional observation battery, growth, development, motor activity, startle response, learning and memory, brain morphometry and histopathology of the central and peripheral nervous systems. Based on these findings and on the presence of both mancozeb and ETU in pup plasma and in milk (investigated in the preliminary study), it can be concluded that mancozeb has been adequately tested for DNT and the results show that at dose levels of up to 30 mg/kg bwt/day mancozeb does not exert any developmental neurotoxicity.

Plasma levels of T4 were not assessed in the main DNT study. However, in the preliminary DNT study, at 30 mg/kg bwt/day there was a significant reduction in mean maternal T4 level. Despite this reduction, there was no evidence of any developmental toxicity. These studies (Anonymous 2008b, 2008c) addressed the concern of the TC C&L specialized experts that transient impairment of maternal thyroid hormone levels in the rat may affect neural brain organization and behavior, giving rise to developmental neurotoxicity and they have demonstrated that such a reduction does not result in developmental neurotoxicity.

There is no evidence, therefore, on which to classify mancozeb for developmental neurotoxicity.

3. Comparative kinetics and metabolism of mancozeb in experimental animals and humans

Toxicokinetic and metabolism studies following oral administration of C14 labelled mancozeb in the rat have shown that approximately 50% of the dose is absorbed, the other 50% is eliminated via the faeces. Metabolic investigations in the rat demonstrated

that there are two routes of elimination, one by conversion to ethylene diamine (EDA) and the other by conversion to ETU. Both of these pathways lead ultimately to the formation of glycine and other natural products. On a weight for weight basis, approximately 7% of mancozeb is converted to ETU. Further studies have shown that these two pathways are common in laboratory and farm animals indicating similar metabolism across species.
The toxicokinetics and metabolism of ETU have been studied in mice, rats, guinea pigs, cats, and monkeys. These studies have shown that ETU is rapidly excreted, primarily in the urine and more quickly by mice than by rats. Half-lives for elimination from maternal blood were 5.5 and 9.4 hours in mice and rats, respectively (Ruddick et al, 1977).
Literature studies indicate that mancozeb and ETU are also rapidly absorbed and eliminated after oral administration in humans. The elimination half-life of ETU in humans following deliberate oral exposure to a low dose of the commercial fungicide Ridomil Gold, containing 64% mancozeb and 4.5% ETU, was estimated to be 17-23 hours (Lindh et al, 2008). The apparently slightly longer half life of ETU in humans compared to rodents may be the result of administering the parent compound in the human study rather than the metabolite as in the rodent study.
Investigations in vitro using either liver S9 (Saghir et al. 2008) or primary hepatocytes (Zhu 2015) as a source of enzymes have allowed comparison of the rate of metabolism of ETU between rat, mouse and human, or rat, mouse, dog rabbit and human respectively. It was found that metabolism by hepatocytes increases in the following order: rat < mice < human, with dog and rabbit being similar to human, indicating that metabolism of ETU in humans may be more efficient than in rodents and thus ETU may be cleared more quickly in humans.

References

Lindh C.H., Littorin M., Johannesson G., Jonsson B.A.G. (2008): Analysis of ethylenethiourea as a biomarker in human urine using liquid chromatography/triple quadrupole mass spectrometry.
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Saghir S.A., Hansen S.C., Lowe E.R., Markham D.A. (2005): Ethylenethiourea: Interspecies comparison of in vitro metabolism by female rat, female mouse and female human liver S9 fractions.

Zhu W. (2015): Ethylene thiourea (ETU): Comparative In vitro Metabolism using Mouse, Rat, Rabbit, Dog and Human Hepatocytes. HLS report.

Dossier Submitter's Response
Thank you. Noted.
RAC's response
Thank you for your comment. RAC notes a mistake in Text Table 2; obviously the intended table content was that of the bottom table on p. 171 of the CLH report. Please see the response to comment No. 22.

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Greece		MemberState	24
Comment received				
The effect of the test substance on pups during lactation should be further evaluated. Labelling of mancozeb with H362 cannot be excluded. The NOAELoffspring is set at 7 mg/kg bw/d based on slight delay in eye opening and decreased pup weight and viability at 65 mg/kg bw/d (Anonymous et al., 1988b). These offspring effects were not observed in the previous 2-generation rats study at doses up to 70 mg/kg bw/day. Please elaborate on this difference. The incidences of the findings should be presented in a table to allow evaluation by the reviewer. Do you consider that there is an effect of the test substance on pups during lactation that should be highlighted by appropriate labelling of mancozeb (H362)? This proposal is substantiated by the observation in the DNT study (Anonymous, 2008b) where it is noted that: "plasma and milk analyses in the high dose animals (60 mg/kg/d) showed that the pups were exposed to residues of both mancozeb and ETU". [See also Mancozeb RAR, Vol. 3, B.6.6.1, Generational studies, p.129 & Vol. 3, B.6.6.2,Developmental neurotoxicity study, p.142]				
Dossier Submitter's Response				
Thank you for your comments. Although ETU was found in rat milk, no specific developmental effects were seen in pups during lactation. Slight delay in eye opening and decreased pup weight and viability were seen at 65 mg/kg bw/d in one multi-generation study (it is unclear why similar effects were not seen in a second multi-generation study). However, these effects were secondary to the maternal toxicity observed in the parental animals. On this basis, classification for adverse effects on or via lactation is not justified.				
RAC's response				
As the assessment in the CLH report is limited to adverse effects on development, classification for effects on or via lactation is outside the mandate of RAC.				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
26.04.2018	Denmark		MemberState	25
Comment received				
Agree that mancozeb should still be considered a skin sensitiser.				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Thank you for your comment. RAC agrees that classification of mancozeb for skin sensitisation should be retained.				

