SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

2-ethylhexyl (2*E*)-3-(4-methoxyphenyl)acrylate

EC No 629-661-9

CAS RN –

(formerly identified as 2-ethylhexyl 4-methoxycinnamate, EC No 226-775-7, CAS RN 5466-77-3 and 2-ethylhexyl trans-4-methoxycinnamate, EC 629-661-9, CAS RN -)

Evaluating Member State(s): Germany (following initial assessment by the UK)

Dated: October 2023

Evaluating Member State Competent Authority

Conclusion of the substance evaluation process – January 2020 to 2022

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Initial evaluation and decision making – March 2016 to December 2019

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Year of evaluation in CoRAP: 2016

Before concluding the substance evaluation a Decision to request further information was issued on: 21 March 2017.

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

2-ethylhexyl (2*E*)-3-(4-methoxyphenyl)acrylate (OMC, "the Substance") was originally selected for substance evaluation in order to clarify concerns about:

- Suspected PBT
- Suspected endocrine disruptor (ED)
- Wide dispersive use
- Consumer use
- Aggregated tonnage

During the evaluation no other concerns were identified. The DE CA did not identify additional concerns during the follow-up assessment.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

OMC is listed in entry 12 on Annex VI of the Cosmetics Regulation (EC) No 1223/2009 (List of UV filters allowed in cosmetic products).[2] The entry specifies that the substance may be used as a UV filter in cosmetic products in a concentration up to 10%.

The European Chemical Agency (ECHA) issued a compliance check (CCH) decision on 10 October 2013 requesting information on the identity of the Substance, which led to a change in the substance identifiers (substance previously registered EC No. 226-775-7). On 7 September 2022, ECHA issued a testing proposal evaluation (TPE) decision requesting a long term toxicity testing on sediment organisms with the Substance by the deadline of 13 March 2024.

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in the table below.

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)	
Restrictions	

[2] Entry on OMC in the Cosmetics Ingredients Database of the European Commission: https://ec.europa.eu/growth/tools-databases/cosing/details/28816

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

Available self-classifications of OMC do not fit to the data available and provided in the SEv report. Therefore, a new entry in CLP Annex VI would be necessary. The eMSCA considers classification as Aquatic Acute 1 (M-factor 10) and Aquatic Chronic 1 (M-factor 1) as necessary for the Substance and intends to submit a proposal for harmonised classification.

With regard to classification of OMC according to the new ED hazard classes, the eMSCA considers that the overall picture of the available data does not allow for classification at the moment. Even though the Substance shows endocrine mediated activity in fish and mammals, endocrine mediated adversity cannot clearly be concluded based on the available data. However, a re-evaluation of classification needs with regard to the ED hazard will be performed after finishing the ongoing substance evaluation of the structural analogue substance, IPMC (EC No 275-702-5; CAS No 71617-10-2).

The Substance does not fulfil the criteria for PBT or vPvB according to Annex XII REACH and hence no classification regarding these hazard classes is forseen.

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

N/A

4.1.3. Restriction

N/A

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 dossier for classification for aquatic chronic toxicity	2023	DE CA

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

2-ethylhexyl (2*E*)-3-(4-methoxyphenyl)acrylate (OMC, "the substance") was originally selected for substance evaluation in order to clarify concerns about:

- Suspected PBT
- Suspected endocrine disruptor (ED)
- Wide dispersive use
- Consumer use
- Aggregated tonnage

During the evaluation no other concerns were identified by the UK CA. The DE CA did not identify additional concerns during the follow-up assessment.

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Endocrine disruption for the environment	Concern unresolved The overall data is insufficient to conclude on ED ENV properties of OMC. However, based on considerations of proportionality, further data have not been requested by the eMSCA to clarify the remaining concern. Data requested and subsequently provided in the substance evaluation of the structural analogue substance IPMC, may contribute to the clarification of the concern about the ED properties of OMC.
PBT properties	Concern removed. The substance does not meet the criteria for PBT or vPvB based on available information.
Persistence	Concern removed OMC is readily biodegradable meeting the 10-day window in a fully valid test. This result is supported by a further well described supporting study in the registrations. The eMSCA does not regard OMC as meeting the P criterion according to REACH Annex XIII.
Bioaccumulation	Concern removed Available information points towards a BCF value clearly below the cut-off value of 2000 for the B criterion according to REACH Annex XIII
Toxicity	Concern confirmed OMC fulfils the T criterion according to REACH Annex XIII based on available information from ecotoxicological studies.
Environmental exposure (wide dispersive use, consumer use, aggregated tonnage)	Concern confirmed. The high aggregated tonnage of OMC and its sole use as an UV filter in cosmetics products for consumers contributes to wide-spread exposure of the environment. Concern confirmed.

7.2. Procedure

CoRAP inclusion and initial substance evaluation by the UK CA:

An Endocrine Disruption screening tool suggests the substance may be an endocrine disruptor. Specific endocrine disruption tests are also available. Given the substance is used as a UV filter in cosmetics and personal care products there is potential for significant exposure and this is investigated further.

A previous Environment Agency scoping assessment of UV Filters for the environment (Brooke et al, 2008) identified the substance as a high priority for further work to investigate potential risks from production, formulation and use of the substance.

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to endocrine disruption, PBT, vPvB properties and wide dispersive / consumer use, OMC was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2016. The updated CoRAP was published on the ECHA website on 22 March 2016. The Competent Authority of the United Kingdom (hereafter called the evaluating MSCA) was appointed to carry out the evaluation.

The initial assessment was initiated on 22 March 2016.

Decision making Process

The UK CA submitted a draft decision with further information requirements on OMC to ECHA. Following registrant's comments and proposals for amendments by other MSCAs and unanimous agreement of the Member State Committee on the amended information requirements, ECHA took the decision and sent it to the registrant on 6 April 2018.[3] A decision on the structurally related substance IPMC (Isopentyl p-methoxycinnamate, EC 275-702-5, CAS 83834-59-7) was taken in parallel.[4]

Hand-over to the DE CA and follow-up assessment:

Following the withdrawal of the United Kingdom from the European Union at the end of 2019, Germany was appointed as the evaluating Member State and the assessment report of the UK CA was handed over to the DE CA.

On 13 July 2021 the registrant provided all the information requested in the decision in a dossier update. The DE CA assessed the new information and concluded the evaluation without requesting further information.

The eMSCA presented the results of the requested studies and available data on OMC to ECHA's Expert Group on Endocrine Disruptors (EDEG) at the 17th and 19th meeting in October 2020 and April 2021, respectively.

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[3] Decision on Substance Evaluation for OMC: https://echa.europa.eu/documents/10162/88e0cc0b-d64c-1b9e-d706-61c0cce731da

[4] Decision on Substance Evaluation for IPMC: https://echa.europa.eu/documents/10162/e1ccd91b-b0ae-e1d2-0c02-0f34ead965f9

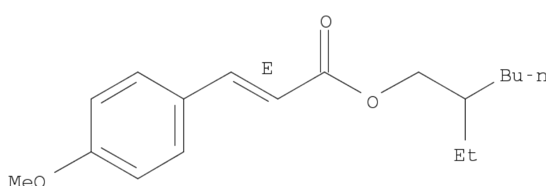
7.3. Identity of the substance

Table 4

SUBSTANCE IDENTITY	
Public name:	2-ethylhexyl (2*E*)-3-(4-methoxyphenyl)acrylate
EC number:	629-661-9* *Substance originally registered under EC 226-775-7, following compliance check by ECHA the identity was clarified and a new index number allocated.
CAS number:	83834-59-7
Index number in Annex VI of the CLP Regulation:	N/A
Molecular formula:	$C_{18}H_{26}O_3$
Molecular weight range:	290.397
Synonyms:	2-Propenoicacid,3-(4-methoxyphenyl)-,2-ethylhexylester,(2E)-; 2-ethylhexyl-4-methoxycinnamate; 2-ethylhexyl trans-4-methoxycinnamate; 2-ethylhexyl 3-(4-methoxyphenyl)acrylate; OMC. The term “OMC” is used in the report.

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

Structural formula:



Grouping

OMC has been evaluated along with a structurally similar cinnamate, isopentyl p-methoxycinnamate (IPMC). Table 5 shows information on IPMC from the ECHA dissemination website.

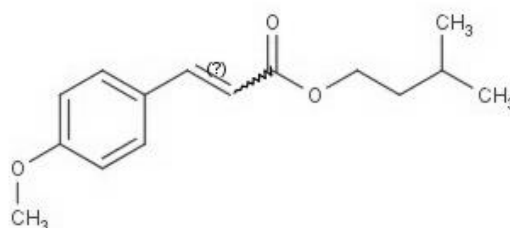
Table 5

SUBSTANCE IDENTITY	
Public name:	Isopentyl p-methoxycinnamate
EC number:	275-702-5
CAS number:	71617-10-2

Index number in Annex VI of the CLP Regulation:	N/A
Molecular formula:	$C_{15}H_{20}O_3$
Molecular weight range:	248.32
Synonyms:	Neo Heliopan® E1000; 3-methylbutyl 3-(4-methoxyphenyl)acrylate; 2-Propenoic acid, 3-(4-methoxyphenyl)-, 3-methylbutyl ester; amiloxate, IPMC.

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

Structural formula:



7.4. Physico-chemical properties

Table 6 lists the physicochemical properties of OMC according to the ECHA dissemination database.

Table 6

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20 °C and 101.3 kPa	According to the product data sheet provided by the producer OMC is a pale yellow liquid.
Melting point/freezing point	-68.3 °C, which is equal to 205.0 K. The estimated accuracy is ± 0.5 K.
Boiling point	382 °C at 1 013 hPa
Density	1.01 at 20 °C
Vapour pressure	0.3 hPa at 154 °C (30 Pa). This is 0.0675 Pa at 25 °C
Water solubility	The water solubility of OMC was investigated according to OECD Guideline 105 and was found to be 0.22 - 0.75 mg/L at 21 °C using the flask method. It is considered to be slightly soluble (0.1-100 mg/L).A new water solubility test was provided according to OECD TG 105. The result was 0.051 mg/L at 20°C and pH of 6.7 using the column elution method.
Partition coefficient n-octanol/water (Log Kow)	>6 at 23 °C (by HPLC) 5.8 (predicted by KOWWIN v1.68)
Surface tension	Waived based on Water solubility <1 mg/L @ 21 °C
Flash point	204 °C

Auto flammability	392 °C at 977 mbar
Viscosity	99.80 mPa·s at 20 °C and 31.60 mPa·s at 40 °C.
Granulometry	n/a, the substance is a liquid.
Flammability	Flammability is deduced from flash point and boiling point; based on chemical structure pyrophoric properties and water reactivity are not to be expected.
Explosiveness	The substance does not contain chemical groups indicating explosive properties.
Oxidising properties	n/a, the test substance molecule contains oxygen which is chemically bonded only to carbon and hydrogen.
Stability in organic solvents and identity of relevant degradation products	The substance is not considered to be unstable or critical in solvents.
Dissociation constant	The substance is not considered to be unstable or critical in solvents.

7.5. Manufacture and uses

7.5.1. Quantities

The tonnage range given on the ECHA dissemination web site (accessed June 2022) is given in Table 7.

Table 7

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

The substance is a UV filter. It is used in the manufacture of cosmetics and personal care products. It is used “as such” in these products, e.g. as a UV filter in sun creams. Therefore, exposure to the environment via this use is anticipated.

Table 8

USES	Use(s)
Manufacture	Manufacturing of the substance
Formulation and repackaging	Cosmetics and personal care products, laboratory chemicals, perfumes and fragrances, pharmaceuticals and photo-chemicals.
Uses at industrial sites	Cosmetics and personal care products, laboratory chemicals, perfumes and fragrances, pharmaceuticals, photo-chemicals and washing & cleaning products

Uses by professional workers	Polishes and waxes, washing & cleaning products, cosmetics and personal care products, laboratory chemicals, perfumes and fragrances, pharmaceuticals and photo-chemicals.
Consumer Uses	Cosmetics and personal care products, perfumes and fragrances, air care products, biocides (e.g. disinfectants, pest control products), polishes and waxes and washing & cleaning products.
Article service life	Paper articles · Plastic articles · Other articles ·

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

OMC does not have a harmonised classification listed in Annex VI of CLP Regulation (Regulation (EC) 1272/2008).

7.6.2. Self-classification

- In the registration(s):

Aquatic Chronic 2 – H411.

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory:

Skin Irrit. 2 – H315
Eye Irrit. 2 – H319
STOT SE 3 – H335
Not classified

7.7. Environmental fate properties

7.7.1. Degradation

7.7.1.1. Photodegradation

A GLP study assessing the photodegradation of OMC in water is provided in the registration dossier. This was performed to an EPA test guideline[5] using radio-labelled (phenyl ring) test substance in a solution of 20% acetonitrile and 80% pH 7 buffer (acetonitrile was used to prevent hydrolysis occurring). Solutions were irradiated with a xenon lamp (290-800 nm) for 23 days, which is indicated to be equivalent to 21 days sunlight (this appears to be corrected to 40°N) and a temperature of 23.2 °C. DT_{50} between 5 and 9 days were determined based on degradation from trans and cis-2-Ethylhexyl 4-methoxycinnamate to transformation products.

7.7.1.2. Hydrolysis

The pH-dependent hydrolysis of the Substance was determined using a SETAC protocol under GLP in a study similar to OECD TG 111 in 2002. The test was performed with radio-labelled (phenyl ring) test substance (known purity) at 20 °C for 30 days using pH 4, 7 and 9 under exclusion of light and oxygen. The test substance concentration was 0.111 mg/L. There was variability in the concentration of OMC, the key degradant and other

[5] EPA Guideline Subdivision N 161-2 (Photodegradation Studies in Water)

degradants at the different sampling points. Generally OMC was between 70-80% at pH 4, 70-90% for pH 7 and 9, throughout the test. The registrant was unable to fit kinetics to the available data and concluded that the half-life of OMC exceeds one year at all pH at 20 °C.

The registrant considered that the the major metabolites most likely to be p-methoxycinnamic acid and ethylhexyl methoxycinnamate cis-isomer. This was based on co-chromatography with reference samples, however, identification could not be confirmed. In the view of the eMSCA the hydrolysis half-life is likely to be less than one year based on the available data. This would also tend to agree with the results of the ready biodegradation study (see below).

