baua:
Bundesanstalt für Arbeitsschutz
und Arbeitsmedizin
Federal Institute for Occupational
Safety and Health

SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

1,3-dihydro-4(or 5)-methyl-2H-benzimidazole-2-thione (EC No. 258-904-8)

and

1,3-dihydro-4(or 5)-methyl-2H-benzimidazole-2-thione, zinc salt (EC No. 262-872-0)

Evaluating Member State(s): Germany

Dated: 4 May 2023

Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2018

Before concluding the substance evaluation, a Decision to request further information was issued on: 15 June 2020

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B, the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

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Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

The Substances 1,3-dihydro-4(or 5)-methyl-2H-benzimidazole-2-thione (hereafter, MMBI) and 1,3-dihydro-4(or 5)-methyl-2H-benzimidazole-2-thione, zinc salt (hereafter, ZMMBI), were originally selected for substance evaluation to clarify concerns about:

- Potential endocrine disruptor
- Exposure of environment

During the evaluation no other concerns were identified.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

In 2017, ECHA performed compliance checks (CCH) and testing proposal evaluation (TPE) for both substances in parallel. A batch of four decisions was issued on 23 March 2017.[2] The following endpoints were covered:

- Under CCH:
  - Description of the analytical methods (Annex VI, Section 2.3.7) and identification and quantification of the main constituent(s)
  - *In vitro* cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2., test method: OECD TG 473) or *in vitro* micronucleus study (Annex VIII, Section 8.4.2, test method: OECD TG 487).
  -
- Under TPE:
  - Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: EU 8.31./OECD TG 414) in a first species (rat or rabbit), oral route.
  - Extended one-generation reproductive toxicity study (Annex IX, Section a.7.3.i test method: EU 8.56./OECD TG 443) in rats, oral route.

Both substances were discussed at the 11th meeting of ECHA's expert group on endocrine disruptors (ED EG) in April 2018 and as well 22nd meeting in April 2022.

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the Substances has led the evaluating Member State to the following conclusions, as summarised in Table 1.

The eMSCA will assess the need for further regulatory measures at EU level following conclusion of the CLH process.

[2] CCH decision on MMBI: https://echa.europa.eu/documents/10162/ada2a712-a49d-1d77-edc2-a009a5d7d2f4
TPE decision on MMBI: https://echa.europa.eu/documents/10162/552e48b5-7117-0f87-ace2-4967037dcd66
CCH decision on ZMMBI: https://echa.europa.eu/documents/10162/ae36bd0a-296b-89b5-651f-bc0009307cd1
TPE decision on ZMMBI: https://echa.europa.eu/documents/10162/e2e4b764-b591-75e1-d62d-d3de13764339

Table 1

CONCLUSION OF SUBSTANCE EVALUATION Conclusions	Tick box
Need for follow-up regulatory action at EU level	x
Harmonised Classification and Labelling	x
Identification as SVHC (authorisation)	(x)
Restrictions	(x)
Other EU-wide measures	
No need for regulatory follow-up action at EU level	

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

4.1.1. Harmonised Classification and Labelling

The eMSCA concludes that MMBI and its zinc salt ZMMBI meet the criteria for an endocrine disruptor (ED) for the environment according to the definition of the World Health Organisation (WHO). Additional hazard classes for ED will be introduced in the CLP regulation, making the harmonised classification as environmental ED in Annex VI CLP possible. The eMSCA considers a harmonised classification and labelling (CLH) proposal concerning the ED properties as the first step of potential further risk management measures. In this context, the existing self-classification for aquatic chronic toxicity for (Z)MMBI would also be addressed by the eMSCA for EU-wide harmonisation.

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

The eMSCA considers that the available data on the ED properties are sufficient to conclude that (Z)MMBI fulfils the WHO/IPCS definition of an endocrine disruptor in the environment. Hence, (Z)MMBI could potentially be identified as a substance of very high concern (SVHC) according to REACH Article 57(f).

The need for SVHC identification or for other regulatory options beyond the classification as ED for the environment via CLH will be scrutinized following the conclusion of the classification procedure.

4.1.3. Restriction

The eMSCA considers environmental ED properties as hazardous properties for which derivation of a safe threshold in the environment is very difficult if possible, at all. Hence, after reaching consensus on the environmental hazard properties at EU level, the eMSCA considers that a restriction might be necessary to further limit emissions of (Z)MMBI to the environment as much as possible. The further need for a restriction may be considered following the outcome of the CLH process considering the use pattern and life cycle of affected mixtures and articles.