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Spain		MemberState	26
Comment received				
Skin sensitization Positive results were obtained in 3 out of the 6 available studies. Based on the results of the guinea pig maximisation test that gave a positive response in 35% of animals at a 50% intradermal induction dose and the Buehler assay that gave a positive response in				

20% of animals at a 50% topical induction dose, mancozeb should be classified for skin sensitisation in at least sub-category 1B. However, data are insufficient for classifications into sub-categories. Since a concentration below 1% was not tested in the maximization test (more sensitive than the Buehler test) then the exclusion of sub-category 1A is not possible. In accordance with the provisions of the 2nd ATP to the CLP Regulation, when Category 1A cannot be excluded, Category 1 should be applied instead of Category 1B. Overall, the Spanish CA supports the proposal of the dossier submitter to confirmed the current classification of mancozeb as Skin Sens 1, H317 – May cause an allergic skin reaction.
Dossier Submitter's Response
Thank you.
RAC's response
Thank you for your comment. RAC agrees that the current classification with Skin Sens. 1 without subcategorization should be retained.

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Germany		MemberState	27
Comment received				
The proposal to retain the current classification (Skin Sens 1, H317) is supported.				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Thank you for your comment. RAC agrees that the current classification with Skin Sens. 1 without subcategorization should be retained.				

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	France		MemberState	28
Comment received				
Proposal for classification Skin Sens 1 H317 (no sub-categorization) is supported				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Thank you for your comment. RAC agrees that the current classification with Skin Sens. 1 without subcategorization should be retained.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Belgium		MemberState	29
Comment received				
BE CA is of the opinion that a STOT RE 2 (thyroid, nervous system) classification is not the most appropriate to address mancozeb endocrine disrupting capability and health				

hazards. Repeated exposure to mancozeb induces in rat, mouse and dog disturbance in T4 (and T3) production balance. The T4 decrease is associated with a physiological response through TSH increase. This endocrine disturbance has been shown to induce thyroid hyperplasia in dog, mouse and rat. Rat being more sensitive to hormonal disturbance of the HPT axis, mancozeb exposure has been therefore demonstrated to result in thyroid follicular cell adenomas and carcinomas in this species and should therefore be discussed for a carcinogenicity classification.

Dossier Submitter's Response

Thank you for your comments. We agree that STOT-RE classification for thyroid effects is not intended to cover the thyroid carcinogenicity effects. However, the DS remains of the view that although hypothyroidism induced by mancozeb is relevant to humans, the WoE supports the contention that thyroid cell proliferation and hyperplasia is unlikely to occur in humans. Therefore, classification for (thyroid) carcinogenicity is not justified.

RAC's response

Thank your for your comment. STOT RE classification is to be assessed separately from the classification for carcinogenicity. RAC considers the mancozeb-induced hypothyroidism indicated by reduced plasma T4 levels in animal studies to be an adverse effect relevant for classification.

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Spain		MemberState	30

Comment received

Specific target organ toxicity- repeated exposure

Significant neurohistopathology findings were seen in the nervous system in a 90 day oral neurotoxicity study in SD rats (Anonymous, 1991e), they started to occur from a dose of 49 mg/kg bw/d, below the guidance value of 100 mg/kg bw/day for classification with STOT-RE 2 and included the following: myelin phagocytosis, Schwann cell proliferation, demyelinated nerves, myelin sheath thickening and myelin bubbles were observed in males and demyelination and myelin ovoids and debris.

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

In addition, statistically significant increased incidence and severity of bilateral retinopathy was observed in a combined chronic toxicity/ carcinogenicity study rats (Anonymous, 1990) from 6.7 mg/kg bw/day in females, below the guidance value of 12.5 mg/kg bw/day. This finding was not mencioned by the dossier submitter regarding the classification with STOT-RE and in our opinion it can be considered as evidence of specific target organ toxicicity after repeated exposure (eyes).

Regarding the thyroid toxicity observed in rats and dogs at dose levels below the guidance values for classification with STOT-RE 2, under our judgment it is not sufficient for classification, however it may be considered to be supporting evidence for classification for STOT-RE.

Therefore, on overall the Spanish CA support the dossier submitter proposal for

classification of mancozeb with STOT-RE 2 (H373) for the oral route.

Dossier Submitter's Response

Thank you for your support. Although a statistically significant increased incidence of bilateral retinopathy was observed in a combined chronic toxicity/carcinogenicity study in rats (Anonymous, 1990) at 6.7 and 40.2 mg/kg bw/day in females (31/50 and 49/50 vs 21/50 in controls), this was only marginally increased above controls at 6.7 mg/kg bw/d and not seen in males at the same dose level. In addition, the severity was not increased at this dose compared to controls and a similar finding was not seen in males and females in a second carcinogenicity study (Anonymous, 1992a) in rats up to a dose of 16.8/20.8 mg/kg bw/d. On this basis, the bilateral retinopathy seen in females at 6.7 mg/kg bw/d was considered part of normal variation. Therefore, classification with STOT-RE for effects on the eye is not justified.