7.7.1.3. Biodegradation

The key ready biodegradability study in the registration dossier is a 28-day manometric respiration test (OECD TG 301F, 1994) performed according to GLP. It used domestic, non-adapted activated sludge and was conducted using 100 mg/L of test substance (known purity) and 30 mg/L (dry weight) activated sludge, in duplicate. Aniline was used as a reference substance, and the test temperature was 20 °C. 78% biodegradation was observed by day 28, and the 10 day window was met (69% biodegradation between days 6 and 16). No inhibition was indicated in the toxicity control. Blank inoculum respiration was 24 mg O_2/L on days 6 and 33 on day 28, which is within the expected levels for the test (20-30 mg/L). The registrant concludes that OMC is readily biodegradable.

Further degradation testing has been waived as a consequence based on the conclusion that the substance is readily biodegradable. The eMSCA can support this conclusion.

7.7.2. Environmental distribution

7.7.2.1. Absorption/desorption

A QSAR using KOCWIN (v2.00) is provided in the registration dossier. Two predictions are provided one using the MCI method: K_{oc} = 8620 L/kg, the other based on the (predicted) log Kow (5.8): K_{oc} = 13290 L/kg.

7.7.3. Bioaccumulation

The key study in the registration dossier is a 14-day GLP aqueous fish bioconcentration test conducted according to OECD TG 305 in *Oncorhynchus mykiss* and flow-through conditions at 15 °C using radio-labelled (phenyl ring) test substance, conducted in 2000. A control and two test concentrations of 0.07 and 0.70 mg/L were used, with ethanol used at a concentration of 0.1 ml/L to help dissolve OMC. Each treatment was run as a single replicate with 40 fish per (stainless steel) vessel. A feeding rate of 1% bw was used. Fish length and weight were measured at study initiation. No further growth measurements were made, being regarded as "irrelevant" due to the short period of the test growth according to the registration dossier. There is no information on the lipid content of the fish. A 5-day uptake and 9-day depuration period was used (NB: the RSS incorrectly states at least an 8 day uptake period ("after 1, 2, 6, 7, and 8 days of exposure and each day during elimination"). The test medium was sampled daily, including two days prior to exposure. This indicated the TWA concentrations (with s.d.) were 84±6.7 µg/L and 731±22 µg/L. Fish were sampled five times during uptake, and four times during depuration. OMC concentrations in fish increased up to 36 µg/g at the lower treatment and 128 µg/g at the upper concentration by the end of uptake. No abnormalities or mortalities were observed during the test.

BCF values were calculated at each time point and the registrant concludes that the BCF was 433 L/kg at the lower concentration and 175 L/kg at the higher concentration based on the values at the final sampling point. In the RSS steady state was indicated to be reached at 0.92 days and 2.25 days (lower and higher concentrations respectively),

although the test report just indicates that the BCF values remain constant after these time points. The DT50 (depuration) was stated to be 1.5 to 1.7 days.

While this study is regarded as Klimisch 1 by the registrants, the eMSCA considers it rather as Klimisch 3 based on deviations from the uptake period of 28 days recommended in the OECD TG 305 test guideline. Nevertheless, the data indicate that OMC is rapidly depurated in fish. Despite the remaining uncertainties, the eMSCA considers no further information on bioaccumulation necessary to clarify the PBT/vPvB properties as OMC is readily biodegradable.

7.8. Environmental hazard assessment

While the registration dossiers contained information on acute toxicity to fish and invertebrates, long-term toxicity had been waived. Following the substance evaluation decision on OMC, an FSDT (OECD TG 234) and a Daphnia Reproduction Test (OECD TG 211) were provided by the registrants in addition to a Growth Inhibition test in algae.

7.8.1. Aquatic compartment (including sediment)

Table 9

Aquatic compartment (including sediment)				
Method	**Species**	**Results [mg/L]***	**Remarks**	**Reference**
OECD TG 203 Range-finding/ limit test	*Cyprinus carpio*	96h-LC_{50} > 100 (nom.)	Klimisch 3 (Registrant: 1) Number of fish per conc. half those required by OECD TG	(NOTOX, 2000a)
OECD TG 203	*Danio rerio*	96h-LC_{50} = 1216.1 (meas.)	Klimisch 3 (Registrant: 1) Turbidity in all test conc.	(Department of Toxicology, 1998)
OECD TG 234	*Danio rerio*	63d-NOEC < 0.0469 (meas.) (gonadal histology, length + weight) 63d-NOEC ≥ 0.0469 (m) (mortality, sex ratio, number of hatch)	Klimisch 1	(Fort Environmental Laboratories, 2020a)
Non-Standard	*Danio rerio*	125d-NOEC = 0.01 (nom.)	Klimisch 2	(Zhou et al., 2019b)
Non-Standard	*Oncorhynchus mykiss*	154d-NOEC < 0.05 (nom.)	Klimisch 2	(Lee et al., 2019)
OECD TG 231	*Xenopus laevis*	21d-NOEC ≥ 0.0442 (meas.)	Klimisch 1	(Fort Environmental Laboratories, 2020b)
OECD TG 202	*Daphnia magna*	48h-EC_{50} > 0.0271 (meas.)	Klimisch 3 (Registrant: 1)	(Department of Ecology, 2003)
OECD TG 202	*Daphnia magna*	48h-EC_{50} = 0.57 (nom.) 48h-EC_{10} = 0.14 (nom.)	Klimisch 2 No analysis	(Sieratowicz et al., 2011)
OECD TG 202	*Daphnia magna*	48h-EC_{50} = 0.29 (nom.)	Klimisch 2 No analysis	(Fent et al., 2010)
Non-standard	*Siriella armata* Mysid curstacean	96h-EC_{50} = 0.199 (nom.) 96h-EC_{10} = 0.081 (nom.)	Klimisch 2	(Paredes et al., 2014)

Aquatic compartment (including sediment)				
Method	**Species**	**Results [mg/L]***	**Remarks**	**Reference**
Non-standard	*Mytilus galloprovincialis* Mussel	48h-EC_{50} = 3.118 (nom.) 48h-EC_{10} = 0.431 (nom.)	Klimisch 2	(Paredes et al., 2014)
Non-standard	*Paracentrotus lividus* Sea urchin	48h-EC_{50} = 0.284 (nom.) 48h-EC_{10} = 0.049 (nom.)	Klimisch 2	(Paredes et al., 2014)
OECD TG 211	*Daphnia magna*	21d-NOEC ≥ 0.06 (nom.)	Klimisch 1	(Fort Environmental Laboratories, 2021a)
OECD TG 211	*Daphnia magna*	21d-NOEC = 0.04 (nom.) (growth)	Klimisch 2 Solvent influence possible	(Sieratowicz et al., 2011)
OECD TG 211	*Daphnia magna*	21d-NOEC ≥ 0.02 (nom.)	Klimisch 2 No analysis	(Fent et al., 2010)
OECD TG 201	*Selenastrum capricornutum* (now: *Raphidocelis subcapitata*)	72h-ErC_{50} > 100 (nom.) 72h-ErC_{10} = 65 (nom.) 72h-NOErC = 32 (nominal)	Klimisch 3 (Registrant: 1) Test conc. much above water solubility limit	(NOTOX, 2000b)
OECD TG 201	*Scenedesmus subspicatus* (now: *Desmodesmus subspicatus*)	72h-ErC_{50} > 100 (nominal) 5.8% effect on growth at 100 mg/L	Klimisch 3 (Registrant: 1) Test conc. much above water solubility limit	(Department of Toxicology and Ecology, 2001)
OECD TG 201	*Desmodesmus subspicatus*	IrC_{50} > 0.25 (nom.) IrC_{10} = 0.07 (nom.)	Klimisch 3 No validity information, fewer replicates used than TG, no analysis	(Sieratowicz et al., 2011)
Non-standard	*Scenedesmus vacuolatus* Uni-cellular chlorophyte	77h-EC_{50} = 0.19 (nom.) Decline of toxicity up to 72h	Klimisch 4 Lack of information on validity criteria, pH changes	(Rodil et al., 2009)
Non-standard	*Isochrysis galbana* Marine uni-cellular microalgae	72h-EC_{50} = 0.075 (nom.) 72h-EC10 = 0.052 (nom.) 72h-NOEC = 0.010 (nom.)	Klimisch 2 Drop in test substance concentration	(Paredes et al., 2014)
Non-standard	*Acropora sp.* Red sea, Egypt Zooxanthellae – form of unicellular algae *Acropora pulchra* Andaman Sea, Thailand 3 experiments	33 µl/L: bleaching initiation after 2h, bleaching rate: 91% at 24 h, 86% Zooxanthellae released 50 µl/L: bleaching initiation after 48 h, bleaching rate: 91% at 96 h, 90% Zooxanthellae released	Klimisch 2	(Danovaro et al., 2008)
OECD TG 221	*Lemna minor*	7d-EC_{50} > 0.0579 (meas.)	Klimisch 1	(Fort Environmental Laboratories, 2021b)

* meas. – measured; nom. – nominal

7.8.1.1. Fish

7.8.1.1.1. Acute fish toxicity

The key acute fish toxicity in the registration dossier (NOTOX, 2000) is a static 96-h test performed according to GLP using *Cyprinus carpio*. This is stated to be a combined range-finding/limit test. Water accommodated fractions were prepared at nominal loadings of 10 and 100 mg/L by stirring nominal concentrations in the dark for 48 hours. Following overnight settling the water phase was decanted for testing. The treatments of 0.1 and 10 mg/L were prepared by diluting the 10 mg/L WAF. Due to a film being observed for the 100 mg/L, this was further settled for an hour and then decanted through glass wool. All final test solutions were observed to be clear and colourless. The test was performed at 20 °C, and pH was between 7.5 and 8.0. Fish loading was below 1 g fish/L. Aeration was introduced after 72 hours of exposure. Seven fish were used for the control and 100 mg/L treatments, with 3 fish used for the 0.1, 1.0 and 10 mg/L treatments. One replicate for each treatment was used. No mortality or behavioural abnormalities were observed in the test. Analytical measurements (HLPC-UV) were only made for the 100 mg/L solution, which showed a marked decline during the test: 0.71 mg/L (t = 0), 0.36 mg/L (t = 24 h), 0.075 mg/L (t = 96 h).

While the registrant assesses the study to be Klimisch 1, the eMSCA considers it as Klimisch 3 based on the number of fish used for the concentrations of 0.1, 1 and 10 mg/L, which is half those required in the OECD TG.

A supporting 96-h OECD TG 203 study performed to GLP is also provided (Department of Toxicology, 1998). This tested *Brachydanio rerio* (now *Danio rerio*, Zebrafish) using a static system. Solutions of nominal concentrations of 0, 100, 215, 464, 1000, 2150, 4640 mg/L were prepared using an Ultra-turrax[6] stirrer for 25 minutes prior to placing the fish in the solutions. Concentrations were chosen based on two range-finding studies. 10 fish were used per vessel, with one replicate per treatment. The RSS indicates all treatments were turbid, with "fat-like" droplets visible on the surface. The level of turbidity increased with concentration to the extent that at the two highest concentrations fish had to be driven to the front of the aquaria to observe them. Analysis was performed on both unfiltered and filtered solutions. In the unfiltered solutions, concentrations were initially between 29.5 % and 59.5 % of nominal concentrations, which fell to between 1.2 and 4.4 % by 96 hours. For the filtered solutions, initial concentrations were between 0.6 and 2.0 % and dropped to between 0.0 - 1.0 % by 96 hours. All fish died at the highest concentration, and two fish died at the second highest concentration. The mean (analysis at start and end of study) of the unfiltered solutions: 16.8, 33.8, 101.5, 271.3, 604.5 and 1422.3 mg/L, which were used to calculate the results: the NOEC was 271.3 mg/L and the LC_{50} was 1216.1 mg/L. The registrant assesses the study to be Klimisch 1 for OMC, but in the view of the eMSCA based on the above concerns the test is Klimisch 3 as the solution preparation is considered to be unsuitable (longer pre-stir period expected, turbidity observations indicate substance in excess of the water solubility limit, effects on the two highest test concentrations with no possibility to determine whether they indicate intrinsic toxicity or physical effects).

7.8.1.1.2. Chronic fish toxicity

See section 7.10.1 where the available fish data on the substance are described.

A short overview of the available data (relevant for classification and labelling) is provided in the following table.

[6] This is a brand of high speed dispersing instrument

Table 10

Aquatic compartment (including sediment)				
Method	**Species**	**Results [mg/L]***	**Remarks**	**Reference**
OECD TG 234	*Danio rerio*	63d-NOEC < 0.0469 (meas.) (gonadal histology, length + weight) 63d-NOEC ≥ 0.0469 (meas.) (mortality, sex ratio, number of hatch)	Klimisch 1	(Fort Environmental Laboratories, 2020a)
Non-Standard	*Danio rerio*	125d-NOEC = 0.01 (nom.)	Klimisch 2	(Zhou et al., 2019b)
Non-Standard	*Oncorhynchus mykiss*	154d-NOEC < 0.05 (nom.)	Klimisch 2	(Lee et al., 2019)

* meas. – measured; nom. – nominal

A fish sexual development test accoding to OECD TG 234 as requested in the first substance evaluation round was conducted (Fort Environmental Laboratories, 2020a) with OMC with the species zebrafish (*Danio rerio*) and a duration of 60 dph (days post-hatch). The limit concentration was nominally 50 µg/L (measured: 46.9 µg/L). The age of the test organisms at intiation of the test was stage 6 and 7. Freshly fertilised zebrafish eggs were exposed in four replicates of 30 empbryos each. The stock solution was prepared using saturator columns. Acetone was used to prepare the stock solutions. A flow-through diluter system was used with a flow rate to each tank of 2.7 L/h (6.5 volumenexchanges/d). Fluorescent lighting was used to provide a photoperiod of 16 h light and 8 h dark at an intensity that ranged from 540 to 1000 lux (lumens/m^2) at the water surface. The water temperature was between 27.1 and 27.3 °C and the pH values were between 7.0 and 7.5. The dissolved oxygen was between 5.3 and 7.4 mg/L.
Survival and hatching success were not affected. The test substance showed significant effects on body weights and length which were both reduced in male and female fish at the single concentration of 46.9 µg/L (measured). Plasma VTG levels in female and male fish as well as of undifferentiated fish were not significantly different from the control. The test revealed decreased mean ovarian stage score (stage 0.0 ovaries in 66 % of treated females, whereas 61% of control females were in ovarian stage 1.0, no statistics). The study authors connected this with treatment-induced decrease in somatic growth. A statistically not significant increase in the ratio of females to males was seen, which according to the study authors was related to the delayed transition from the female to male phenotype and treatment-induced decrease in somatic growth. The test is rated by the eMSCA with Klimisch 1.