4.1.4. Other EU-wide regulatory risk management measures

N/A.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

N/A.

5.2. Other actions

N/A.

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

Indication of a tentative plan is not a formal commitment by the evaluating Member State. A commitment to prepare a REACH Annex XV dossier (SVHC, restrictions) and/or CLP Annex VI dossier should be made via the Registry of Intentions.

Table 2

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
CLH	t.b.d.	eMSCA
Potential further RMM (SVHC identification and/or restriction) based on outcome of CLH	t.b.d.	eMSCA

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

The Substances MMBI and ZMMBI were originally selected for substance evaluation in order to clarify concerns about:

- Potential endocrine disruptor
- Exposure of environment

During the evaluation no other concerns were identified.

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Endocrine disrupting properties in the environment	The data obtained from the requested AMA study show clear anti-thyroidal activity of MMBI plausibly leading to the observed adverse effects on thyroid histology and development. Available *in vitro* and *in vivo* studies from close structural analogues indicate that MMBI and ZMMBI interact with the HPT axis in fish. According to the ECHA/EFSA Guidance these effects can be considered as population relevant. Hence, it is concluded that MMBI and ZMMBI fulfil the WHO/IPCS definition for endocrine disruptors for the environment. **Concern confirmed.**
Aquatic toxicity	The data available for (Z)MMBI warrant a (harmonised) classification for Aquatic chronic 1 (as also done in the registration dossiers).
Wide dispersive use and exposure of the environment	A release into the environment at the production site cannot be excluded entirely based on available information. A release to the environment is likely by widespread use of materials and articles. **Concern confirmed.**

7.2. Procedure

The registration data was evaluated based on the Chemical Safety Report (CSR) provided by the lead registrant and the aggregated registration dossier. The evaluation covered all environmental endpoints (physico-chemical data, ecotoxicity data, exposure, fate, and behaviour).

The eMSCA conducted a comprehensive and structured literature research to gather information on the ecotoxicity to different organism groups, endocrine mode of actions, endocrine effects, exposure, fate, and monitoring results of MMBI and ZMMBI using defined keywords and synonyms.

The concern for endocrine disrupting properties for the environment was evaluated considering the available *in vitro* and *in vivo* data.

ECHA's Endocrine Disruptors Expert Group was consulted in April 2018. The received comments have been considered.

A substance evaluation decision was sent to the registrants with the information request for an Amphibian Metamorphosis Assay (AMA) according to OECD TG 231 with MMBI.

The full study report for the requested study was provided in December 2021 by the registrants and the registration dossier was updated subsequently.

The ED EG was again consulted in April 2022 to present the newly generated information from the AMA at the 22nd ED EG Meeting before the eMSCA formally concluded the evaluation.

7.3. Identity of the substance

Table 4

SUBSTANCE IDENTITY (MMBI)	
Public name:	1,3-dihydro-4(or 5)-methyl-2H-benzimidazole-2-thione
EC number:	258-904-8
CAS number:	53988-10-6
Index number in Annex VI of the CLP Regulation:	N/A
Molecular formula:	$C_8H_8N_2S$
Molecular weight range:	164 g/mol
Synonyms:	MMBI, Vulkanox MB 2

Type of substance ☐ Mono-constituent ☒ Multi-constituent ☐ UVCB

Structural formula:

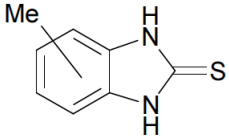
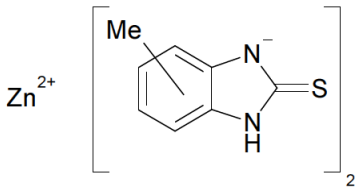


Table 5

SUBSTANCE IDENTITY (ZMMBI)	
Public name:	1,3-dihydro-4(or 5)-methyl-2H-benzimidazole-2-thione, zinc salt (2:1)
EC number:	262-872-0
CAS number:	61617-00-3
Index number in Annex VI of the CLP Regulation:	N/A
Molecular formula:	$C_{16}H_{14}N_4S_2$ Zn; $C_8H_7N_2S$ * ½ Zn
Molecular weight range:	391 g/mol; 195.5 g/mol
Synonyms:	ZMMBI, Vulkanox ZMB 2