RAC's response

Thank your for your comments.

Your support for classification in Category 2 due to neurotoxicity and mortality is noted. RAC is of the view that the effects on both the nervous system and the thyroid are sufficient for classification and both organs should be listed as targets. Mortality, although not considered by RAC sufficient for classification on its own, provides additional support.

RAC agrees with the DS that classification for effects on the eye is not warranted for reasons outlined in the DS's response.

RAC does not agree that the oral route should be specified, as no dog studies via the dermal and inhalation routes are available. In addition, neurotoxicity was seen at doses relevant for classification after inhalation exposure in the rat (Lu and Kennedy 1986).

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

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Germany		MemberState	31

Comment received

The proposal to add a classification as STOT-RE2, H373, based on effects on the thyroid and the nervous system through prolonged oral exposure is supported.

Dossier Submitter's Response

Thank you for your support.

RAC's response

Thank you for your comment. RAC agrees that classification with STOT RE 2 based on effects on the thyroid and the nervous system is warranted.

RAC does not agree that the oral route should be specified, as no dog studies via the dermal and inhalation routes are available. In addition, neurotoxicity was seen at doses relevant for classification after inhalation exposure in the rat (Lu and Kennedy 1986).

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

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	France		MemberState	32
Comment received				
Proposal for classification STOT RE2 H373 thyroid and nervous system is supported				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you for your comment. RAC agrees that classification with STOT RE 2; H373 (thyroid, nervous system) is justified.				

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Belgium		MemberState	33
Comment received				
BE CA is of the opinion that a STOT RE 2 (thyroid) classification is not the most appropriate to address mancozeb endocrine disrupting capability and health hazards. Repeated exposure to mancozeb induces in rat, mouse and dog disturbance in T4 (and T3) production balance. The T4 decrease is associated with a physiological response through TSH increase. This endocrine disturbance has been shown to induce thyroid hyperplasia in dog, mouse and rat. Rat being more sensitive to hormonal disturbance of the HPT axis, mancozeb exposure has been therefore shown to result in thyroid follicular cell adenomas and carcinomas. BE CA believes that thyroid follicular cell hyperplasia in rat is a pathological adaptative response that might be considered to some extend as a potential pre-neoplastic indication, therefore discussed for a carcinogenicity classification.				
Dossier Submitter's Response				
This is a repeat of comment 29.				
RAC's response				
Please see the response to comment No. 29.				

Date	Country	Organisation	Type of Organisation	Comment number
26.04.2018	Denmark		MemberState	34
Comment received				
Agree to STOT RE 2, both for thyroid and nervours system. Although for the thyroid it should be checked if the short-term study in dog from 1987 is valid and a STOT RE 1 should perhaps be considered.				
Dossier Submitter's Response				
Thank you for your comments. The thyroid histopathology findings seen in a 3-month study in dogs at 5.7 mg/kg bw/d (Anonymous, 1987c) was not accompanied by weight changes or changes in thyroid hormones. In addition, thyroid effects started to appear in numerous other studies in dogs (including those of longer duration) only from higher dose levels (around 23-28 mg/kg bw/d). Therefore, the reliability of the thyroid findings from this study is low. On this basis, classification with STOT-RE 1 is not warranted.				

RAC's response
Thank you for your comment. RAC agrees that classification with STOT RE 2 for both the thyroid and the nervous system is warranted. RAC also agrees with the DS's response regarding the potential classification with STOT RE 1 based on thyroid findings in the study Anon. (1987c).

Date	Country	Organisation	Type of Organisation	Comment number
20.04.2018	Netherlands	EU Mancozeb Task Force	Company-Manufacturer	35

Comment received
The EU MTF disagrees with the position of the RMS that Mancozeb should be classified as a STOT-RE. A background document is provided in the file attached. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Mancozeb_ECHA Comments_EU MTF.zip
Dossier Submitter's Response
Noted.
RAC's response
Thank you for your comments. <u>Thyroid toxicity</u> Mancozeb-induced hypothyroidism is considered an adverse effect. Both irreversible and reversible effects are relevant for STOT RE classification (CLP, Annex I, 3.9.1.1). Although the T4 reduction in the dog at doses causing severe general toxicity might be partly an adaptive response to chronic stress, a T4 reduction was also observed at lower doses. RAC acknowledges that the effects in the dog at doses below the guidance values (GVs) for classification are weaker than in the rat, but this is partly due to the dose selection. Human non-relevance of the mancozeb-induced reduction in thyroid hormone levels in the rat studies is not supported by the available data. Humans do have a larger reservoir of thyroid hormones compared to rats, but if thyroid hormone synthesis is reduced for prolonged periods of time, the reservoir will ultimately be depleted and thyroid hormone levels will decrease. In the monkeys, whose hypothalamus-pituitary-thyroid physiology axis is similar to that of humans, T4 reduction began after 5 months of exposure to ETU, and the LOAEL was not markedly higher than the LOAELs in rat studies with ETU. In addition, disruption of thyroid hormone synthesis is a mode of action of drugs used to treat hyperthyroidism in humans (e.g., methimazole). Two *in vitro* comparative metabolic studies (Saghir *et al.* 2005; Zhu 2015) indicate that ETU might be more readily metabolised in humans than in rats while metabolism in the dog appeared similar to humans. 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 in-built 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 a similar metabolic rate to humans *in vitro*. Epidemiology studies generally address risk rather than hazard. The absence of effects in the epidemiology studies on EBDCs can be explained by low systemic exposure. For these reasons, RAC agrees with the DS that classification of mancozeb in Category 2 for thyroid effects is justified.