The study by Zhou et al. (2019b) was conducted with Zebrafish (*Danio rerio*). The nominal concentrations were 0.001, 0.010 and 0.100 mg/L and not analytically verified. However, the study by Zhou et al. (2019a) showed that the measured concentrations could be maintained at about 80 % or higher of nominal using a similar exposure media preparation. In the actual study the exposure media was even replaced twice a day, whereas in the study by Zhou et al. (2019a) it was replaced once a day. Zebrafish embryos were exposed to OMC from 2 hpf (hours post-fertilisation) for 4 months until sexual maturation. At 120 dpf (days post-fertilisation) male and female fish were paired. F1 eggs were divided into 2 groups: with and without continued OMC exposure until 5 dpf.
The malformation rate after 5 dpf was significantly increased at 0.100 mg/L in the F0 generation. Body weight decreased dose dependently (significantly decreased at 0.100 mg/L at 40 dpf (F0)). The 3-d hatching rates were significantly decreased in the F0 generation and in the not further exposed F1 generation at 0.010 and 0.100 mg/L, whereas in the further exposed F1 groups the hatching rate was significantly decreased at 0.001, 0.010 and 0.100 mg/L (see Table 16). In the blank and solvent control the hatching rates were > 80% fulfilling the validity criterium of OECD TG 234. 5-day survival was decreased at 0.100 mg/L in the F0 generation, but not in the F1 generations.
In the F1-group with continued exposure 5-day malformation rates were increased at 0.010 and 0.100 mg/L, whereas no effect on malformation was seen in the F1-group without

continued exposure. In the F0 generation the 5-day malformation rates were increased at 0.100 mg/L.

Lee et al. (2019) conducted a 2-generation study with *O.latipes* (OECD CF level 5 study). The test concentrations were 0.05, 0.158, 0.500, 1.580, 5.000 mg/L (nominal concentrations; no chemical analysis). This study was conducted according to OECD TG 234 with slight modifications. During the exposure, the test solution was renewed three times per week, and water quality parameters (dissolved oxygen (DO), pH, temperature, and conductivity) were measured shortly before and after the renewal. Exposure was conducted under the following conditions: 6.9 ± 1.2 mgO2/L (DO), 7.5 ± 0.3 (pH), 26 ± 2 °C (temperature), 158 ± 18 µS/cm (conductivity) and under 15:9 h light:dark photoperiod. The eggs were randomly distributed into the glass beakers of 50 mL volume with 20 eggs per replicate and four replicates per control, solvent control (0.01% DMSO) or each treatment. The parents (F0) were exposed from 24 hpf (hours post-fertilisation) until 154 dpf (days post-fertlisation). At 106 dpf, the fish were paired and a mating period of 49 d began, the number of eggs was determined until 154 dpf. At 120 dpf eggs (F1) were further exposed and the F1 generation was examined until 38 dpf. There were no significant effects on hatchability and survival. However, effects on reproduction appeared: The number of eggs (per brood per day) was significantly decreased at 0.050 mg/L and higher concentrations. Growth was decreased at 0.500 and 1.580 mg/L at 38 dpf (only compared to solvent control).

7.8.1.2. Aquatic invertebrates

7.8.1.2.1. Acute toxicity

A 48-h GLP study using *Daphnia magna* (Department of Ecology, 2003) according to OECD TG 202 was performed using static conditions. Very limited information about the test is available in the RSS. Nominal concentrations of 12.5, 25, 50 and 100 mg/L together with a control were prepared. Five animals per vessel and four replicates per treatment were used and the test medium was prepared with (q.v.) *an aqueous extract of the test substance (eluate)*. To prepare the test solutions, OMC was stirred for 20 hours at 20 °C in M4 medium. Undissolved test substance was subsequently removed by centrifuging (20 minutes at 17700 G) to provide a stock solution of nominal concentration 100.2 mg/L. Further M4 medium was added to dilute the stock solution to provide the remaining treatments. Chemical analysis was only performed on the control and highest concentration, at the start and end of the test. This shows that the starting concentration for the nominal 100 mg/L treatment was 0.035 mg/L, and at 48 hours was 0.0191 mg/L (mean measured: 0.0271 mg/L). The report indicates that all four test guideline validity criteria were met, and the test substance showed no inhibitory effects at any concentration (apart from one Daphnid mortality at 12.5 mg/L).

The registrant assesses the study to by Klimisch 1. According to the updated water solubility value (0.051 mg/L) cited in the registration dossier, the measured solubility in the study is not as far from this water solubility value as previously assumend (with the old data). Therefore, the eMSCA agrees to the rating of the study.

<u>Other information found by the eMSCA</u>

Sieratowicz et al. (2011)

Sieratowicz et al (2011) performed a non-GLP 48-h acute toxicity Daphnia study according to OECD TG 202 using a number of UV filters including OMC. This was part of a battery of three ecotoxicity tests (also including chronic toxicity Daphnia and algal inhibition studies) conducted to provide data for a risk assessment of the substances. The source of OMC was the University of Göttingen, and the purity was not stated. This was a static test using nominal concentrations of 0.08, 0.16, 0.31, 0.63, 1.25 and 2.5 mg/L and M4 test media.

A range-finding test is not mentioned. Ethanol (0.05%) was used as a solvent, but a solvent control is not mentioned for this experiment (it was used for the other studies performed by the authors in the same paper, so this may just be an editorial omission). There were four replicates per treatment with five animals per replicate. Immobilisation was observed at 24 and 48 hours. The results are stated as 48-h EC_{50} = 0.57 mg/L and 48-h EC_{10} = 0.14 mg/L. No analysis was conducted to verify the nominal concentrations, but some information can be taken from the chronic Daphnia test which was performed in the same research lab (and described below). This had significant issues for the concentration maintenance in the limited analysis performed. The results would suggest that significant test substance loss is likely to have occurred in the acute study as well, albeit the test period was half the renewal period of the chronic test. On this basis the quoted 48-h EC_{50} value may well be lower than 0.57 mg/L. The reliability of the study is assessed with Klimisch 2 by the eMSCA.

Fent et al (2010)

In Fent et al (2010) the authors report the findings of another paper they wrote[7] which included an acute Daphnia study. There are few details in Fent et al (2010) itself but it can be summarised as a 48-h non-GLP acute Daphnia toxicity study performed in accordance with OECD TG 202 for four UV filters including OMC. For OMC[8] the test used five concentrations between 0.1 and 1 mg/L. It appears to have been performed under static exposure conditions without chemical monitoring. The 48-h EC_{50} is stated as 0.29 mg/L, and so it could have been lower.

Paredes et al. (2014) - *Siriella armata* (mysid crustacean 96-h)

The test was performed according to a method described in another reference (Perez & Beiras, 2010), which has not been reviewed by the eMSCA. Single newly released neonates were incubated in 20 mL glass vials for 96 h at 20 °C using a 16/8 light/dark photo period. 20 animals were used per concentration and they were fed daily for the first half of the test. Dead neonates were counted every 24 h.
Results were stated as EC_{50} = 0.199 mg/L; EC_{10} = 0.081 mg/L, NOEC = 0.063 mg/L and LOEC = 0.125 mg/L.

Table 11

Nominal and measured concentrations for the 96h-time period (Paredes et al., 2014)

Nominal concentration, mg/L	Measured concentration, µg/L	
	0 h	96 h
0.050	0.015	n.d.
0.200	0.027	n.d.
0.800	0.126	0.018

n.d. = not detected

Commentary

The paper contains limited information about the test methods, validity criteria and controls. In addition significant losses are suggested from the analytical work conducted in parallel to the experiment with the animals. This makes it very difficult to assess the concentration at which effects occurred. However, the available data suggest a significant sensitivity of a number of the organisms to OMC, particularly if measured concentration

[7] Cited as "Fent, K., Zenker, A., Rapp, M., 2010. Environmental Pollution, in press", but the UK CA was unable to locate this paper

[8] The quoted concentration range is small compared to the the ranges used for the other three chemicals in the test.

means the actual results would be lower than suggested by the nominal values. Without better information on the controls and validity criteria, it is not possible to use the data as they stand for firm PNEC derivation.

The UK CA also asked the registrant for their view of the paper. They provided a robust study summary which indicates all four studies had good concentration-effect curves. They conclude the assays are all of Klimisch reliability 2. They highlight the following specific points:

- *Comments to Materials and methods*
    - Not all nominal test concentrations are given (should have been 5 to 7 concentrations according to concentration-effect curves), only the analysed concentrations are listed in Table 11.
    - Recoveries of OMC at test start were highly variable between the different tests: 56-151% for algae, mussel, and sea urchin; measured start concentrations were much lower for the mysid test (recovery 14-30%). The latter test was performed under light/dark conditions. However, this would not explain the lower start concentrations. Another explanation might be that possibly the same stock solutions were used over a longer period of time and that the mysid test was performed at the end of the project.

- *Comments to Results*
    - The authors present a reasoning why nominal concentrations should be used for expressing the toxicity:
        - it is likely that the degradation products (not included in the analyses) show similar toxicity as the parent substance
        - the same photodegradation and chemical degradation processes taking place in the test are likely to take place in the natural environment.

The eMSCA agree with the reliability assessment of the study with Klimisch 2.

Paredes et al. (2014) - Mytilus galloprovincialis (mussel 48-h test)

The mussels were induced to spawn by thermal stimulation, and subsequently fertilised eggs were transferred to the experimental vials and incubated at 20 °C until the second larval stage at 48-h. 40 eggs/ml were used, but the paper does not indicate the volume. Toxicity was assessed based on percentage of normal (second stage) larvae at test completion. The test was performed according to a method described in another reference (Bellas et al, 2005), which has not been reviewed by the eMSCA. The test appears similar in principle to a US EPA test guideline[9] where the test commences 4-h after fertilisation and continues for 48-h. Results were stated as EC_{50} = 3.118 mg/L, EC_{10} = 0.431 mg/L, NOEC = 0.500 mg/L and LOEC = 1 mg/L. The eMSCA notes that the NOEC is above the EC_{10}, although this may well be an outcome of the statistical derivation.

Table 12

Nominal and measured concentrations for the 48h-time period (Paredes et al., 2014)		
Nominal concentration, mg/L	Measured concentration, mg/L	
	0 h	48 h
0.050	0.039	0.023
0.200	0.111	0.044
0.600	0.463	0.093

[9] OPPTS 850.1055 Bivalve Acute Toxicity Test (Embryo-Larval), 1996.

Paredes et al. (2014) - Paracentrotus lividus (sea urchin 48-h test),

The test was performed according to a method described in another reference (Saco-Alvarez et al, 2010), which has not been reviewed by the eMSCA. Fertilized eggs (density 40/mL) were incubated in vials at 20 °C for 48-h. At test completion these were fixed with formalin, and viewed under a microscope. Toxicity was assessed using larval growth (by subtracting the average diameter of the fertilized eggs from the maximum dimension of the first 35 larvae in each vial at 48-h. Results were stated as EC_{50} = 0.284 mg/L; EC_{10} = 0.049 mg/L, NOEC = 0.600 mg/L and LOEC = 0.800 mg/L. The eMSCA notes that the EC_{50} and EC_{10} are below the values of the NOEC and LOEC, suggesting a possible error.

Table 13

Nominal and measured concentrations for the 48h-time period (Paredes et al., 2014)		
Nominal concentration, mg/L	Measured concentration, mg/L	
	0 h	48 h
0.050	0.039	0.023
0.200	0.111	0.044
0.600	0.463	0.093

- *Comments to Results*
  - Large difference between NOEC and EC_{10} in the sea urchin test. Apparently, effects at 600 µg/L were not significantly different from control; yet > 80% effect. It results in a NOEC that is markedly higher than the EC_{50}.
  - The authors present a reasoning why nominal concentrations should be used for expressing the toxicity:
    - it is likely that the degradation products (not included in the analyses) show similar toxicity as the parent substance
    - the same photodegradation and chemical degradation processes taking place in the test are likely to take place in the natural environment.

7.8.1.2.2. Chronic toxicity

A chronic Daphnia test with OMC was conducted in 2021 due to request in the substance evaluation decision (Fort Environmental Laboratories, 2021a). The robust study summary is available. The test was conducted according to OECD TG 211. The purity of the Substance was 99.8%. Analytical monitoring was conducted using LC-MS/MS (LOQ= 0.00324 mg/L). The test was semi-static with renewal of the test solutions in 24-hour intervals. The test solution were prepared using saturator columns. There was no evidence of undissolved material. M4 Medium was used. The exposure duration was 21 d. The nominal concentration was 0.06 mg/L (limit test). No vehicle was used. The measured concentrations were initial 0.06 mg/L and old test solution 0.0582 mg/L (arithmetic mean). The nominal concentration can be used. The hardness was 216 to 224 mg $CaCO_3$/L, the test temperature 20.6 to 20.9 °C, the pH values were between 6.5 and 9 and the dissolved oxygen was above 3.0 mg/L. One organism per test vessel and 10 replicates were used. The light intensity was 1000 to 1500 lux with 16 hours light per day. The validity criteria of the OECD TG 211 were fulfilled. No effects on mortality, reproduction and growth appeared up to 60 µg/L. The registrants assigned this test as Klimisch 1 and the eMSCA agrees with this.