Type of substance ☐ Mono-constituent ☒ Multi-constituent ☐ UVCB

Structural formula:



7.4. Physico-chemical properties

Table 6

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES (MMBI)	
Property	**Value**
Physical state at 20°C and 101.3 kPa	*oil-coated granules, microgranules, white to beige/yellowish, odourless, inspected visually*
Vapour pressure	*QSAR prediction; EPI Suite v4.1 – model MPBPWIN; 0.00181 Pa at 25 °C*
Water solubility	*0.12 g/L at 20 °C, pH 5.7*
Partition coefficient n-octanol/water (Log P_{ow})	*HPLC method, log P_{ow} 0.3 fraction 1; log P_{ow} 0.4 fraction 2 at 25 °C pH 5.8,*
Flammability	*non-flammable solid, no pyrophoric properties, does not emit flammable gases in contact with water*
Granulometry	*All particles with a mean diameter less than 50 µm have a mass fraction of 0.37 %. 0.024 % / 0.027 % (spherical/ cubical) particles of this mass fraction have a mean diameter less than 4 µm.*
Dissociation constant	*QSAR prediction ACD 7.0; at the environmentally relevant pH, i.e., 5-9, the substance will be present in protonated form.*

Table 7

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES (ZMMBI)	
Property	**Value**
Physical state at 20°C and 101.3 kPa	*visual inspection; powder, off white, odourless*
Vapour pressure	*EPIWIN MPBPVP v1.43 model, 1.87E-011 Pa at 25 ºC*
Water solubility	*32 mg/L at 20°C pH ca 5.9-7.0*
Partition coefficient n-octanol/water (Log P_{ow})	*log P_{ow} 3.07 at 20.5°C pH ca. 6.3-6.5*
Flammability	*non-flammable solid, no pyrophoric properties, not emit flammable gases in contact with water*
Granulometry	*D_{50} 96.44 µm*

7.5. Manufacture and uses

7.5.1. Quantities

Both MMBI and ZMMBI are registered at the tonnage band 100 to 1000 tpa.

Table 8

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☒ 10 – 100 t	☒ 100 – 1000 t	☒ 1000- 10,000 t	☒ 10,000-50,000 t
☒ 50,000 – 100,000 t	☒ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☒ > 1000,000 t	☐ Confidential

7.5.2. Overview of uses

MMBI and ZMMBI are used for the industrial production of technical plastic and rubber goods. The substances are included into a matrix, serve as reactive processing aids, and are used as process regulators for polymerisation processes in production of resins, rubbers, polymers. The substances are also used as antioxidant and anti-aging additives in diverse rubber and plastic goods. The articles and materials made with MMBI and ZMMBI are mainly articles with long-life outdoor use. They are used in articles, in formulation or re-packing, at industrial sites and in manufacturing. Both substances are manufactured in the EU, used in the EU, and exported from the EU. Articles and materials made with MMBI and ZMMBI may be reimported into the EU.

Table 9

USES (MMBI)	Use(s)
Uses as intermediate	N/A
Formulation	Formulation of preparations: Stabilizers
Uses at industrial sites	Production of tyres, rubber, and plastic goods: process regulator for polymerisation processes
Uses by professional workers	N/A
Consumer Uses	N/A
Article service life	Production and use of tyres, rubber, and plastic goods: Stabilizers

Table 10

USES (ZMMBI)	Use(s)
Uses as intermediate	N/A
Formulation	Formulation of preparations: Stabilizers
Uses at industrial sites	Production of tyres, rubber, and plastic goods: process regulator for polymerisation processes
Uses by professional workers	Professional handling of rubber and plastic goods

Consumer Uses	Consumer handling of rubber and plastic goods
Article service life	Production and use of tyres, rubber, and plastic goods: Stabilizers

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

Neither MMBI nor ZMMBI are listed in Annex VI CLP. No CLH proposal has been submitted for either substance.

7.6.2. Self-classification

For MMBI:

- In the registration(s):

Acute Tox. 4	H302
Skin Sens. 1B	H317
Acute Tox. 4	H332
Repr. 1B	H360
STOT RE 2	H373 (Liver, spleen)
Aquatic Chronic 1	H410, M(chronic) = 1

- No additional hazard classes are notified among the aggregated self-classifications in the C&L Inventory.