Nervous system toxicity
Although neurotoxicity was only observed in the rat and not in the mouse or the dog, non-relevance of the rat findings to humans has not been demonstrated. Thus, the rat findings are considered relevant to humans. Negative epidemiology data cannot be used to overrule the animal findings; epidemiological studies have their limitations and the exposure levels are unlikely to have reached the levels causing neurotoxicity in the rat.

RAC considers the histopathological findings at the mid-dose in the 90-day neurotoxicity study (Anon., 1991e) sufficient for classification, 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 at doses above the guidance values, are considered to provide additional support for classification.

Lack of neurotoxicity in animal studies with ETU cannot negate the findings with mancozeb. Mancozeb-induced neurotoxicity may be caused by other metabolites.

Date	Country	Organisation	Type of Organisation	Comment number
04.04.2018	United Kingdom		Individual	36

Comment received

STOT-RE is assigned on the basis of findings of 'significant' or 'severe' toxicity which is of relevance to human health.

With regard to thyroid toxicity, thyroid follicular cell hyperplasia and changes in thyroid hormones have been observed with exposure to mancozeb in 90-day studies in SD rats and dogs at levels below, or just above, the guidance value of 100 mg/kg bw/d for classification with STOT-RE 2. However, some dog studies have resulted in equivocal findings.

Mancozeb affects the thyroid hormonal system via its main metabolite and driver of toxicity, ethylenethiourea (ETU). ETU affects the production of thyroid hormone (T4) through inhibition of thyroid peroxidase (TPO) enzyme. This can lead, via a homeostatic loop, to hypertrophy and hyperplasia of the thyroid follicular cells. ETU does not bind with the thyroid receptor, have any direct interaction with the thyroid stimulating hormone (TSH) pathway or inhibit iodothyronine deiodinase. It only affects TPO and this inhibition is transient and reversible, since ETU is metabolised in binding to TPO.
Rats appear to be particularly sensitive to thyroid peroxidase inhibitors and most susceptible to the thyroid disrupting effect of ETU. Metabolism of ETU seems much less efficient in rats than in dogs and humans. As the CLP guidance document notes, humans, unlike rodents, possess a T4 binding protein which greatly reduces susceptibility to plasma T4 depletion and thyroid stimulation; such a mechanism/effect cannot therefore be directly extrapolated to humans. It concludes that these thyroid effects observed in rodents are therefore of insufficient concern for classification.

Since they have a relatively large reservoir of thyroid hormones and more quiescent follicular cells, humans are less responsive to chemicals which can affect the thyroid. Although hypertrophy of follicular cells has been observed in humans, hyperplasia is rare and there is no evidence that hypothyroidism (goiter) in humans progresses to neoplasia; so, it is unlikely that humans would ever develop thyroid tumours after exposure to mancozeb, even at very high doses.

Occupational monitoring studies under 'worst case' exposure scenarios indicate that urinary ETU concentrations are several times higher than in general populations. In a study of pregnant workers from a banana plantation, the median chronic Estimated Daily Intake (EDI) from each woman's average urinary creatinine corrected ETU concentration was 0.12 µg ETU/kg/day (with low variability), approximately 17 times lower than the reference Acceptable Daily Intake (ADI) of 2.0 µg/kg/day and several orders of magnitude lower than the effect levels from animal studies. Moreover, studies of exposed workers have not found any evidence for effects from mancozeb.

In summary, the observations from the rat studies can be discounted for the purposes of STOT-RE classification; those from the dog studies are somewhat equivocal; and the observed thyroid effects are not relevant to human health. On this basis, therefore, the classification of mancozeb as STOT-RE for thyroid toxicity is not supported.

With regard to neurotoxicity, effects have been observed in studies of mancozeb on SD rats; hind limb ataxia and paralysis were seen in 28-day oral studies, at below the guidance value of 300 mg/kg bw/day for classification with STOT-RE 2; myelin phagocytosis, Schwann cell proliferation, demyelinated nerves, myelin sheath thickening, myelin bubbles and myelin ovoids were observed in a 90-day oral neurotoxicity study in SD rats below the guidance value of 100 mg/kg bw/day for classification with STOT-RE 2. However, neurotoxicity was not seen in any repeat dose studies with ETU and no developmental neurotoxicity was seen in an extended one-generation study in rats exposed to ETU up to the top dose, 10 mg/kg bw/d, which resulted in systemic toxicity. No evidence has been found of delayed neurotoxicity being caused by mancozeb.

Several studies have investigated possible links between pesticide use and the appearance of neurological effects, such as state of mind as suicide, in humans. No association has been found between pesticide exposure and subsequent incidence of suicide in pesticide applicators and their spouses in the US and this was consistent across use of any pesticide, individual pesticides including maneb/ mancozeb, functional or chemical classes, and cumulative lifetime use of pesticide. Studies of Parkinson's Disease and pesticide exposure have not found any evidence that exposure to mancozeb was associated with increased incidences of the illness. No evidence has been found that mancozeb can cause neural tube defects in humans.