Other information found by the eMSCA

Sieratowicz et al. 2011

Sieratowicz et al (2011) performed a non-GLP 21-d *Daphnia magna* reproduction toxicity study according to OECD TG 211 for four different UV filters including OMC. The test was performed under semi-static conditions with renewal twice a week. There were 10

replicates with one Daphnid per replicate. Reproductive output (number of neonates) as well as length was assessed. Nominal OMC concentrations were 0.005, 0.01, 0.02, 0.04 and 0.08 mg/L, together with a control and solvent control (ethanol 0.05%) in M4 media. Chemical analysis was HPLC with UV detection. The paper indicates that the method achieved a 51.9% recovery rate for OMC. The results of the analysis of the two concentrations 0.05 and 0.8 mg/L indicate significant decreases in test substance concentration: ~100% at the lowest concentration (as it could not be detected after 4 days) and 93% at the highest concentration. The authors note analytical difficulties during the study. They suggest that the exposure conditions would require amendment and advise caution for the interpretation of the calculated "time-weighted mean concentrations" calculated from the fresh and expired media in the publication. No statistically significant effects on reproduction were observed for OMC. The paper notes that that non-solvent control had a significantly increased parental length compared to the solvent control for this chemical (compared to the other three which were tested) at the highest treatment. A graph for these data is provided in the paper, and it can be estimated graphically that the control length was 4.1 mm, the solvent control length was 3.8 mm and the highest concentration length was 3.7 mm. The standard deviation of the solvent control and highest concentration may coincide. Results are stated as being based on nominal concentrations: 21-d NOEC = 0.04 mg /L and 21-d LOEC 0.08 mg/L. An EC_{10} could not be calculated. The paper cautions about the possibility of a solvent induced effect because of the length difference between the control and solvent control. Therefore, the reliability is assessed with Klimisch 2 by the eMSCA.

Fent et al (2010)

Fent *et al* (2010) report the findings of another paper they wrote[10] which included a chronic toxicity Daphnia study. There are few details in Fent et al (2010) itself but the test can be summarised as a 21-d non-GLP Daphnia reproduction toxicity study performed in accordance with OECD TG 211 for four UV filters including OMC. It used 48-h static renewal. Four concentrations of OMC were used (0.00128, 0.0032, 0.008 and 0.02 mg/L). The exposure concentrations were based on the acute test described above (Fent *et al*, 2010). No effects on reproduction or body length were observed for OMC. There is no mention of chemical analysis, so the eMSCA assumes the quoted values are nominal concentrations. The apparent lack of analysis in this study makes interpretation of the results difficult as it may be that little if any test substance was present particularly towards the end of the renewal period for such low concentrations. The eMSCA rated the study with Klimisch 2.

7.8.1.3. Algae and aquatic plants

7.8.1.3.1. Toxicity to algae

A 96-h algal inhibition toxicity test was performed according to GLP using *Selenastrum capricornutum*, now *Raphidocelis subcapitata* (Notox, 2000) according to OECD TG 201. The first 24-h were performed in the absence of light, and the remaining 72-h were performed in light (TLD lamp yielding 3100 – 3300 lux). Water accommodated fractions (WAF) were prepared for nominal concentrations of 10, 18, 32, 56 and 100 mg/L OMC together with a control. The test was originally a range-finding-limit test, with the range-finding using 0.1, 1, 10 and 100 mg/L WAFs. It then appears to have been extended to include loadings at 18, 32 and 56 mg/L. The study report describes the preparation of the 0.1, 1, 10 and 100 mg/L loading WAFs. The 10 and 100 mg/L loadings were prepared by 48-h stirring in a closed vessel in the dark (because of known photodegradation of OMC). After settling the aqueous phase was decanted off for the test. However, as an oily layer remained, a volumetric pipette was used to remove portions of the middle of the solution.

[10] Cited as "Fent, K., Zenker, A., Rapp, M., 2010. Environmental Pollution, in press", but the UK CA was unable to locate this paper

The 0.1 and 1 mg/L WAFs were prepared by dilution of the 10 mg/L WAF. It is unclear how the concentrations at 18, 32, 56 mg/L were prepared (dilution of the 100 mg/L WAF, or preparation of individual WAFs). Final test solutions are described as clear and colourless to opalescent. According to the study report there were "3 +2" replicates of each test concentration, "6+3" replicates of treatment control with algae, 1 replicate of each test concentration without algae and 2 extra replicates of the highest concentration without algae. The pH ranged from 7.9 to 9.7, with the temperature around 23 °C. Chemical analysis was conducted at 0, 24, 48 and 96-h. At the start of the test OMC concentrations exceeded the water solubility limit of 51 µg/L for all treatment levels. After 24-h in the dark, measured concentrations had fallen significantly, with further reductions indicated at 48 and 96-h so that all treatments were below the detection limit (0.001 mg/L) except 56 mg/L (measured as 0.0603 mg/L. The validity criteria were fulfilled (study report), for example exponential growth occurred in the controls within three days. In the registration dossiers, no information is given on fulfilment of validity criteria.

The results are expressed based on nominal concentrations for the light exposure (24-96-h). The 72-h NOE_rC was 32 mg/L, E_rC_{10} was 65 mg/L and the 72-h E_rC_{50} >100 mg/L.

The test is assigned by the registrant to be Klimisch 1. The eMSCA assigns this test to be Klimisch 3, due to very high test concentrations above solubility limit of 0.051 mg/L (and also the former valid solubility limit) and the use of WAF.

A second, supporting, algal inhibition study is a 96-h OECD TG 201 test performed according to GLP using *Scenedesmus subspicatus,* now *Raphidocelis subcapitata* (BASF 2001). Nominal concentrations of 6.25, 12.5, 25, 50, 100 mg/L OMC and a control were tested, but the number of replicates is not stated. Solutions were prepared in the same way as the acute Daphnia study (20-h stirring, and centrifuging at 17700 G). This provided a stock solution of nominal concentration 125 mg/L. This was diluted to 100, 50, 25, 12.5 and 6.25 mg/L nominal concentrations. No analytical monitoring was conducted. Validity criteria (study report) were indicated to have been met as the control growth was exponential, pH change was "less than 2 pH units", and the positive control results were in line with expectation. In the registration dossier, no information is given on fulfilment of validity criteria. Contrary to the pH rise in the other algal test, pH at the end of the study was pH 8.0. As a result of the test, 5.8% effect on growth was observed at 100 mg/L. However, there is no analysis to indicate whether this was statistically significant. No effects on growth were observed for the remaining concentrations.

The registrant assesses this study to be Klimisch 1. In the view of the eMSCA based on the above lack of information the test is Klimisch 3. This is mainly because the absence of analysis means it is unclear what concentration the algal were exposed to. The effect values based on nominal concentrations given in the registration dossier do not represent the reality as they are highly above the very low solubility limit. Furthermore information on number of replicates is lacking.

Other information found by the eMSCA

Sieratowicz et al (2011)

Sieratowicz et al (2011) performed a non-GLP 72-h algal inhibition test according to OECD TG 201 using *Desmodesmus subspicatus*. This used nominal concentrations of 0.015, 0.03, 0.06, 0.13 and 0.25 mg/L, together with a control and solvent control (ethanol 0.05%). There were 5 replicates for the control and 3 replicates per treatment and solvent control. Cell density was assessed at 24, 48 and 72 hours. The maximum observed inhibition of the growth rate was 23.9%, therefore the IrC_{50} > 0.25 mg/L. The IrC_{10} was 0.07 mg/L. No validity information, such as confirmation of exponential growth, is provided in the paper. This test used fewer replicates than the OECD TG 201 guideline. Hence, the statistical confidence of the results is lower. No analysis was conducted to verify the measured concentrations, but some information can be taken from the chronic Daphnia test performed by the same authors in the same paper (described above). The Daphnia study

had significant issues for the concentration maintenance in the limited analysis performed, which suggests that significant test substance loss is likely to have occurred in the algal study as well. Therefore the stated results should be treated with caution. The test is assigned to Klimisch 3, as the fulfilment of validity criteria was not stated and fewer replicates are used.

Rodil et al (2009)

As part of their investigation of several UV filters Rodil et al (2009) assessed the phytotoxicity of OMC[11] to the uni-cellular chlorophyte *Scenedesmus vacuolatus*. The cells were cultured using a 14/10 light/dark cycle so that a full reproduction cycle occurred after 24 h. A 24-h test was performed using a concentration range for OMC of 0.024 to 0.400 mg/L together with three solvent controls (DMSO, 0.1%) and three controls. No further details on the exact concentrations or number of replicates of these are provided. A second test was performed using OMC solutions exposed to UV light for 14, 28, 42 and 77 h. The irradiated solutions were diluted with algal medium and contained then 5.6, 11.3, 22.5, 45 and 90% of the original volume. Per dilution level were six controls and two replicates. For both experiments cell density was determined after 24 h and used to assess inhibition. No information is provided in the paper about validity criteria such as exponential growth in the controls, or pH changes (although the study is otherwise well reported, for example the initial cell density is provided). The eMSCA rated the study with Klimisch 4.

Results: The 24-h EC_{50} was stated to be 0.19 mg/L although it was not stated whether this is a growth or biomass result. For the UV-degraded solutions, toxicity declined with time (up to 72 h) and the authors conclude that the degradants of OMC are less toxic than the parent substance.

Paredes et al (2014)

A test with *Isochrysis galbana*, marine unicellular microalgae, with exposure duration of 72 h exists (Paredes et al. 2014). The test was performed according to a method described by Perez et al. (2010 a) at 20 °C with a 24-h light cycle and an initial cell density of 7000 cells/ml. 3 replicates per concentration and three controls were used. Cell density and growth rate were calculated at 72 h. The calculation of growth rate (comparing initial and final numbers of cells) is stated in the publication with reference to Perez et al. (2010 b). Therefore it is assumed that the results stated in the paper are based on growth rate. These were EC_{50} = 0.075 mg/L, EC_{10} = 0.052 mg/L, NOEC = 0.010 mg/L and LOEC = 0.030 mg/L (all based on nominal concentrations). The eMSCA notes that the values of EC_{10} and EC_{50} suggest a very steep dose-response curve. There was a 99% drop in test substance concentration over the duration of the test. Therefore the real effect values are much lower than specified above, since nominal values were used. The eMSCA rated the study with Klimisch 2.

Summary of algal toxicity data

A summary of available toxicity data for algae is provided in table 14. Effects are observed in all available studies, but there is significant variation in the severity. It is not clear if this is due to the actual exposure concentration (which were not analytically monitored in most of the studies) or differences in the quality of the study, or a difference in species sensitivity. As in two of the three academic studies solvents were used, the concentration maintenance was better than in the GLP lab ones. In the academic study where no solvent was used, a drop in test concentration was observed. In the GLP lab studies no analytical verification was conducted.

[11] The earlier part of their work assessing the photodegradation is described in section 7.7.1.1.

Table 14

Summary of available toxicity data for algae				
Reference	**Algae species**	**EC_{50} / mg/L**	**EC_{10} / mg/L**	**Nominal / measured concentration**
Notox, 2000	*Selenastrum capricornutum*	>100	65	nom.
BASF, 2001	*Selenastrum capricornutum*	>100	50 (?)	nom.
Sieratowicz et al, 2011	*Desmodesmus subspicatus*	>0.25	0.07	nom.
Rodil et al, 2009	*Scenedesmus vacuolatus*	0.19	?	nom.
ECOSAR	-	0.096	n/a	n/a
Paredes et al, 2014	*Isochrysis galbana*	0.075	0.052	nom.

7.8.1.3.2. Toxicity to aquatic plants other than algae

Fulfiling the data requirements from the substance evaluation decision, a 7-d-toxicity test on *Lemna minor* according to OECD TG 221 was performed using a semi-static test type (Fort Environmental Laboratories, 2021b). GLP compliance was stated. The substance purity was 99.8%. Analytical monitoring was conducted using LC-MS (LOQ = 0.00324 mg/L). The limit concentration was 0.0637 mg/L (measured: 0.0579 mg/L). No solvent was used. The test temperature was 24 ± 2°C and the pH 6.5 to 9 (with a variability less than 1.5 units). 16 hours light per day with a intensity of 6500 to 10000 lux were provided. Six replicates per treatment and control were used. The initial density was 10 fonds per replicate. No significant effects on dry weight, growth rate, or fond area were observed. There were no visual signs of phytotoxicity. The validity criteria described in OECD TG 221 were fulfilled. The substance was stable in the test medium (94.3 to 100.4 % measured compared to nominal concentrations). The study is assigned by the registrant to be Klimisch 1. The eMSCA agrees on this rating.

7.8.1.4. Sediment organisms

No data in registration dossier available.

7.8.1.5. Other aquatic organisms

A 21-d Amphibian Metamorphosis Assay (AMA) as requested in the first substance evaluation round with the African Clawed Frog (*Xenopus laevis*) was conducted with OMC (Fort Environmental Laboratories, 2020b). Three concentrations up to 0.0442 mg/L (measured) were tested. The median developmental stage, snout-vent length, hind limb development and body weight were not statistically different from control on SD day 7 and 21. No treatment-related histopathologic findings in the thyroids of tadpoles exposed to OMC were seen. In summary no thyroid effects were seen and no other signs of toxicity. The eMSCA rated the study with Klimisch 1, GLP compliance was stated.

<u>Other information found by the eMSCA</u>

Danovaro et al (2008)

Danovaro et al (2008) investigated coral bleaching resulting from exposure to a number of sun screens and individual UV filters including OMC. They conducted in-situ studies in four locations in the world: Siladen, Celebes Sea (Indonesia, Pacific Ocean), Akumal, Caribbean Sea (Mexico, Atlantic Ocean), Phuket, Andaman Sea (Thailand, Indian Ocean) and Ras Mohammed, Red Sea (Egypt, Indian Ocean). Three species of hard coral were investigated: *Acropora* (different species of this genus: *A. divaricata, A. cervicornis,*

pulchra, A. aspera, A. intermedia), *Stylophora pistillata* and *Millepora complanata*. OMC was only assessed in two locations: Phuket (*Acropora pulchra)* and Ras Mohammed (*Acropora spathulata)*. Nubbins of *Acropora* were collected and incubated in-situ in polyethylene bags containing 2-L of virus-free seawater. The test used concentrations of 10, 33, 50, 100 µl/L seawater together with a control of untreated seawater. Three replicates each containing more than 300 polyps were used for each treatment. The exact length of the experiments is not specified but appears to be 96 h, with observations made periodically during that time. No chemical analysis was performed.