For ZMMBI:

- In the registration(s):

Acute Tox. 4	H302
Skin Sens. 1B	H317
Acute Tox. 4	H332
Repr. 1B	H360
STOT RE 2	H373 (Liver, spleen)
Aquatic Chronic 1	H410, M(chronic) = 1

- No additional hazard classes are notified among the aggregated self-classifications in the C&L Inventory.

7.7. Environmental fate properties

In the tests with MMBI for ready biodegradation as well as tests with adapted inoculum, no signs for biodegradation were observed.

ZMMBI dissociates to MMBI and zinc ions within seconds when ZMMBI is introduced to an aqueous environment.

A study according to OECD TG 309 "Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test" was conducted using ZMMBI. ^{14}C-labelled test item [phenyl-U-14C] zinc-4- and 5-methyl-2-mercaptobenzimidazole under aerobic conditions at 12°C was used in the dark. The DT_{50} value of ZMMBI at 12 °C is 12.9 days (low concentration) and 24.6 days (high concentration).

7.7.1. Degradation

Table 11

Summary of hydrolysis and screening tests on ready biodegradability		
Name	**Abiotic degradation**	**Biodegradation**
MMBI	hydrolytically stable	OECD 301 B: 0 % (O_2-consumption) after 28 d No simulation test available
ZMMBI	hydrolytically stable	OECD 301 C: 27 % (CO2-evolution) after 28 d OECD TG 309: aerobic conditions at 12°C in the dark DT_{50} (ZMMBI) is 12.9 days (low concentration) and 24.6 days (high concentration) (Registration dossier, 2019)

7.7.2. Environmental distribution

An OECD TG 121 study was performed for MMBI, and an adsorption coefficient was determined: $logK_{oc}$ = 1.9 or K_{oc} = 79 L/kg.

7.7.3. Bioaccumulation

Based on the low octanol/water partition coefficient ($logP_{ow}$ = 0.3-0.4) of MMBI and the dissociation of ZMMBI to MMBI and zinc ions in aqueous solution, bioaccumulation testing in fish has been waived for both substances by the registrants.

Based on the physico-chemical properties, the eMSCA considers it unlikely that MMBI or ZMMBI fulfil the criterion for bioaccumulation according to REACH Annex XIII.

7.8. Environmental hazard assessment

The following chapter contains ecotoxicological information on both evaluated substances (MMBI and ZMMBI).

7.8.1. Aquatic compartment (including sediment)

Summary

The most sensitive test organism from all available short-term and long-term studies with MMBI (cf. Tables 12 to 16) were aquatic invertebrates (*Daphnia magna*). No long-term toxicity test on fish is available with MMBI.

The lowest effect concentration for acute toxicity from all available short-term studies with ZMMBI was observed for aquatic invertebrates (*Daphnia magna*) (Table 15). For fish and aquatic invertebrates no long-term toxicity study is available.

7.8.1.1. Fish

Table 12

Summary of results for fish short-term toxicity				
Substance	**Method, Species**	**Effect conc. [mg/L]**	**Reference**	**Comment**
MMBI	*Danio rerio* (proposal by UBA 5/1984)	96h-LC_{50}= 37.2 (nominal)	Registration dossier	geometric mean: 22 and 63 mg/L Bayer AG (1990)
ZMMBI	*Oncorhynchus mykiss* (OECD 203)	96h-LC_{50}= 5.6 (nominal)	Registration dossier	Safepharm Lab. Ltd (2003b)

Table 13

Summary of results for fish long-term toxicity				
Substance	**Method, Species**	**Effect conc. [mg/L]**	**Reference**	**Comment**
MMBI	Data waived			
ZMMBI	QSAR (ECOSAR)	9.8 mg/L (ChV; Class Thioureas) 3.5 mg/L (ChV; Neutral organic)	Registration dossier	US EPA (2009)

7.8.1.2. Aquatic invertebrates

Table 14

Summary of results for aquatic invertebrate short-term toxicity				
Substance	**Method, Species**	**Effect conc. [mg/L]**	**Reference**	**Comment**
MMBI	*Daphnia magna* (OECD 202)	48h-EC_{50}= 1.9 (nominal)	Registration dossier	Currenta (2011b)
ZMMBI	*Daphnia magna* (OECD 202)	48h-EC_{50}= 1.4 (geo. mean)	Registration dossier	Safepharm Lab. Ltd (2003c)