While animal studies have suggested that neurotoxic effects can occur at exposures to mancozeb below the STOT-RE guideline levels, such effects occurred only in a small number of test animals. Mancozeb's principal metabolite, ETU, is not neurotoxic and there is no indication of neurotoxic effects in humans from exposure to mancozeb.

So, although the reported observations of effects at doses of mancozeb below the guideline level point towards its classification for STOT-RE (Category 2) for neurotoxicity, it remains unclear whether the findings are reliable and of relevance to human health.

On this basis, it cannot be definitively resolved that mancozeb should be classified as STOT-RE 2 for neurotoxicity.

Dossier Submitter's Response

Thank you for your comments. As stated in the CLH report, significant thyroid toxicity occurred in rats and dogs at dose levels below the guidance values for classification with STOT-RE 2. Although there is evidence that rats are more sensitive than humans to perturbation of thyroid homeostasis, this evidence is less clear for the dog. In addition, it

is noted that in a 6-month study in monkeys with ETU, thyroid toxicity was observed from the relatively low dose of 2.5 mg/kg bw/day. Overall, therefore, it is concluded that mancozeb could induce thyroid toxicity in humans at dose levels relevant for classification. Hence, classification of mancozeb with STOT-RE 2 for effects on the thyroid is justified. This conclusion may appear in conflict with the conclusion that mancozeb-induced thyroid tumours in rats (and not mice) are unlikely to occur in humans and therefore that classification of mancozeb for carcinogenicity is not appropriate. The DS considers that there is no inconsistency as although it is plausible that mancozeb would induce thyroid toxicity in humans at dose levels relevant for classification, it is highly unlikely that mancozeb would cause thyroid hyperplasia and tumours in humans since there is no clear evidence that hypothyroidism (goitre) in humans progresses to neoplasia and because whilst thyroid hypertrophy has been observed in humans, thyroid hyperplasia is rare.

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

In addition, in a 90 day oral neurotoxicity study in SD rats (Anonymous, 1991e), myelin phagocytosis, Schwann cell proliferation, demyelinated nerves, myelin sheath thickening and myelin bubbles were observed in males and demyelination and myelin ovoids and debris were noted in females at 750 ppm (49/63 mg/kg bw/day; M/F) (below the guidance value of 100 mg/kg bw/day for classification with STOT-RE 2). Therefore, based on these findings, mancozeb should be classified for STOT-RE category 2 for effects on the nervous system.

The DS disagrees that these neurotoxicity findings are not reliable just because they were not seen with ETU, mancozeb's major metabolite. The neurotoxicity of mancozeb might not be caused by ETU. The DS also disagrees that these findings are not relevant to humans based on the absence of neurological effects in some very limited epidemiological studies.

RAC's response

Thank you for your comments. Please see the response to comment No. 35.

Date	Country	Organisation	Type of Organisation	Comment number
05.03.2018	United Kingdom		Individual	37

Comment received

Consideration of Thyroid Toxicity

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

was observed in 3/4 males and 2/4 females.

These results therefore raise concern with respect to the classification of mancozeb as a STOT-RE, Category 2. The salient question is whether the observed changes are, in the words of the guidance, significant and of relevance to human health. Certainly there is evidence that rats are more sensitive than humans to perturbation of thyroid homeostasis, but this is less clear for the dog. With respect to thyroid toxicity in rats, the CLP guidance document states: "It is known that rodents are highly sensitive to a reduction in thyroid hormone levels (T4), resulting in thyroid toxicity (e.g. hypertrophy, hyperplasia) after repeated stimulation/exposure of this organ. This in turn is related to an increase in the activity of hepatic UDPG-transferase. Humans, unlike rodents, possess a T4 binding protein that greatly reduces susceptibility to plasma T4 depletion and thyroid stimulation. Thus, such a mechanism/effect cannot be directly extrapolated to humans, i.e. these thyroid effects observed in rodents… are therefore considered of insufficient concern for classification". It would appear, therefore, that the observed thyroid changes in rats can be dismissed for the purposes of classification as not relevant to human health. Findings in dogs were equivocal, with one study (of 8 animals) but not another (of 12 animals) finding thyroid changes at dose levels below the guidance value of 100 mg/kg bw/d. Again the question must be asked whether these findings of increased thyroid follicular hyperplasia are both significant and of relevance to human health.

The effects of mancozeb on the thyroid are driven by its major metabolite, ETU, which interferes with the production of thyroid hormone T4 by inhibition of the thyroid peroxidase (TPO) enzyme. This mechanism can lead to hypertrophy and hyperplasia of the thyroid follicular cells through a feedback mechanism (homeostatic loop) mediated by thyroid stimulating hormone (TSH) released by the pituitary: as T4 levels fall the pituitary releases more TSH which, if T4 levels do not rise, eventually causes the thyroid to become hypertrophic and/or hyperplastic.

Effects of ETU on TPO are transient and reversible, and ETU is metabolised as it binds to TPO. The metabolism of ETU is higher in humans and other higher animals than in lower animals. Also, humans are known to be less responsive to thyroid active chemicals as they have a large reservoir of thyroid hormones compared to rats for example, and the follicular cells are more quiescent. In humans, follicular cell hypertrophy has been observed, but hyperplasia is rare. It is generally acknowledged that a challenge in the safety evaluation of compounds that produce thyroid hyperplasia in laboratory animals is the assessment of probable risk for thyroid changes in humans, as species differences in the sensitivity of thyroid tissues to such effects is likely. Many drugs are capable of inducing changes to thyroid morphology and function in laboratory animals, particularly rats, but do not cause significant adverse effects on thyroid function in clinical practice. Also, there is no evidence that hypothyroidism (goitre) in humans progresses to neoplasia and it is thus unlikely that thyroid tumours would ever develop in humans exposed to mancozeb.