Bleaching was assessed using a colorimetric analysis from digital photographs taken during the study. These were analysed using photo-editing software to assess colour composition (cyan, magenta, yellow and black). Changes in each colour were assessed relative to the control to establish the level of bleaching. Adverse effects to the Zooxanthellae (protozoa which live on the coral) was also assessed. These microalgae were extracted using artificial seawater, and the number of cells counted and their health assessed (based on colour and condition). A final aspect of the study was the effect of sunscreen on causing viral infections. However this aspect used only (unspecified) sunscreen mixtures, rather than specific chemicals such as OMC.

The results for OMC are in the following Table.

Table 15

Information on coral bleaching of OMC				
Coral	**Quantity**	**Bleaching initiation / h**	**Bleaching rate hr (%)**	**% Zoozanthellae released**
Phuket (*Acropora pulchra)*	33 µl/L	2	24 (91)	86
Ras Moohammed (*Acropora sp.)*	50 µl/L	48	96 (91)	90

Commentary

It can be seen that there was a marked difference between the bleaching initiation time, for the two corals. Although the paper compares the control Zooxanthellae cell condition with the exposed cells, specific quantification of toxicity to Zooxanthellae from OMC is not reported.

While there are no standard guidelines for this type of test, on the basis of comparison to the controls, effects were observed. It is known that many causes have been suggested for coral bleaching (for example temperature changes due to global warming). It is beyond the scope of this evaluation to assess this impact. However, the eMSCA does note that the toxic effects on the Zooxanthellae (which are a form of unicellular algae) do not in principle appear to be out of step with other studies described above where OMC caused adverse effects on algae.

7.8.2. Terrestrial compartment

Not assessed.

7.8.3. Microbiological activity in sewage treatment systems

Not assessed.

7.8.4. PNEC derivation and other hazard conclusions

Not part of the evaluation.

7.8.5. Conclusions for classification and labelling

The eMSCA considers the classification of OMC as Aquatic Acute 1, H400 (M=10) and Aquatic Chronic 1, H410 (M=1) as necessary based on the available information (acute: E_rC_{50} from the algae toxicity test of 0.075 mg/L (nom.) for the algae *Isochrysis galbana*; chronic: ready biodegradability and F1-$NOEC_{hatching\ rates}$ of 0.01 mg/L (nom.) for the fish *Danio rerio* from the study conducted by Zhou et al. (2019b)) and will prepare a corresponding CLH dossier.

7.9. Human Health hazard assessment

Not part of the assessment.

7.10. Assessment of endocrine disrupting (ED) properties

Endocrine disruption potential was a key concern highlighted in the CoRAP for OMC. Data (including public domain research studies) investigating the ED properties of OMC were taken from the IUCLID REACH registration dossier for the substance, including its CSR documentation. In addition a literature search was performed by the eMSCA to identify papers on the ED potential of OMC (and IPMC) published since 2005.

The general approach taken in the following assessments is to consider the available data, using weight-of-evidence, according to the different 'Levels' of testing outlined in the OECD Conceptual Framework (CF) for Testing and Assessment of Endocrine Disrupters (OECD, 2012) and provisionally against the World Health Organisation/International Programme on Chemical Safety working definition of an endocrine disruptor (WHO/IPCS, 2002), i.e.:

"*An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations.*"

Based on recommendations from the European Commission's Endocrine Disrupters Expert Advisory Group (JRC, 2013) key aspects to take from this definition when identifying an ED substance are:

i) The presence of adverse health effects in an intact organism or (sub)population (the environmental assessment being conducted at the population level);

ii) The presence of an endocrine mode of action or activity (in which disruption of the endocrine system is not a secondary consequence of other non-endocrine-mediated systemic toxicity) and;

iii) A plausible or demonstrated causal relationship between the endocrine activity and the adverse effect;

iv) Relevance to humans or wildlife populations

An adverse and population-relevant effect is assumed based on the following WHO definition: A change in the morphology, physiology, growth, development, reproduction, or, life span of an organism, system, or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences (WHO/IPCS, 2009)[12]. The elements of the WHO definition have also been taken into account in the newly established hazard classes for ED under the CLP regulation (Regulation (EC) No 1272/2008).

[12] WHO/IPCS (2009): Principles and methods for the risk assessment of chemicals in food. Environmental health criteria 240. https://www.who.int/publications/i/item/9789241572408

The OECD Conceptual Framework (CF) for the Testing and Assessment of Endocrine Disrupting Chemicals provides a guide to relevant data and the standardised OECD test guidelines available (or then under development) for the evaluation of chemicals for endocrine disrupting properties. The purpose of the CF is further described in the OECD Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption (OECD GD 150, 2012). Importantly, the CF is not a testing strategy that should be followed linearly from Level 1 to Level 5. Data from the various CF levels have different applications and implications, e.g. providing mechanistic information (Levels 2 and 3) or providing data on adverse effects on endocrine relevant endpoints (Levels 4 and 5). Therefore, no single assay is likely to provide all the information required to conclude on whether a substance is an endocrine disruptor, because of the need to provide both mechanistic and apical information. All the available information on adversity and endocrine activity should be considered together, by adopting a weight-of-evidence approach. Typically Level 1 data are *in silico* models and estimates of ED activity, Level 2 includes *in vitro* studies and Levels 3 to 5 are *in vivo* tests of increasing duration and complexity in terms of the endpoints and life stages examined.

The CF is a useful tool to frame the available data in the assessment of potential endocrine disruption. The evaluation of data within this framework determines whether the weight-of-evidence is sufficient to make a conclusion on endocrine disrupting properties or whether further testing is required.

Whilst the human health and environmental ED assessments are described separately, they are considered together in the overall summary of ED potential given in Section 7.10.3 of this report. Results of the human health ED assessment (see Section 7.10.2) are of relevance to wildlife and would also be considered in relation to potential adverse impacts on wild mammals at the population level. A primary focus is however, also on the potential for adverse population-level ED effects on aquatic species, in particular fish, amphibians and invertebrates.

The reliability of studies was assessed according to the criteria described by Klimisch *et al.* (1997). Each study was assigned to one of four possible categories on the basis of compliance with the stated Klimisch criteria, i.e.:

1. Reliable without restrictions
2. Reliable with restrictions
3. Not reliable
4. Not assignable

7.10.1. Endocrine disruption – Environment

In vitro mechanistic data

Several *in vitro* studies are available that tested OMC regarding the EAST (oestrogen-androgen-steroidogenesis-thyroid) modalities. The studies are listed in Table 17 (Annex 1). Binding studies invariably could not demonstrate binding of OMC to mammalian oestrogen receptors (ER) (Morohoshi et al., 2005; NTP, 2020; Seidlova-Wuttke et al., 2006a). Furthermore, the evidence from transactivation assays for interaction of OMC with ER signalling is considered inconclusive. Transactivation assays based on human (h)ER are either negative (Kunz and Fent, 2006; Kunz et al., 2006; NTP, 2020) or report very weak/ambiguous oestrogenic activity (Gomez et al., 2005; Schreurs et al., 2002; Schreurs et al., 2005). Similarly, OMC displayed no activity at rainbow trout (rt) ERα as reported by Kunz et al. (2006), which is the only study utilising a non-mammalian receptor. Very weak anti-oestrogenic activity of OMC at hERα was detected by Kunz and Fent (2006) and Morohoshi et al. (2005), and partial agonistic activity at hERα is reported by Schreurs et al. (2002). On the other hand, OMC displayed weak oestrogenic activity with regard to proliferation or pS2 expression/secretion in MCF-7 cells (Heneweer et al., 2005; Schlumpf et al., 2001). For pS2 expression, the EC_{50} was 1.04 µM OMC. For cell proliferation by

OMC, the EC50 was 2.37 µM, while the maximal cell increase was 77 % in relation to proliferation by 17β-oestradiol (E2).

OMC was tested for binding to the human androgen receptor (hAR) and is considered a non-binder (NTP, 2020). The majority of transactivation assays reports no androgenic or anti-androgenic activity of OMC (Ma et al., 2003; Nashev et al., 2010; NTP, 2020; Schreurs et al., 2005). However, anti-androgenic activity of OMC at hAR (IC50 of 0.3 mM) was detected in a yeast-based transactivation assay (Kunz and Fent, 2006). In this assay, OMC was 37-fold less potent than the model anti-androgen flutamide.

OMC displayed antagonistic activity (IC_{50}: 0.5 µM) at the human progesterone receptor (hPR) in a transactivation assay (Schreurs et al., 2005), and was shown to activate the CatSper Ca^{2+} channel and to increase Ca^{2+} influx in human sperm in a similar fashion as progesterone (Rehfeld et al., 2016; Rehfeld et al., 2018). No anti-gestagenic activity regarding SULT1E1 mRNA-expression was observed in Ishikawa cells (Yin et al., 2015).

Regarding effects on steroidogenesis, OMC at 10 µM increased the production of corticosteroids and modulated the expression of steroidogenic enzymes (increased expression of 3β-hydroxysteroid dehydrogenase (HSD3β2), CYP21A2, CYP11B1, CYP11B2; decreased expression of steroidogenic acute regulatory protein (STAR) and CYP17A1) in H295R cells (Strajhar et al., 2017). No inhibition of catalaytic activity of 17β-hydroxysteroid dehydrogenases (17β-HSD) 1, 2, 3, 5 could be detected by Nashev et al. (2010). Recent testing by NTP demonstrated neither inhibition of aromatase, nor interference with E2 and testosterone production in H295R cells (NTP, 2020).

There is indication for a potential interaction of OMC with the central regulation of the hypothalamus-pituitary-gonad (HPG) axis: OMC inhibited the *in vitro* release of luteinising hormone-releasing hormone (LH-RH; aka gonadotropin-releasing hormone (GnRH)), and of amino acid neurotransmitters from hypothalamic fragments of rats (Carbone et al., 2010; Szwarcfarb et al., 2008).

For OMC, several *in vitro* studies on thyroidal activity are available. OMC has been tested negative for inhibition of thyroid peroxidase (TPO) (Schmutzler et al., 2007a; Schmutzler et al., 2004), and high-throughput screenings provided no evidence that OMC inhibits (iodothyronine) deiodinases (DIO) (Olker et al., 2019), iodotyrosine deiodinase (YTD) (Olker et al., 2021) or the Na^{+}/I^{-}-symporter (NIS) (Wang et al., 2021). However, Song et al. (2013) observed downregulation of DIO3 mRNA-expression by 50 µM OMC in human neuroblastoma cells (SH-SY5Y), and Schmutzler et al. (2007b) reported in a review paper inhibition of NIS (by determining iodide-uptake of FRTL-5 cells) by 0.1 and 1 µM OMC. Furthermore, there is some evidence that OMC has limited capacity for interaction with thyroid hormone receptor (TR)-signalling *in vitro*: in a TRα transactivation assay, OMC tested positive at a concentration of 1 µM (1.5-fold induction over vehicle control vs 122-fold induction by triiodothyronine (T3) at 0.1 nM (Hofmann et al., 2009)). No antagonistic activity was observed by Hofmann et al. (2009) up to 10 µM OMC, while a high-throughput screening employing higher concentrations reported borderline antagonistic activity of OMC in the GH3-TRE-Luc assay (AC50 of CAS No. 5466-77-3 and CAS No. 83834-59-7: 58.7 µM and 107 µM, respectively; only highest concentration above baseline; less than 50% efficiency; no cytotoxicity (Paul-Friedman et al., 2019)).

Other *in vitro* studies not directly related to the EATS-modalities are available for OMC: OMC did not activate the pregnane X receptor (PXR) in a transactivation assay (Mnif et al., 2007) but modulates aryl hydrocarbon receptor (AhR) signalling by inhibition of CYP1A1 and CYP1B1 in keratinocytes (Phelan-Dickinson et al., 2020).

Mammalian in vivo studies

In vivo studies in mammals comprising OECD levels 3-5 are available for OMC (see Table 18 to Table 20**Error! Reference source not found.** in Annex 1). Mechanistic studies provide rather weak evidence for EAS-related activity. OMC was negative in two Hershberger assays with castrated male rats (BASF-SE, 2003; NTP, 2020) and two

Uterotrophic assays with either immature or ovarectomised female rats (BASF-SE, 2001; NTP, 2020) up to a dose of 1000 mg/kg bw/d. On the other hand, OMC increased uterus growth slightly at doses > 1000 mg/kg bw/d in another Uterotrophic assay (Schlumpf et al., 2001). Studies in ovarectomised rats demonstrated slight effects of OMC on uterus and vaginal growth and/or histology, changes in gene expression in uterus, vagina, liver and pituitary, and effects on fat deposition, plasma lipids, leptin and luteinising hormone (LH) levels (Klammer et al., 2005; Seidlova-Wuttke et al., 2006a; Seidlova-Wuttke et al., 2006b). Together, these studies indicate weak endocrine endocrine activity with some effects similar but others distinct from the positive control E2.

In intact animals, guideline repeated dose-toxicity studies testing OMC in rats and rabbits by oral and dermal exposure (Hoffmann-La Roche, 1984; Hazleton, 1980a; Hazleton, 1980b; Lab. Pharm., 1979) did not detect any EAS-sensitive or EAS-mediated adversity (see Table 19). Similarly, in guideline prenatal developmental toxicity studies with OMC (Hoffmann-La Roche, 1983a; Hoffmann-La Roche, 1983b), no EAS-sensitive adversity was observed. However, these studies do not meet current standards with regard to dosing period and investigation of endocrine mediated/sensitive parameters. A guideline 2-generation toxicity study with OMC reported reduced ovary weight (without histological findings), marginally reduced epididymal sperm counts, and reduced implantations and litter size at the highest dose (1000 mg/kg bw/d), but the effects were slight and sperm counts were within the historical control range (BASF-SE, 2007; Schneider et al., 2005). On the other hand, a non-guideline study in rats pre-/perinatally exposed to OMC reported effects on epididymal sperm counts, decreased testes and prostate weight (accompanied with histological changes), and changes in levels of testosterone, E2, and progesterone (Axelstad et al., 2011). A recent, modified one-generation study testing OMC via diet in rats could not reproduce any effects on epididymal sperm counts, implantation sites, or litter size (NTP, 2022). Only a few treatment-related changes in EAS-mediated/sensitive parameters were observed. These included a slight delay in pubertal development (balanopreputial separation (BPS) in males and vaginal opening (VO) in females). This finding is in agreement with observations also reported in other studies (Axelstad et al., 2011; BASF-SE, 2007; Schneider et al., 2005) and is considered an unspecific effect due to lower pre- and postweaning body weights. Furthermore, F1 females spent a slightly but significantly longer time in oestrous (significant in all dose groups; no dose-response) while the overall cycle length remained unchanged (NTP, 2022). This finding cannot readily be explained by lower body weights because lower body weight usually is expected to lead to an increase in overall cycle length.