Table 15

Summary of results for aquatic invertebrates' long-term toxicity				
Substance	**Method, Species**	**Effect conc. [mg/L]**	**Reference**	**Comment**
MMBI	*Daphnia magna* (OECD 211)	21d-$NOEC_{Repro}$= 0.0346 (measured) 21d-$NOEC_{Mortality}$= 0.104 (measured)	Registration dossier	Currenta (2012b)
ZMMBI	QSAR (ECOSAR)	0.08 mg/L (ChV; Class Thioureas) 2.78 mg/L (ChV; Neutral organic)	Registration dossier	US EPA (2009)

7.8.1.3. Algae and aquatic plants

Table 16

Summary of results for algae toxicity				
Substance	**Method, Species**	**Effect conc. [mg/L]**	**Reference**	**Comment**
MMBI	*Desmodesmus subspicatus* (OECD 201)	72h-E_rC_{50}= 62.2 (nominal) 72h-E_rC_{10}= 36.1 (nominal) 72h-NOE_rC= 25 (nominal)	Registration dossier	Currenta (2011c)
ZMMBI	*Desmodesmus subspicatus* (OECD 201)	72h-E_rC_{50}= 10 (nominal) 72h-NOE_rC= 0.69 (nominal)	Registration dossier	Bayer AG (1992b)

7.8.1.4. Sediment organisms

No data available.

7.8.1.5. Other aquatic organisms

No data available.

7.8.2. Terrestrial compartment

No data available (waived).

7.8.3. Microbiological activity in sewage treatment systems

Table 17

Summary of results for toxicity to microorganisms				
Substance	**Method, Species**	**Effect conc. [mg/L]**	**Reference**	**Comment**
MMBI	*Activated sludge of a predominantly domestic sewage* (OECD 209)	3h-E_rC_{50} > 10000 (nominal; respiration rate)	Registration dossier	(1989)
ZMMBI	*No data available (read across to MMBI)*			

7.8.4. PNEC derivation and other hazard conclusions

Not relevant here.

7.8.5. Conclusions for classification and labelling

As MMBI is not readily biodegradable and the lowest reliable NOEC is below 0.1 mg/L (21d-$NOEC_{reproduction}$ of 0.0346 mg/L for *Daphnia magna*), a classification as Aquatic Chronic 1 would be necessary. This corresponds to the self-classification applied in the registrations of both MMBI and ZMMBI.

7.9. Human Health hazard assessment

Not part of the substance evaluation.

7.10. Assessment of endocrine disrupting (ED) properties

The scope of the ED assessment performed under substance evaluation by the eMSCA was limited to the environment.

***In vitro* tests on endocrine activity**

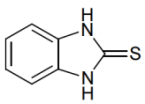
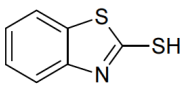
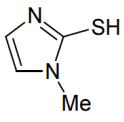
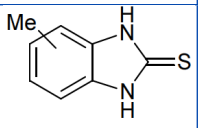
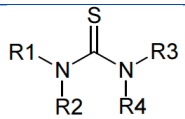
Sakemi et al. (2002) studied the inhibition of lactoperoxidase (LPX) by thioureylenes and their desulfurized metabolites (see Table 18) testing, among others, MBI (CAS 583-39-1), MMBI mix (CAS 53988-10-6; 1:1 mix of 4-methyl and 5-methyl isomers), as well as 5-MMBI (CAS 27231-36-3), 4-MMBI (CAS 27231-33-0) and 5-MeBI (CAS 614-97-1). Methimazole (MMI) was used as positive control. Structures and identifiers for these structurally similar substances are listed in Table 18. LPX is a known proxy for the enzyme thyroid peroxidase (TPO) and hence an inhibition of LPX can be taken as in vitro evidence for the interaction of the test substance with the hypothalamus-pituitary-thyroid (HPT) axis in vertebrates.