Studies in exposed workers have found no evidence for effects of mancozeb, and human monitoring studies (investigating occupational exposure to ethylene bisdithiocarbamates, including mancozeb), have shown ETU levels in all cases to be well below established reference values, with calculated Estimated Daily Intakes (EDIs) several orders of magnitude lower than effect levels in experimental animals.

The principal conclusions to be drawn from the available evidence regarding thyroid toxicity are that:
• Effects of mancozeb on the thyroid are due to the principal metabolite ETU.

• The mode of action of ETU is through a transient, reversible inhibition of the enzyme TPO which interferes with production of thyroxin which in turn impacts the homeostatic feedback loop that regulates and maintains circulating thyroxin levels.
• The metabolism of ETU is particularly efficient in humans.
• Humans are far less susceptible than rats and other experimental animals to thyroid effects.
• Human epidemiology and medical studies have shown no demonstrable effects of mancozeb (including effects on the thyroid).
• Estimated ETU intakes in highly exposed workers are several orders of magnitude lower than derived NOAELs in experimental studies and, similarly, considerably lower than derived reference values.

Thus it would appear that the thyroid effects observed in experimental animals are of questionable toxicological significance and are not relevant to human health. For these reasons it is inappropriate to classify mancozeb as STOT-RE for thyroid toxicity.

Consideration of Neurotoxicity

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

In addition, in a 90 day oral neurotoxicity study in SD rats (10 males and 10 females per group), myelin phagocytosis, Schwann cell proliferation, demyelinated nerves, myelin sheath thickening and myelin bubbles were observed in occasional males, and demyelination and myelin ovoids and debris were noted in two females at 750 ppm (49/63 mg/kg bw/day in males and females respectively) - below the guidance value of 100 mg/kg bw/day for classification with STOT-RE 2. None of the findings were seen in control animals apart from myelin bubbles in the lumbar, dorsal root ganglion sections, which were also observed in one control female.

Interestingly, there is no evidence that ETU, the principal metabolite of mancozeb, causes neurotoxicity. Neurotoxicity was not reported in any of the repeated dose toxicology studies on ETU and no developmental neurotoxicity was seen in an extended one-generation study in rats up to the top dose of 10 mg/kg bw/d at which systemic toxicity occurred.
There is no evidence that mancozeb causes delayed neurotoxicity.

The possible appearance of neurological effects in humans exposed to pesticides has been investigated in several studies. Effects on state of mind, as suicide, have been determined. No association was established between pesticide and subsequent incidence of suicide in pesticide applicators and their spouses in the US - this finding was consistent for use of any pesticide, individual pesticides including maneb/ mancozeb, for functional or chemical classes, and for cumulative lifetime days of pesticide use. Possible associations between pesticide use and Parkinson's disease (PD) have been studied but none found evidence that exposure to mancozeb was associated with increased incidences of PD. The possible association between mancozeb exposure and neural tube defects has been investigated in two studies; the overall conclusion from these studies is that there is no evidence that mancozeb has caused neural tube defects in humans.

Thus the evidence for significant and human health-relevant neurotoxicity is equivocal. Animal studies have indicated that neurotoxic effects occur at exposure levels somewhat

below the guidelines, but only in a small number of animals. ETU, the principal metabolite of mancozeb, is demonstrably not neurotoxic. Moreover, there is no human evidence for neurotoxic effects.

While the finding of hindlimb ataxia and/or paralysis in treated rats at a dose below the guideline level in a small number of animals indicates that mancozeb should probably be classified as STOT-RE Category 2 for neurotoxicity, there is uncertainty regarding the reliability and the human relevance of this finding.

Summary Conclusions

On a weight-of-evidence basis it can be concluded that mancozeb should not be classified as STOT-RE for thyroid toxicity.

It is not possible to conclude with any degree of certainty that mancozeb should be classified as STOT-RE (Category 2) for neurotoxicity.

Dossier Submitter's Response

See DS response to comment 36 above.

RAC's response

Thank you for your comments.

Thyroid toxicity

The CLP guidance refers to thyroid toxicity via liver enzyme induction, which is not the case here. On the contrary, inhibition of thyroid hormone synthesis is a mechanism relevant to humans as indicated by the therapeutic use of a TPO inhibitor methimazole in the treatment of hyperthyroidism. Human relevance is further supported by results of the study with ETU by Leber *et al.* (1978) in monkeys, whose hypothalamus-pituitary-thyroid physiology axis is similar to that of humans. The LOAEL for T4 reduction in this monkey study was not markedly higher than LOAELs in the rat studies with ETU.

Two *in vitro* comparative metabolic studies (Saghir *et al.* 2005; Zhu 2015) indicate that ETU might be more readily removed in humans than in rats while metabolism in the dog appeared similar to humans. 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 in-built 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 metabolic rate to humans *in vitro*.

Risk-based arguments cannot be used for hazard classification.

As human non-relevance of the rat and dog thyroid findings has not been demonstrated, RAC agrees with the DS that classification of mancozeb in Category 2 for thyroid effects is justified.