Increased postimplantation and perinatal losses associated with considerable maternal toxicity (decreased body weight gain) were observed after OMC exposure in a feasibility study regarding thyroid disruptor testing up to a dose of 750 mg/kg bw/d (Axelstad et al., 2019). Similarly, a dose range-finding study for a subsequent modified one-generation study reported lower birth weight and postnatal mortality associated with pronounced maternal toxicity (NTP, 2022). Although the doses were higher (up to 1841 mg /kg bw during gestation) as in Axelstad et al. (2019), no postimplantation losses were observed in the NTP dose range-finder study (NTP, 2022). It is concluded by the eMSCA that, if observed, the increased postimplantation losses, decreased birth weigh, decreased postnatal growth, and increased postnatal mortality are possibly a consequence of unspecific effects on development because they were associated with considerable maternal toxicity.

Regarding the T modality, a number of studies in repeated-dose toxicity studies in ovarectomised or intact rats, or in developmental settings demonstrate effects of OMC on the thyroid hormone system (Axelstad et al., 2011; Axelstad et al., 2019; BASF-SE, 2004; Ferraris et al., 2019; Klammer et al., 2007; Schmutzler et al., 2004; Seidlova-Wuttke et al., 2006a). Frequently, T3 and thyroxin (T4) levels were decreased although sometimes no changes or increases were observed. If performed, thyroid histology did not show signs of follicular hypertrophy/hyperplasia indicating no activation of an adequate feedback response despite sometimes severely reduced T4 levels (e.g. Axelstad et al. (2011)). This is in line with inconsistent effects on thyroid-stimulating hormone (TSH) levels, showing

mostly mild changes (both increases or decreases are observed) or remaining unchanged. The lack of effects on thyroidal TPO activity and expression of NIS (both usually upregulated by TSH) determined after *in vivo* exposure similarly indicated no increased TSH stimulation of the thyroid gland (Klammer et al., 2007). Two studies detected decreased hepatic DIO1 activity *in vivo* in ovarectomised rats (Klammer et al., 2007; Schmutzler et al., 2004). Whether this is a cause or consequence of decreased thyroid hormone levels (as seen e.g. in Klammer et al. (2007)) remains unclear in an *in vivo* setting. While hepatic DIO1 activity is known to be upregulated by thyroid hormones, it can also be modulated by oestrogens (keeping in mind that OMC displays some oestrogenic activity *in vivo* in some of the mechanistic studies). OMC, however, does not seem to be a direct inhibitor of DIOs when tested *in vitro* (Olker et al., 2019).

In the developmental toxicity study by Axelstad et al. (2011), severely reduced T4 levels were detected in dams, but T4 was much less affected in offspring. Neurodevelopmental testing revealed no effects of OMC which could be related to gestational or perinatal hypothyroidism (Axelstad et al., 2011). In the study by Axelstad et al. (2019), developmental exposure of OMC did not increase the occurence of cortical heterotopias as it was observed in 6-propyl-2-thiouracil (PTU)-induced hypothyroid animals in the same study. It should be noted that the observed OMC-induced changes in T3 and T4 in Axelstad et al. (2019) were complex (decreases or inceases depending on the time-point) and generally mild, somehow contradicting the findings of severely reduced T4 reported before by Axelstad et al. (2011). Nonetheless, OMC induced some changes in the expression of thyroid hormone-dependent genes in the brain showing a pattern comparable to that of PTU treatment in the same study (Axelstad et al., 2019). Interestingly, OMC induced lower brain weight in offspring, an effect not seen by PTU treatment, indicating potential developmental neurotoxicity independent of effects on thyroid hormone levels.

In conclusion, OMC displays some endocrine activity with regard to the EAS-modality *in vitro* and *in vivo*. However, the effect size of apical findings related to the EAS modalities is usually of low magnitude, and the findings are inconsistent between studies, often lack a dose-response, or occur together with unspecific toxicity. Regarding the T modality, the available data clearly show impacts of OMC on the thyroid hormone system (endocrine activity) in mammals (in particular on T4 levels), but potential adverse effects (neurodevelopmental toxicity) cannot be plausibly related to T-mediated endocrine activity. Furthermore, the populational relevance of these findings is unclear.

Ecotoxicological studies

Several endocrine-relevant *in vivo* fish studies have been conducted on OMC (for more details see Annex 2, Table 17).

Studies in general examining HPG axis in fish at Level 3 according to OECD CF for Testing and Assessment of Endocrine Disrupters (OECD, 2012): Studies by Christen et al. (2011), Inui et al. (2003), Zucchi et al. (2011); at Level 5: Lee et al. (2019), Zhou et al. (2019b), Zhou et al. (2019a) (level 3, 21 d, adult fish), (BASF-SE, 2020) (level 4).

Studies examining HPT axis in fish: Chu et al. (2021); partly Lee et al. (2019) (level 5)

In the study conducted by Christen et al. (2011) adult male and female fathead minnow (*Pimephales promelas*) were exposed to mean measured concentrations of 5.4, 37.5, 245 and 394 µg OMC/L for 14 days. ERα at 394 µg/L OMC in females, AR at 37.5, 245 and 394 µg/L OMC in females, 3β-HSD at 37.5, 245 and 394 µg/L OMC in males and at 245 and 394 µg/L OMC in females were statistically significant down-regulated in the liver. These effects indicated potential anti-oestrogenic (down-regulation of ERα), anti-androgenic activity (down-regulation of AR) and oestrogenic activity (down-regulation of 3β-HSD) following exposure to OMC. Changes in gene expression were organ specific as there was no significant effect on ERα, AR or 3β-HSD in the brain or ovary of female fish and there was no effect of ERα or AR in male fish (any organ). Plasma vitellogenin (VTG) levels were

significantly increased in male fish exposed at 245 µg OMC/L but this was not dose-dependent as no significant effects were seen at the highest test concentration. There was no significant effect of OMC on the number or score of nuptial tubercles. Significant effects were seen at 394 µg/L on the histology of male gonads, where significant alterations in the frequencies of different spermatogenic stages in testes appeared. The spermatogenesis appeared to be inhibited as spermatogonia did not develop to spermatocytes (sign. reduced presence of spermatocytes, and enlarged areas of mature spermatids were seen in testes). These effects were interpreted by the authors as consistent with an oestrogenic or anti-androgenic effect. The significantly decreased proportion of spermatocytes is a secondary criteria for histopathological change due to endocrine activity according to OECD TG 123. In females exposed to 394 µg/L the proportion of vitellogenic oocytes was significantly increased, and the proportion of primary oocytes was decreased, however not significantly. An alteration of frequency of cells at the different stages of oogenesis was shown. The authors concluded from the study that OMC exerts multiple hormonal activities.

Inui et al. (2003) investigated the potential oestrogenic effects of OMC on adult male Japanese Medaka (*Oryzias latipes*). The fish were exposed to nominal concentrations of 0.034, 0.34, 3.4 and 34 mM OMC (9.87, 98.7, 987, 9877 mg/L) and a solvent control (ethanol: 0.1%) for seven days, but maintenance of the concentrations was not analytically verified. There were indications that plasma VTG levels were slightly elevated in a dose-dependent manner, but no level of statistical significance was given. There were, however, significant effects reported in a dose-dependent manner at all concentrations on mRNA expression of oestrogen mediated genes for VTG and also for choriogenin (CHG) proteins and for ERα. CHG is a precursor protein of the fish egg envelope. The results of this study indicate that OMC can significantly affect mRNA expression of oestrogen-regulated genes encoding for VTG and CHG proteins. There are also indications that OMC effects mRNA expression of ERα, which would be consistent with positive autoregulation of this receptor following exposure to oestrogenic compounds.

In the study by Zucchi et al. (2011), adult male zebrafish (*Danio rerio*) were exposed to median measured concentrations of 2.2 and 890 µg/L OMC for 14 days. OMC caused slight but statistically significant up- and down-regulation of key genes associated with hormonal pathways with some evidence for oestrogenic activity. Up-regulated were ERα in the whole body; ERβ in the whole body and liver and VTG1 in the liver, suggesting an oestrogenic activity, whereas down-regulation of AR in the liver suggests antiandrogenic activity. Conversely, there was down-regulation of VTG1 in all other tissues except the liver. The authors concluded that OMC weakly affects genes involved in hormone pathways, but they also reflected that it is difficult to link the results of this study to a specific mode of action. OMC may act through several mechanisms/modes of action involved in the sex hormonal pathways, and this may explain the varied changes in gene expression observed.

Zhou et al. (2019a) exposed adult *D. rerio* for 21 days at the concentrations 1, 10, 100 µg OMC/L (nominal). The measured concentrations were 0.87, 8.5, 79.5 µg/L (3, 29.3, 273.7 nmol/L). E2, testosterone and VTG were determined in the visceral mass of fish. The gene expression levels of ER, AR, PR, VTG1, CYP17a1, CYP19a1, 17β-HSD1, and 17β-HSD3 were also determined in the visceral mass. Additional oxidative stress markers were examined. The contents of VTG and E2 in visceral mass significantly decreased and testosterone level significantly increased at all concentrations after 21 days. After 21 days exposure, gene expression of VTG1, CYP19a1, ER, PR and 17β-HSD1 were down-regulated at all concentrations, whereas AR and 17ß-HSD3 mRNA were up-regulated at all concentrations. No effects were seen on CYP17a1. Oxidative stress: Catalase and superoxide dismutase were increased at all concentrations (already after 7 days), the other indicators (malondialdehyde, glutathione, glutathione reductase) were increased at higher concentrations. The concentration of OMC in fish muscle tissue exposed to 100 µg/L OMC increased with exposure time from about 3000 ng/g wet weight after 7 days to about 17500 ng/g wet weight after 21 days. At exposure to 10 µg/L the OMC concentration increased from about 300 ng/g wet weight (7 d) to 1500 ng/g wet weight (21 d).

The study by Zhou et al. (2019b) was conducted with Zebrafish (*D.rerio*). The nominal concentrations were 1, 10 and 100 µg/L and not analytical verified. However, the study by

Zhou et al. (2019a) showed, that the measured concentrations could be maintained at about 80 % or higher of nominal using a similar exposure media preparation. In the actual study the exposure media was even replaced twice a day, whereas in the study by Zhou et al. (2019a) it was replaced once a day. Zebrafish embryos were exposed to OMC from 2 hpf (hours post-fertilisation) for 4 months until sexual maturation. At 120 dpf (days post-fertilisation) male and female fish were paired. F1 eggs were divided into 2 groups: with and without continued OMC exposure until 5 dpf. VTG and E2 levels significantly decreased in the visceral mass at 1, 10 and 100 µg/L at 40 dpf. Aromatase activity was not decreased. There were also effects on gene expression at 40 dpf exposure measured in the visceral mass: VTG1 was down-regulated at 1, 10 and 100 µg/L. No effect appeared on VTG2. AR was up-regulated at 10 and 100 µg/L. ER, PR, CYP19a, CYP19b, 17ß-HSD1, and 17ß-HSD3 were downregulated at 1, 10 and 100 µg/L. The malformation rate after 5 dpf was significantly increased at 100 µg/L in the F0 generation. Body weight decreased dose dependently (significantly decreased at 100 µg/L at 40 dpf (F0)).The 3-d hatching rates were significantly decreased in the F0 generation and in the not further exposed F1 generation at 10 and 100 µg/L, whereas in the further exposed F1 groups the hatching rate was significantly decreased at 1, 10 and 100 µg/L (see Table 16). In the blank and solvent control the hatching rates were > 80% fulfilling the validity criterium of OECD TG 234. 5-day survival was decreased at 100 µg/L in the F0 generation, but not in the F1 generations.

Table 16

3-d hatching rates (%) (Zhou et al., 2019b)								
F0	BC	82.1	F1 (without further expo)	BC	84.7	F1 (with further expo)	BC	-
	SC	84.3		SC	82.1		SC	80.7
	1 µg/L	80.2		1 µg/L	78.7		1 µg/L	72.4*
	10 µg/L	76.5*		10 µg/L	72.9*		10 µg/L	70.5*
	100 µg/L	74.8*		100 µg/L	72.1*		100 µg/L	68.3*

Asterisk indicates significant differences between the exposure group and the control group.

In the F1-group with continued exposure 5-day malformation rates were increased at 10 and 100 µg/L, whereas no effect on malformation was seen in the F1-group without continued exposure. In the F0 generation the 5-day malformation rates were increased at 100 µg/L.

Lee et al. (2019) conducted a 2-generation study with *O.latipes* (OECD CF level 5 study). The test concentrations were 50, 158, 500, 1580, 5000 µg/L (nominal concentrations; no chemical analysis). This study was conducted according to OECD TG 234 with slight modifications. During the exposure, the test solution was renewed three times per week, and water quality parameters (dissolved oxygen (DO), pH, temperature, and conductivity) were measured shortly before and after the renewal. Exposure was conducted under the following conditions: 6.9 ± 1.2 mgO2/L (DO), 7.5 ± 0.3 (pH), 26 ± 2 °C (temperature), 158 ± 18 µS/cm (conductivity) and under 15:9 h light:dark photoperiod. The eggs were randomly distributed into the glass beakers of 50 mL volume with 20 eggs per replicate and four replicates per control, solvent control (0.01% DMSO) or each treatment. The parents were exposed from 24 hpf (hours post-fertilisation) until 154 dpf (days post-fertilisation). At 106 dpf the fish were paired and a mating period of 49 d began, the number of eggs was determined until 154 dpf. At 120 dpf eggs (F1) were further exposed and the F1 generation was examined until 38 dpf. There were no significant effects on hatchability and survival. However effects on reproduction appeared: the number of eggs (per brood per day) was signicantly decreased at 50 µg/L and higher concentrations. Growth was decreased at 500 and 1580 µg/L at 38 dpf (only compared to solvent control). There were no significant effects on E2 level in plasma of adult males and females or on mRNA expression of ERα, ARα and VTG1 in the liver of males and females at 154 dpf. The

ARα gene was down-regulated (not significantly) at all concentrations at 38 dpf in F0 and F1, except at 50 µg/L in the F0 generation. A down-regulation trend on HSD3β and 17β-HSD3 genes (but no significant differences) was observed. Also thyroid related effects appeared: The DIO2 gene was down-regulated at the concentrations 158 to 5000 µg/L. The whole-body T3 content was decreased at 500 to 5000 µg/L, whereas T4 contents were not significantly decreased. A decreasing trend was seen for both T3 and T4 levels (F1, whole-body, 38 dpf), however the T4 levels were rather fluctuating. Several T3 and T4 measurements at 38 dpf of F1 did not fall within the detection range of the the ELISA kit (the non-detected values were substituted with minimum detectable level divided by 2). However, the authors stated that a decrease of TH levels with increasing OMC concentrations was seen.