In the study of Sakemi et al. (2002) excess hydrogen peroxide was added to a mixture of LPX, test chemicals, and guaiacol in 0.1 M phosphate buffer (pH 7.0). The initial concentrations of hydrogen peroxide, LPX and guaiacol were 0.2 mM, 60 nM, and 33 mM, respectively. Test chemicals were dissolved in ethanol and added to the reaction mixtures (1% v/v). The rate of guaiacol oxidation was followed on a recording spectrophotometer (Shimadzu UV-1600PC) at 470 nm using a cuvette with a 1.0-cm light path with a total volume of 3.0 ml reaction mixture at room temperature. The enzyme activity at 30 s after initiation was compared with that of the control reaction mixture containing 1% ethanol. The IC_{50} was calculated from the plots of percentage enzyme inhibition rate versus inhibitor concentrations using the linear part of the concentration ranges near 50% inhibition. The measured IC_{50} values of MBI, 4-MMBI, and 5-MMBI were 20.6 µM, 45.6 µM and 31.6 µM, respectively (Table 188).

Table 188

IC_{50} values for inhibition of LPX (proxy for TPO) by thioureylenes in rats (Sakemi et al., 2002)	
Compounds	**IC_{50} (µM)**
MMI (Methimazole), positive control	11.9
MBI (2-mercaptobenzimidazole)	20.6
MMBI mix (mixture of methyl isomers of MBI)	43.7
MMBI mix (1:1 w/w mixture of 4-MMBI and 5-MMBI)	42.1
4-MMBI (2-mercapto-4-methylbenzimidazole)	45.6
5-MMBI (2-mercapto-5-methylbenzimidazole)	31.6
BI (benzimidazole)	> 4000
5-MeBI (5-methylbenzimidazole)	> 4000
Thiourea	> 4000

Table 199

Similar substances used in *in vitro* tests					
	MBI	**MBT**	**MMI**	**MMBI**	**Thiourea**
CAS	583-39-1	149-30-4	60-56-0	53988-10-6	General structure
Structure					

Conclusions for *in vitro* tests

In vitro data show that on the molecular level 4-MMBI and 5-MMBI have significant inhibitory effects on LPX and hence TPO. Their determined IC_{50} values are in the same order of magnitude as the ones of MBI and the known TPO-inhibiting antithyroidal drug MMI (Cooper 2005).

7.10.1. Endocrine disruption – Environment

An Amphibian Metamorphosis Assay with the test species *Xenopus laevis* was conducted with MMBI according to OECD TG 231 fulfilling the information requirement from the substance evaluation decision (Registration dossier, 2021).

The nominal concentrations used (0.0048, 0.024, 0.12, 0.60, 3.0 and 15 mg/L) were verified analytically (HPLC, GC- MS; LOQ = 0.0045 mg/L) (mean measured concentrations: 0.0052, 0.027, 0.15, 0.72, 3.3 and 16 mg/L). The test was conducted under flow-through conditions with a 12h photoperiod (600 to 2000 lux) at 22 ± 1°C. In the test system 20 tadpoles per vessel and 4 vessels per control/ concentration (replicates) were exposed to MMBI up to NF stage 51 (21 days). The endpoints measured were the hind limb length (HLL), snout to vent length (SVL), developmental stage, wet weight, and thyroid histology.

In thyroid histopathology, thyroid hypertrophy was observed starting at 0.12 mg/L, follicular cell hypertrophy starting at 0.60 mg/L as well as dilatation of follicles. Follicular cell hyperplasia (starting at 0.12 mg/L) was observed up to a severe degree at doses of 3.0 and 15 mg/L. Apical endpoints affected were: wet weight (significantly reduced at 15 mg/L at day 7 and 21), snout-vent length (SVL) (significantly reduced at 15 mg/L at day 7, significantly increased at 3.0 mg/L and reduced at 15 mg/L at day 21), hind limb length (HLL) (significantly reduced at 3.0 mg/L at day 7 and 21). The reduced HLL (normalised by SVL) and a significantly reduced NF-stage points towards TPO-inhibition (as described in AOP 175; https://aopwiki.org/aops/175). No signs for asynchronous development were observed.

For MMBI and ZMMBI, there are no studies available investigating the effect in fish.

However, there are some studies available investigating the effects of the structurally similar thiourylene compounds MBT (2-mercaptobenzothiazole; CAS 149-30-4) and MMI on the development of the swim bladder of fathead minnow and zebrafish. In zebrafish, the posterior swim bladder development starts at 3 dpf and completes to develop by 5 dpf, while the anterior swim bladder completes the development by 21 dpf. The impaired posterior swim bladder inflation may result in reduced swimming capacity, an adverse outcome that can affect feeding behaviour and predator avoidance, ultimately resulting in lower survival probability and population trajectory decline (Czesny et al., 2005; Woolley and Qin, 2010).