Neurotoxicity

RAC considers the histopathological findings at the mid-dose in the 90-day neurotoxicity study (Anon., 1991e) sufficient for classification, 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 guidance values for classification, are considered to provide additional support for classification.

Negative epidemiology data cannot be used to overrule the animal findings; epidemiological studies have their limitations and the exposure levels are unlikely to have reached the levels causing neurotoxicity in the rat. As human non-relevance of the rat findings has not been demonstrated, these are considered relevant to humans.

Lack of neurotoxicity in animal studies with ETU cannot negate the findings with mancozeb. Mancozeb-induced neurotoxicity may be caused by other metabolites.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Belgium		MemberState	38

Comment received

BE CA supports the proposed environmental classification as Aquatic Acute 1, H400 (Macute=10) and Aquatic Chronic 1, H410 (Mchronic=10).

Based on the results of the aquatic acute toxicity test on the most sensitive species performed with the substance as such, Algae (Pseudokirchneriella subcapitata) with 72hErC50=0.0509 mg/L(geom mean m), it is warranted to classify as Aquatic Acute 1, H400 with M=10 (0.01mg/l <LC50 ≤0.1 mg/l).

Chronic data on the substance as such are not available on all 3 trophic levels, therefore the surrogate approach is also used and both outcomes result in the same classification and M-factor.
Classification as Aquatic Chronic 1, H410 is warranted based on :
- Fish (Pimephales promelas) with 215d EC10=0.00127 mg/l (mm) and the fact that the substance is not rapidly degradable. A M=10 can be assigned (0.001mg/l <NOEC ≤0.01mg/l)
- Surrogate approach : Daphnia magna : 48hEC50=0.073 mg/L(mm) and not rapidly degradable. A M=10 can be assigned (0.01mg/l <LC50 ≤0.1 mg/l)

Dossier Submitter's Response

Noted – thank you for your support.

RAC's response

Thank you. Noted.

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Finland		MemberState	39

Comment received

FI CA supports the conclusions that mancozeb is neither rapidly degradable or potentially bioaccumulative.

FI CA supports the use of key study (Anonymous 2012) for classification purposes. Thus the most relevant and reliable chronic endpoint is the EC10 value of 0.00127 mg a.s./L for Pimephales promelas.

Based on classification criteria FI CA supports the proposed environmental classification Aquatic Acute 1, H400 with M-factor of 10 and Aquatic Chronic 1, H410 with M-factor of 10 for mancozeb.

Dossier Submitter's Response
Noted – thank you for the support.
RAC's response
Thank you. Noted.

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	France		MemberState	40
Comment received				
FR agrees with the classification and M factors (acute and chronic) proposals.				
Dossier Submitter's Response				
Noted, thank you for the support.				
RAC's response				
Thank you. Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2018	Belgium		MemberState	41
Comment received				
BE CA supports the proposed environmental classification as Aquatic Acute 1, H400 (Macute=10) and Aquatic Chronic 1, H410 (Mchronic=10). Based on the results of the aquatic acute toxicity test on the most sensitive species performed with the substance as such, Algae (Pseudokirchneriella subcapitata) with 72hErC50=0.0509 mg/L(geom mean m), it is warranted to classify as Aquatic Acute 1, H400 with M=10 (0.01mg/l <LC50 ≤0.1 mg/l). Chronic data on the substance as such are not available on all 3 trophic levels, therefore the surrogate approach is also used and both outcomes result in the same classification and M-factor. Classification as Aquatic Chronic 1, H410 is warranted based on : - Fish (Pimephales promelas) with 215d EC10=0.00127 mg/l (mm) and the fact that the substance is not rapidly degradable. A M=10 can be assigned (0.001mg/l <NOEC ≤0.01mg/l) - Surrogate approach : Daphnia magna : 48hEC50=0.073 mg/L(mm) and not rapidly degradable. A M=10 can be assigned (0.01mg/l <LC50 ≤0.1 mg/l)				
Dossier Submitter's Response				
Noted – thank you for your support.				
RAC's response				
Thank you. Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
20.04.2018	Netherlands	EU Mancozeb Task Force	Company-Manufacturer	42
Comment received				
Aquatic toxicity: Page 109, Table 25, 2nd row and page 114, 1st study summary: The Patel (1998) study should be disregarded as it is strictly not valid (not according to OECD 202). It was				