A fish sexual development test accoding to OECD TG 234 as requested in the first substance evaluation round was conducted (Fort Environmental Laboratories, 2020a) with OMC with the species zebrafish and a duration of 60 dph. Survival and hatching success were not affected. The test showed significant effects on body weights and length which were both reduced in male and female fish at the single concentration of 46.9 µg/L (measured). Plasma VTG levels in female and male fish as well as of undifferentiated fish were not significantly different from the control. The test revealed decreased mean ovarian stage score (stage 0.0 ovaries in 66 % of treated females, whereas 61% of control females were in ovarian stage 1.0, no statistics). The study authors connected this with treatment-induced decrease in somatic growth. A statistically not significant increase in the ratio of females to males was seen, which according to the study authors was related to the delayed transition from the female to male phenotype and treatment-induced decrease in somatic growth. The test is rated by the eMSCA with Klimisch 1.

There were some studies showing no effects. Kunz et al. (2006) exposed zebrafish for 14 d to OMC at 8, 889, 5025 µg/L (measured) and no effects on VTG production were recorded. Schreurs et al. (2002) reported no oestrogenic activity in transgenic zebrafish at nominal 10 µM OMC (equal to 2.9 mg/L) after 96 h exposure. In Soto and Rodriguez-Fuentes (2014), the there was no induction of the synthesis of VTG in the liver of juvenile *A. saxatilis* after exposure for 96 h observed (some effects were noted after exposure to E2) (Soto, 2014). In Søgaard et al. (2021), no effect on VTG in plasma was seen after intraperitoneal injections of OMC in juvenile rainbow trout on days 0, 3, 6 and 10, as measured using blood sampling on days 0, 3, 6, 10 and 14.

Studies examining HPT axis:

In a study with *Danio rerio* conducted by Chu et al. (2021) two tests were performed: with adult male fish exposed for 1 d and with larvae exposed from 4 hpf to 5 dpf. The exposure concentrations were in both tests 1, 3, 10, 30 µM OMC. Effects appeared on the the thyroid system: In male adults the plasma T3 level decreased, however not significantly. In larvae the whole-body content of T3 significantly decreased at 3 µM (871 µg/L) and higher, and the T4 level significantly decreased at 1 µM (290 µg/L) and higher concentrations. Also the gene expression regarding the thyroid system was affected in several cases. The most pronounced were the TRß down-regulation at 1 µM (290 µg/L) and higher and the DIO1 down-regulation at 3 µM (871 µg/L) and higher in the liver of adult fish.

A 21-d Amphibian Metamorphosis Assay (AMA) with the African Clawed Frog (*Xenopus laevis*) was conducted with OMC according to OECD TG 231 (Fort Environmental Laboratories Inc., 2020b). Three concentrations were tested: 5.97, 18.1, and 44.2 µg/L (measured). The age of the organisms at test initiation was Nieuwkoop and Fabe (NF) stage 51. They were exposed for 21 days. Twenty tadpoles were exposed per test tank and four replicates per concentration and water control, were used. The median developmental stage, snout-vent length, hind limb development and body weight were not statistically different from control on SD day 7 and 21. No treatment-related histopathologic findings in the thyroids of tadpoles exposed to OMC were seen. In summary no thyroid effects were seen and there were no other signs of toxicity. The eMSCA rated

the study with Klimisch 1, GLP compliance was stated.

Summary of effects of OMC in *in vivo* ecotoxicity studies:

There were oestrogenic effects at high concentrations (plasma VTG level increased at 244 µg/L after 14 day exposure of adult male fathead minnow (Christen et al., 2011)). Also effects on the histology of male gonads were seen, as spermatogenesis seemed to be inhibited (proportion of spermatogonia increased, and significantly less spermatocytes were seen).

In the studies by Zhou et al. (2019a) and Zhou et al. (2019b), the amount of VTG and E2 were decreased (beginning with zebrafish embryos) after 40 dpf (Zhou et al., 2019b) and in adult zebrafish after 21 day exposure at 1, 10, 100 µg/L (Zhou et al., 2019a). In the study from (Zhou et al., 2019a) also testosterone was determined which was increased at 1, 10, 100 µg/L.

From these studies it seems that at lower concentrations there are anti-oestrogenic effects and at higher concentrations the effects were oestrogenic.

Effects on reproduction appeared in a two-generation reproduction study with Japanese medaka (Lee et al., 2019), where the number of eggs was sign. decreased at 50 µg/L and higher concentrations. In this study also some effects on thyroid hormones were observed.

A 21-d Amphibian Metamorphosis Assay (AMA) with the African Clawed Frog (*Xenopus laevis*) was conducted with OMC according to OECD TG 231 (Fort Environmental Laboratories Inc., 2020b). In this assay, no treatment-related effects on metamorphosis nor on histopathologic findings in the thyroids of tadpoles exposed to OMC were seen. In summary no thyroid effects were seen and no other signs of toxicity.

There were several effects on gene expression:

Effects on VTG gene expression were: upregulation in one study in adult medaka (Inui et al. 2003) at high concentrations (8.4 to 8443 mg/L); down-regulation in three studies with zebrafish in the visceral mass in adults and juveniles (Zhou et al., 2019a; Zhou et al., 2019b); down-regulated in whole fish, upregulated in liver of adult male zebrafish (Zucchi et al., 2011) at lower concentrations (from 1 – 840 µg/L).

Effects on gene expression of the ER and AR were not consistent in the several studies, partly up-regulated or down-regulated or without effects.

In summary it can be seen that OMC shows some endocrine activity in fish related to the HPG and HPT axis. The available data with respect to HPG axis activity are not clearly consistent with some data supporting an oestrogenic activity whilst some data pointing more to an anti-androgenic activity of OMC in the respective test systems. From the study performed by Lee et al. (2019) where a decrease in the number of eggs per female/day was observed, no clear conclusion on the underlying mode of action for this adverse effect can be drawn. The available AMA study did not show any T-mediated activity or adversity up to the highest testes concentration of 44 µg/L. Thus, even though there are hints for an endocrine activity of OMC in fish and adverse effects on reproduction that can be endocrine mediated were observed in one study, the eMSCA considers the overall database inconclusive. Taking the aspect of the poor water solubility of OMC into account and the ongoing substance evaluation of the close structural analogue IPMC (EC No 275-702-5; CAS No 71617-10-2), further long-term testing requests to clarify the concern for ED ENV are judged to be disproportionate at the moment by the eMSCA.

7.10.2. Endocrine disruption - Human health

The scope of the eMSCA assessment (as a follow-up to the initial assessment by UK) is focused on the endocrine disruption concern for the environment. Available mammalian studies were checked whether they would inform on the identified concern for endocrine disruption in the environment.

There is limited evidence from mechanistic *in vitro* and *in vivo* studies for EAS-related endocrine activity possibly pointing to some oestrogenicity of OMC (see section 7.10.1). Furthermore, the evidence for adversity related to the EATS modalities in intact animals is ambiguous. The *in vivo* studies in mammals comprising OECD levels 3-5 for OMC are presented in Table 18 to Table 20**Error! Reference source not found.** in Annex 1. The most relevant findings observed include:

<u>Epididymal sperm count</u>

Significantly decreased epididymal sperm counts in adult F1 Wistar rats were observed by Axelstad et al. (2011) after pre- and postnatal (gavage) exposure at all doses (-25.8%, -15.5% and -25.3% compared to controls at 500, 750 and 1000 mg/kg bw/d, respectively). There was no dose response, and testes and cauda epididymis weights remained unchanged in F1 adults. Also in a dietary two-generation study with Wistar rats, a marginal but significant reduction (-8% compared to controls) in epididymal sperm count in F1 males at the high dose of 1000 mg/kg bw/d (700 spermatids/g vs 763 spermatids/g in controls) was reported (BASF-SE, 2007; Schneider et al., 2005). Sperm counts were within the historical control range (517-727 spermatids/g, mean 625, N=19) while the concurrent control values were unusually high. Notably, effects on epididymal sperm counts could not be reproduced in a recent (dietary) modified one-generation study with Sprague Dawley rats (NTP, 2022), and sperm motility or morphology was not influenced in any study.

<u>Prostate weight and histology</u>

Absolute and relative prostate weight was significantly lower at the high dose in F1 males on PND16 (absolute: -31.5%, relative: -18.5% vs controls) and in F1 adults (absolute: -37.4%, relative: -22% vs controls) in the study by Axelstad et al. (2011). Histology on PND16 revealed a reduced presence of large fluid-filled acini and an increased presence of intermediate size acini at the high dose, indicating delayed development (probably related to reduced growth performance of these animals). In adult males, increased acinar atrophy was reported at the mid and high dose, interpreted by the authors as a typical finding in rats exposed to oestrogenic substances. In NTP (2022), reduced absolute and relative prostate weight at the high dose was reported for the F1 subchronic cohort. However, there were no histological correlates, and no effects on prostate weight were observed in the other cohorts with higher animals numbers. Therefore, this finding in one cohort is not considered treatment-related. In agreement with this, no effect on prostate weight or histology were reported in the OECD TG 416 study (BASF-SE, 2007; Schneider et al., 2005).

<u>Oestrous cycle</u>

In the modified one-generation study performed by NTP (2022), high-dose F1 females spent a slightly but significantly longer time in oestrous (significant in all dose groups; no dose-response) while the overall cycle length remained unchanged (days in oestrus: 1.1, 1.3, 1.3, and 1.3 at 0, 1000, 3000, and 6000 ppm, respectively). Time in di-oestrus was correspondingly reduced. According to the authors, this finding cannot readily be explained by lower body weights since lower body weight usually is expected to lead to an increase in overall cycle length. Since no specific effects of OMC on other female reproductive parameters were reported in this study, the eMSCA considers this finding as being of questionable adversity.

<u>Implantation sites and litter size</u>

A significantly reduced number of implantation sites was reported in the two-generation study (BASF-SE, 2007; Schneider et al., 2005) in the P0 (12.0, 12.3, 11.3, and 10.0 at 0, 150, 450, and 1000 mg/kg bw/d, respectively) and F1 generation (12.4, 11.5, 10.7, 10.3 at 0, 150, 450, and 1000 mg/kg bw/d, respectively), reaching statistical significance at the high dose in P0 animals and at the mid and high doses in F1 animals. Ovarian follicle count revealed no changes upon treatment. F1 litter size was significantly reduced at the high dose (11.0, 11.9, 10.9, and 9.2 at 0, 150, 450, and 1000 mg/kg bw/d, respectively) but no effect was observed on F2 litter size. The implantation rate in P0 and F1 dams was at the lower end of the historical control range (10.2-11.5 implants per dam, N = 12 experiments from the years 2000-2002), or just outside, and the concurrent control values exceeded the historical control range. Given the rather mild body weight effects in females (generally not exceeding 10% difference to controls), the finding of reduced implantations and litter size cannot be completely dismissed as a consequence of unspecific toxicity. Nonetheless, the reduced implantations and litter size seem to be occasional findings since none of the other reproductive toxicity studies reported significant effects of OMC on these parameters.

In conclusion, the evidence for adversity related to the EAS modalities is ambiguous.

Thyroid hormone system

OMC clearly displays endocrine activity *in vivo* with regard to the T-modality by inducing changes in levels of thyroid hormones. (Axelstad et al., 2011; Axelstad et al., 2019; BASF-SE, 2004; Ferraris et al., 2019; Klammer et al., 2007; Schmutzler et al., 2007a; Seidlova-Wuttke et al., 2006a). In particular, T4 levels were affected with sometimes severe reductions, although in some studies no changes or increases were observed. No study detected thyroid hypertrophy/hyperplasia and effects on TSH were either absent or rather mild and of varying effect-directions (both, increases and decreases were observed).