(Nelson et al., 2016; Stinckens et al., 2016) examined the effects of disruption of thyroid hormone (TH) synthesis on swim bladder inflation during fathead minnow and zebrafish early-life stages using 2-mercaptobenzothiazole (MBT; CAS 149-30-4). The fishes were exposed continuously from just hours post fertilisation (hpf) through 21 dpf. Three concentrations of MBT (0.25, 0.5, 1 mg/L), as well as a control were tested in four replicates per treatment. No solvent was used. The concentrations were determined at least every 4 days using HPLC. Other conditions: 25 ± 1°C; 16 hours light per day, flow-through. On days 6, 14, and 21, larval fish were sampled from each tank (n = 20, 14, and 10, respectively). Fish were anesthetized with MS-222 (100 mg/L buffered with 200 mg $NaHCO_3$/L), observed for general health and swim bladder inflation, and photographed. Individuals were split into two even groups, one for RNA isolation and the other for whole-body T_3/T_4 analysis. On days 14 and 21 fish allocated for RNA isolation were sectioned to allow the subsequent RNA sample to be more specific to the thyroid (located in headsection) or swim bladder (located in abdomen section). A subset of larval fish from the control and each MBT treatment (n = 8 per treatment) were sampled on days 14 and

21 for histopathological assessment of the thyroid follicles. At 1 mg/L nearly half of the exposed fish did not develop an anterior swim bladder and those that did, had significantly smaller anterior swim bladders than controls.

Investigations of the effects of the TPO inhibitor MMI (CAS 60-56-0) on the swim bladder development of zebrafish embryos have been conducted (Godfrey et al., 2017). AB wild type zebrafish embryos (4.5 hpf) were randomly placed into petri dishes containing 25 mL of test solution (semi-static renewal). Each petri dish contained 20 embryos and a minimum of three replicates per treatment were used with experiments repeated three times. The zebrafish embryos were exposed for 6 days (0 – 6 dpf) or for 28 days (0 – 28 dpf). For the chronic exposure, larvae were moved after 6 days to a 500 mL glass mason jar containing 200 mL solution. Test temperature was 28 ± 1 °C and photoperiod was 14 hours light per day. After 6 days exposure, close to 60% of the fish exposed to 30 nM MMI did not develop an anterior swim bladder. *TPO* and *sp-a* expression levels were down-regulated in these fish. After 28 days exposure, MMI caused a significant inhibition of larval length compared to controls.

The impaired posterior swim bladder inflation in fish has further been investigated by using *in chemico* enzyme inhibition assays to measure the molecular initiating events for an array of 51 chemicals (Stinckens et al., 2018). Zebrafish embryos were then exposed to 14 compounds and the effects on posterior swim bladder inflation were evaluated. Among others they used MMI, MBI and MBT (CAS 149-30-4). The zebrafish embryo experiments based on OECD TG 236 are directly observing possible effects on posterior swim bladder chamber inflation. Zebrafish embryos were exposed until 120 and/or 168 hpf to at least six concentrations of the test compounds compared to control and solvent control conditions. The study authors found that MBT and MMI showed weaker or no DIO inhibition. According to Stinckens et al., it seems that MMI does not directly affect DIO activity in fish, but causes hypothyroidism due to TPO inhibition, possibly activating a compensation mechanism at the level of DIO1 and DIO2 activity to increase the conversion of T_4 to T_3.

In addition to the *in vitro* data of MMBI and MBI and the *in vivo* data of MMBI with amphibia, there are two studies with fish conducted with MBT and two with MMI showing a clear anti-thyroidal effect (delayed development of the swim bladder and T_4/T_3 level reduction in the blood).

Conclusions for *in vivo* tests

The eMSCA considers the effects on thyroid histology and metamorphosis observed in the AMA test as adverse and population relevant as they were seen together with effects on development (in line with the ECHA/EFSA guidance on the identification of ED) (EFSA 2018).

7.10.2. Endocrine disruption - Human health

The evaluation was centered on the assessment of available data on amphibians and fish (see above). The eMSCA did not consider mammalian data to conclude on the concern for endocrine disruption in the environment.

7.10.3. Conclusion on endocrine disrupting properties and classification and labelling

In vitro data for MMBI reveal an inhibition of LPX activity (proxy for TPO activity) in the same order of magnitude as it can be observed for the structurally similar antithyroidal compounds MBI and MMI.