therefore also not considered within the original Annex I process. Moreover, mancozeb was analytically not detected in this study, neither at test start (0 h) nor at test end (24 h). There are also only few details on the analytical method given and no validation data are available. Moreover, 24-h values should not be used in daphnid risk assessments - especially when valid 48-h values are available - as they underlie considerable variability (see also ECHA Guidance Document R.7b: Endpoint specific guidance, page 29: For daphnids, a test duration of 48 hours is standard. However, 24 hour LC50 or EC50 values are often reported for this study. 24 hour values can have considerable variability in the repeatability of results and should not be compared to 48 hour values. The standard 48 hour reported values are favoured over 24 hour values for these reasons. 24 hour values should be considered only in the absence of good quality 48 hour values and in conjunction with other available date (non-testing, read-across, information on time-dependence of effects etc).). Therefore this endpoint should not be used in the CLH assessment. Instead the Douglas (1988) study provides the most sensitive endpoint (LC50 = 0.073 mg a.s./L). Biodegradation: It is the view of the EU MTF that Mancozeb can be assessed as rapidly biodegradable. Please refer to the file attached for further information. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Mancozeb_ECHA Comments_EU MTF.zip
Dossier Submitter's Response
Regarding degradation of EBIS, the EU Mancozeb Task Force states that maximum half-lives of <1 day are reported for the water phase. The Dossier Submitter calculated water phase dissipation rates for EBIS in the OECD 308 water/sediment studies. Three half-live (SFO DT50) values were reported, these being 0.59 days, 0.85 days and 1.2 days. Thus it is not strictly correct that all half-lives were <1 day. The Dossier Submitter must point out that EBIS was found in sediment in the OECD 308 water/sediment studies, albeit 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. With this in mind, the Dossier Submitter is of the opinion that the whole system half-lives are more representative of degradation and the water phase values are strictly representative of dissipation from the water phase, not degradation in the water phase. Our understanding is that for classification purposes, degradation rather than dissipation is considered. Regarding "aquatic toxicity". This issue has also been raised during the formal commenting on the RAR for the active substance. Mixed opinions were aired on whether the use of the endpoint from this study is appropriate. The RMS (Dossier submitter) has suggested expert discussion on this point. We therefore consider that the relevance of this study is open for interpretation. For the purpose of the CLH, the Dossier submitter has provided a full consideration of this study, its short-comings, and support for use in the risk assessment. It is, however, noted that this will have no outcome on the ultimate acute classification (or M-factor) for mancozeb. Regarding "biodegradation". The RMS notes the additional document submitted by EU MTF. However, it is considered that the consideration of toxicity of degradants made in the CLH report still stands. The Dossier submitter considers that, according to the Guidance on the Application of the CLP Criteria (ECHA, 2015) mancozeb should not be classified as rapidly degradable based on the classification of the degradant EBIS.
RAC's response
Thank you. Noted.

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2018	Netherlands		MemberState	43

Comment received

Degradation:
Mancozeb is not readily biodegradable (5-6% mineralization after 36 days). Hydrolysis is rapid, ranging 0.6 hours to 6.0 days. Several hydrolysis products were detected including EBIS with maximum concentrations of 33% at pH 4, 41% at pH 7 and 30% at pH 9. Primary degradation in surface water was rapid (OECD TG 309), i.e. mancozeb was not detected after 3 days. Normalized to 12 °C, a DT90 of <5.7 days was obtained. Mineralization reached a maximum of 16.8% after 60 days. Sampling was not conducted during first three days, possibly missing transformation products, including EBIS. Two water sediment simulation studies were also available. DT50 normalized to 12 °C ranged 0.59 to 3.41 days. Mineralization ranged from 17.6 – 57.8% applied radioactivity after 105/106 days. Degradants were detected, with maximal concentrations for EBIS reaching 8.9 to 30.9% applied radioactivity early during testing (0 – 0.25 days). EBSI has a harmonized classification as Aquatic Acute category 1 and Aquatic Chronic category 1. Therefore, agreed that mancozeb is to be considered not rapidly degradable.

Bioaccumulation:
No fish bioaccumulation study is available and the log Kow is 2.3, which is below trigger. Therefore, agreed with low bioaccumulation potential.

Ecotoxicity:
Studies conducted with technical mancozeb available for three taxa with respects to acute ecotoxicity and for fish and algae with respect to chronic ecotoxicity. Studies conducted with ~80% w/w wettable powder formulations of mancozeb are available for all three taxa with respect to acute ecotoxicity, and for aquatic invertebrates and aquatic plants for chronic ecotoxicity.
Points worth nothing:
• For the algal key study (Forbis, 1990), endpoints based on mean measured data were calculated instead of the initially reported endpoints based on nominal/initial measured concentrations. The newly calculated endpoints reflect the actual concentration of the parent substance during the static exposure of 120 hours..
• With respect to chronic fish toxicity data. It can be interpreted in the conclusion that the Dossier submitter states that the EC10 of 0.00127 mg a.s./L for Pimephales promelas is preferred above the NOEC of 0.000918 mg a.s./L for Cyprinodon variegates. At first sight it thus appears that they prefer an EC10 from one study above a lower NOEC from another study, which is not correct (EC10 is only preferred above NOEC if both can be derived from same study). However, in the summary of paragraph 11.6.1 it is clear that for Cyprinodon variegates a NOEC of 0.918 µg/L and an EC10 growth of 2.878 is µg/L are reported, and thus they have compared EC10 values from the different studies with each other. Agreed with this approach.

Dossier Submitter's Response

Regarding "Degradation":
Noted, thank you for your support. We agree with the observation that the absence of sampling between 0 and 3 days in the OECD 309 study may mean that the formation of EBIS and other metabolites may have been missed. In attempting to interpret the results of the OECD 309 study, the results of the water/sediment studies have to be taken into consideration where EBIS peaked at major quantities within the first day after treatment.

Regarding "bioaccumulation":

Noted, thank you. Regarding Ecotoxicity: Noted, thank you – and apologies for any confusion caused by the wording of the conclusion.
RAC's response
Thank you. Noted.

PUBLIC ATTACHMENTS
1. Mancozeb_ECHA Comments_EU MTF.zip [Please refer to comment No. 11, 22, 35, 42]
2. MANCOZEB WEIGHT OF EVIDENCE CARCINOGENICITY - QBECHA April 2018.pdf [Please refer to comment No. 12]