There are two studies with fish conducted with the structurally similar substances MBT and MMI showing also a clear anti-thyroidal effect (delayed development of the swim bladder and T_4/T_3 level reduction in the blood). For MMBI, there are no studies available investigating the effect in fish.

The Amphibian Metamorphosis Assay (AMA) (OECD TG 231) conducted with MMBI provides clear evidence for a thyroid antagonistic activity (with effects on metamorphosis and thyroid histopathology) of MMBI. This evidence fits to the available *in vitro* data for MMBI (TPO inhibition), and to the effects observed in fish for structural analogues.

MMBI and ZMMBI have adverse and population-relevant effects in the environment, which can be plausibly linked to an endocrine MoA.

The eMSCA considers that the available data on the ED properties are sufficient to conclude that MMBI and ZMMBI fulfil the WHO/IPCS definition of endocrine disruptors in the environment.

As above described, there is animal data providing evidence that the substances show endocrine activity and an adverse effect in an intact organism (or its offspring) with a biologically plausible link, the eMSCA considers that the criteria for classifying (Z)MMBI as endocrine disruptor category 1 are fulfilled.

Additionally, both substances fulfil the the criteria for classification as Aquatic Chronic 1.

7.11. PBT and vPvB assessment

Not part of the evaluation.

7.12. Exposure assessment

7.12.1. Human health

Not part of the evaluation.

7.12.2. Environment

MMBI as well as ZMMBI are manufactured in the European Economic Area in 100 - 1000 tonnes per year. About the half of the manufactured substance is exported outside the European Economic Area.

According to the manufacturer, there is no release of MMBI as well as ZMMBI into the environment during manufacturing and formulation. Monitoring data are available from a wastewater treatment plant at the production site. In a very low number of samples MMBI and ZMMBI was detected. However, the limit of detection was relatively high (5ppm). Therefore, an environmental exposure cannot be excluded at the production site.

During use, MMBI and ZMMBI are incorporated into rubber and plastic matrices as well as used as an antioxidant additive. These rubber and plastic goods are long-life materials with low to high release rates. The substances may be found in complex articles with no release intended. It is likely that articles containing MMBI and ZMMBI are reimported into the European Economic Area. Therefore, a release of MMBI and ZMMBI to the environment is likely by widespread use of articles.

7.12.3. Combined exposure assessment

Not part of the evaluation.

7.13. Risk characterisation

Not part of the evaluation.

7.14. References

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7.15. Abbreviations

5-MeBI	5-methylbenzimidazole
4-MMBI	2-mercapto-4-methylbenzimidazole
5-MMBI	2-mercapto-5-methylbenzimidazole
AMA	Amphibian metamorphosis assay
AOP	Adverse Outcome Pathway
CCH	Compliance check
CoRAP	Community rolling action plan
DIO	Deiodinase
Dpf	Days post fertilisation
EC_{50}	Half maximal effect concentration
ED	Endocrine disruptor
eMSCA	Evaluating Member State Competent Authority
GC-MS	Gas chromatography–mass spectrometry
HLL	Hind limb length
Hpf	hours post fertilisation
HPLC	High performance liquid chromatography
HPT	Hypothalamus-pituitary-thyroid
IC_{50}	Half maximal inhibitory concentration
LAGDA	Larval amphibian growth and development assay
LOQ	Limit of quantification
LPX	Lactoperoxidase
LOEC	Lowest observed effect concentration
MBI	2-mercaptobenzimidazole
MBP	Myelin basic protein
MBT	2-mercaptobenzothiazole
MMI	Methimazole
MoA	Mode of action
MSCA	Member State Competent Authority
OECD	Organisation for Economic Co-operation and Development
RMOA	Risk management option analysis
SEv	Substance evaluation
sp-a	surfactant protein A
SVHC	Substances of Very High Concern
SVL	snout-vent length
T	Testosterone
T_3	Triiodothyronine
T_4	Thyroxin
TG	Test guideline
TH	Thyroid hormone
TPE	Testing proposal evaluation
TPO	Thyroid peroxidase
vPvB	Very persistent, very bioaccumulative
ZMMBI	1,3-dihydro-4(or 5)-methyl-2H-benzimidazole-2-thione, zinc salt (2:1)