In the developmental toxicity study by Axelstad et al. (2011), T4 was severely decreased in dams at all dose groups at GD15 and PND16 (PND16: -70%, -97% and -100% at 500, 750 and 1000 mg/kg bw/d, respectively). In offspring, T4 was decreased only in F1 male pups on PND16 but to a lesser extent than in dams (-14%, -34% and -35% at 500, 750 and 1000 mg/kg bw/d, respectively compared to controls). Despite the pronounced effect on T4 in dams, no histological changes were observed in the thyroid glands reminiscent of TSH feedback (although thyroid weight was increased in F1 animals on PND16 at the mid and high dose). Extensive behavioural testing was performed including recording of motor activity, radial arm maze (RAM) test, acoustic startle response and testing of auditory function. Significant decreases were observed in motor activity in females (at post-natal week (PNW) 9 (≥ 750 mg/kg bw/d) and at PNW 17 (1000 mg/kg bw/day)). In the RAM test, less errors were made by males (PNW 22-25) at the low and high dose but not at the mid dose. The decrease in errors was seen only during the first week of the three-week testing. Therefore, it is unclear to the eMSCA whether this observation is treatment-related. No effects were observed in the auditory startle response and in the hearing test. The authors concluded that the changes observed in the behavioural testing were rather different from what is expected from gestational or perinatal hypothyroidism (e.g. by PTU treatment). For example, it would be expected that motor activity becomes increased instead of decreased, and that spatial learing is impaired in the RAM test. Since T4 was only mildly affected in F1 males, and no effects were seen in F1 females, the OMC-induced changes observed in the behavioural testing might not be a consequence of a thyroid hormone-related mechanism. However, it should be kept in mind that plasma thyroid hormone levels are not necessarily a good predictor of tissue levels e.g. in the brain (see Gilbert et al. (2021))

In a feasibility study, Axelstad et al. (2019) investigated the formation of brain cortical heterotopias as an apical neurodevelopmental read-out for perinatal hypothyroidism (see also e.g. O'Shaughnessy et al. (2019)) after exposure to substances affecting the thyroid

hormone system via different modes of action. The test chemicals included PTU, DE-71 (a commercial mixture of polybrominated diphenyl ethers), and OMC (intitially at doses of 500 and 750 mg/kg bw/d). The high dose of 750 mg/kg bw/d was reduced after 15 days of dosing to 375 mg/kg bw/d due to excessive maternal toxicity (about -50% body weight gain compared to controls between GD7 and GD21) resulting in a rather complex dosing regime (see also Table 20). At the new high dose of 500 mg/kg bw/d, there was still maternal toxicity and increased postimplantation and perinatal losses. OMC induced changes in T3 and T4 in dams and offspring. However, these changes were less pronounced than in the study by Axelstad et al. (2011) reported before and did not show a clear pattern (decreases or increases were observed depending on the time-point), and a dose-response was often lacking. In contrast to PTU, there was no increase of heterotopia incidence or volume by OMC (investigated in male pups only). Despite the absence of heterotopia formation, OMC induced changes in the cortical expression of genes known to be regulated by thyroid hormones. In part, the expression pattern resembled the one observed for PTU in the same study. The adversity associated with these changes in gene expressions is unknown. OMC additionally induced lower brain weight in offspring on PND22 (-9.6% and -13.2% compared to controls at 375, and 500 mg/kg bw/d). This effect cannot be readily explained by lower body weights of pups, because brain weight remains usually stable unless body weight reduction is severe. Furthermore, this effect was not seen by PTU or DE-71 treatment causing similar reductions in body weight (about 8-15% lower body wight compared to controls) but induced severe reductions of T4 levels. Thus, this might indicate potential developmental neurotoxicity by OMC probably independent of effects on thyroid hormone levels.

In conclusion, OMC clearly displays endocrine activity with regard to the thyroid hormone system. However, although there are some indications for neurodevelopmental toxicity, these findings cannot plausibly related to T-mediated endocrine activity.

7.10.3. Conclusion on endocrine disrupting properties for the environment

Regarding the environment the eMSCA considers the available data inconclusive. It is noted that there are various hints for an endocrine activity of OMC in fish, however no clear conclusion on the endocrine mode of action can be drawn. Adversity (reduced number of eggs) was observed in one long-term fish study but no clear mechanistic data are obtained in this study that would allow the conclusion that OMC is an ED based on the WHO/IPCS definition of an endocrine disruptor in the environment. The data from mammalian studies does not provide conclusive evidence for EATS-mediated adverse effects. However, some concerns remain, in particular regarding the potential neurodevelopmental effects of OMC (dependent or independent of the T-modality). The available AMA study did not show T-mediated activity or adversity.

The request for further data in the course of the present substance evaluation process is considered disproportionate based on all the available data and the ongoing substance evaluation for the close structural analogue IPMC addressing the environmental ED concern. Data provided and assessed during the substance evaluation of IPMC might also be conclusive for the environmental ED concern of OMC.

7.11. PBT and VPVB assessment

7.11.1. Persistence (P)

OMC is readily biodegradable. The eMSCA considers that it is not persistent and does not fulfill the P criterion according to Annex XIII REACH or the CLP hazard class for PBT/vPvB.

7.11.2. Bioaccumulation (B)

Based on available information, OMC is not bioaccumulating. Despite remaining uncertainties regarding the available bioconcentration study, the eMSCA does not consider further information necessary to assess whether OMC fulfils the B criterion according to Annex XIII REACH or CLP since it is not considered persistent.

7.11.3. Toxicity (T)

Available information on OMC suggests that the substance fulfils the T criterion according to REACH Annex XIII or CLP based on aquatic toxicity data. The eMSCA plans to submit a CLH dossier to classify the substance as Aquatic Chronic 1 based on its effects observed in long-term studies in fish.

Overall, OMC is not considered to be PBT or vPvB as it does not screen as persistent.

7.12. Exposure assessment

Additional information with regard to environmental exposure assessment was required in the substance evaluation decision on OMC and consequently was provided by the registrants. The information was assessed to comply with the information requirement in the substance evaluation decision. However, a refined exposure assessment was not part of the follow-up assessment by the DE CA.

7.13. Risk characterisation

Not part of the follow-up assessment by the DE CA.

7.14. References

Axelstad M., Boberg J., Hougaard K.S., Christiansen S., Jacobsen P.R., Mandrup K.R., Nellemann C., Lund S.P., and Hass U. (2011): Effects of pre- and postnatal exposure to the UV-filter octyl methoxycinnamate (OMC) on the reproductive, auditory and neurological development of rat offspring. Toxicol Appl Pharmacol 250 (3), 278-290. DOI: 10.1016/j.taap.2010.10.031

Axelstad M., Ramhoj L., Scholze M., Martin O., Köhrle J., and Kortenkamp A. (2019): Development of a study protocol for thyroid disruptor testing in the mammalian system - Deliverable 16: Final feasibility study report. Project leader: Brunel University, Institute of Environment, Health and Societies. Commision E. DOI: doi: 10.2779/194307

BASF-SE (2001): Uvinul MC 80; Uterotrophic Assay in immature female Wistar rats; Oral administration (gavage). 07R0228/99121. BASF SE, Experimental Toxicology and Ecology. Unpublished study report.

BASF-SE (2003): Uvinul MC 80 N; Hershberger Assay in Wistar rats. 4750495/00149. BASF SE, Experimental Toxicology and Ecology. Unpublished study report.

BASF-SE (2004): Uvinul MC 80 N; Hormone study in female Wistar rats; Administration in the diet over 4 weeks. 4800371/01137. Unpublished study report.

BASF-SE (2007): Uvinul MC 80 N - Two-Generation Reproduction Toxicity Study in Wistar Rats - Continuous Dietary Administration. 70R0371/01057. BASF SE. Unpublished study report.

Cahova J., Blahova J., Marsalek P., Doubkova V., Franc A., Garajová M., Tichy F., Mares J., and bodova Z. (2021): The biological activity of the organic UV filter ethylhexyl methoxycinnamate in rainbow trout (Oncorhynchus mykiss). Sci Total Environ 774, 145570. DOI: 10.1016/j.scitotenv.2021.145570

Carbone S., Szwarcfarb B., Reynoso R., Ponzo O.J., Cardoso N., Ale E., Moguilevsky J.A., and Scacchi P. (2010): In vitro effect of octyl - methoxycinnamate (OMC) on the release of Gn-RH and amino acid neurotransmitters by hypothalamus of adult rats. Exp Clin Endocrinol Diabetes 118 (5), 298-303. DOI: 10.1055/s-0029-1224153

Christen V., Zucchi S., and Fent K. (2011): Effects of the UV-filter 2-ethyl-hexyl-4-trimethoxycinnamate (EHMC) on expression of genes involved in hormonal pathways in fathead minnows (Pimephales promelas) and link to vitellogenin induction and histology. Aquat Toxicol 102 (3-4), 167-176. DOI: 10.1016/j.aquatox.2011.01.013

Chu S., Kwon B.R., Lee Y.M., Zoh K.D., and Choi K. (2021): Effects of 2-ethylhexyl-4-methoxycinnamate (EHMC) on thyroid hormones and genes associated with thyroid, neurotoxic, and nephrotoxic responses in adult and larval zebrafish (Danio rerio). Chemosphere 263, 128176. DOI: 10.1016/j.chemosphere.2020.128176

Danovaro R., Bongiorni L., Corinaldesi C., Giovanelli D., Damiani E., Astolfi P., Greci L., Pusceddu A. (2008): Sunscreens Cause Coral Bleaching by Promoting Viral Infec-tions. Environ Health Perspect. 2008, 116(4), 441-447. DOI: 10.1289/ehp.10966

Department of Toxicology (1998): Uvinul MC 80: Acute toxicity study on the zebra fish (Brachydanio rerio RAM. and BUCH.) in a static System (96 h). 17F0653/965182. Department of Toxicology. Unpublished study report.

Department of Toxicology and Ecology (2001): Uvinul MC 80 - Determination of the inhibitory effect on the cell multiplication of unicellular green algae. 01/0111/60/2. Department of Toxicology and Ecology. Unpublished study report.

Department of Ecology (2003): Uvinul MC 80 N - Determination of the acute effect on the swimming ability of the water flea, Daphnia magna Straus. 01/0371/50/2. Department of Ecology. Unpublished study report.

Fent K., Kunz P.Y., Zenker A., Rapp M. 2010: A tentative environmental risk as-sessment of the UV-filters 3-(4-methylbenzylidene-camphor), 2-ethyl-hexyl-4-trimethoxycinnamate, benzophenone-3, benzophenone-4 and 3-benzylidene cam-phor. Mar Environ Res. 2010, 69, S4-6. DOI: 10.1016/j.marenvres.2009.10.010

Ferraris F.K., Garcia E.B., Chaves A.D.S., de Brito T.M., Doro L.H., Félix da Silva N.M., Alves A.S., Pádua T.A., Henriques M., Cardoso Machado T.S., and Amendoeira F.C. (2019): Exposure to the UV Filter Octyl Methoxy Cinnamate in the Postnatal Period Induces Thyroid Dysregulation and Perturbs the Immune System of Mice. Front Endocrinol (Lausanne) 10, 943. DOI: 10.3389/fendo.2019.00943

Fort Environmental Laboratories (2020a): 60-dph Fish Sexual Development Test (FSDT) of 2-Ethylhexyl trans-4-methoxycinnamate (OMC) with Zebrafish, Danio rerio. 56F0153/18X092. Fort Environmental Laboratories, Inc. (In-Life Testing), 515 South Duncan Street, Stillwater, OK 74074, USA. Unpublished study report.

Fort Environmental Laboratories (2020b): 21-d Amphibian Metamorphosis Assay (AMA) of 2-Ethylhexyl trans-4-methoxycinnamate (OMC) with African Clawed Frog, Xenopus laevis. 90F0153/18X09. Fort Environmental Laboratories, Inc. (In-Life Testing), 515 South Duncan Street, Stillwater, OK 74074, USA. unpublished study report.

Fort Environmental Laboratories (2021a): 21-d Daphnia magna Reproduction Test of 2-Ethylhexyl trans-4-methoxycinnamate (OMC). 51E0153/18X003. Fort Environmental Laboratories, Inc. (In-Life Testing), 515 South Duncan Street, Stillwater, OK 74074, USA. unpublished study report.

Fort Environmental Laboratories (2021b): 7-d Lemna minor Growth Inhibition Test of 2-Ethylhexyl trans-4-methoxycinnamate (OMC). 63E0153/18X004. Fort Environmental Laboratories, Inc. (In-Life Testing), 515 South Duncan Street, Stillwater, OK 74074. unpublished study report.

Gilbert M.E., O'Shaughnessy K.L., Thomas S.E., Riutta C., Wood C.R., Smith A., Oshiro W.O., Ford R.L., Hotchkiss M.G., Hassan I., and Ford J.L. (2021): Thyroid Disruptors: Extrathyroidal Sites of Chemical Action and Neurodevelopmental Outcome-An Examination Using Triclosan and Perfluorohexane Sulfonate. Toxicol Sci 183 (1), 195-213. DOI: 10.1093/toxsci/kfab080

Gomez E., Pillon A., Fenet H., Rosain D., Duchesne M.J., Nicolas J.C., Balaguer P., and Casellas C. (2005): Estrogenic activity of cosmetic components in reporter cell lines: parabens, UV screens, and musks. J Toxicol Environ Health A 68 (4), 239-251. DOI: 10.1080/15287390590895054

Hazleton (1980a): 28-day percutaneous toxicity study in the rat. 2113-161/26, Hazleton Laboratories Europe Ltd, unpublished study report

Hazleton (1980b): A three-week dermal toxicity study in the rabbit. 2178-161/27. Hazleton Laboratories Europe Ltd. , unpublished study report

Heneweer M., Muusse M., van den Berg M., and Sanderson J.T. (2005): Additive estrogenic effects of mixtures of frequently used UV filters on pS2-gene transcription in MCF-7 cells. Toxicol Appl Pharmacol 208 (2), 170-177. DOI: 10.1016/j.taap.2005.02.006

Hoffmann-La Roche (1983a): Embryotoxicity and teratogenicity study in rats with oral administration of 2-ethyl-p-methoxy cinnamate. Segment II-teratological study. 104'958. F. Hoffmann-la Roche & Co, Ltd. Unpublished study report.

Hoffmann-La Roche (1983b): Embryotoxicity Study in Rabbits with Oral Administration of 2-Ethylhexyl-p-methoxy cinnamate. Segment II-Teratological study. F. Hoffmann-La Roche & Co. Ltd. IM 104'752. Unpublishd study report.

Hoffmann-La Roche (1984): Ethylhexyl Methoxycinnamate: 13-week oral dietary toxicity study in the rat. F. Hoffmann-La Roche & Co. Ltd. IM104812. Unpublished study report.

Hofmann P.J., Schomburg L., and Köhrle J. (2009): Interference of endocrine disrupters with thyroid hormone receptor-dependent transactivation. Toxicol Sci 110 (1), 125-137. DOI: 10.1093/toxsci/kfp086

Inui M., Adachi T., Takenaka S., Inui H., Nakazawa M., Ueda M., Watanabe H., Mori C., Iguchi T., and Miyatake K. (2003): Effect of UV screens and preservatives on vitellogenin and choriogenin

production in male medaka (Oryzias latipes). Toxicology 194 (1-2), 43-50. DOI: 10.1016/s0300-483x(03)00340-8

JRC (2013). Key Scientific issues relevant to the identification and characterisation of endocrine disrupting substances – Report of the Endocrine Disruptors Expert Advisory Group (ED EAG). Eds. Munn S. and Gourmenou M. Pp 32. Available at: https://ec.europa.eu/jrc/en/publication/eur-scientific-and-technical-research-reports/key-scientific-issues-relevant-identification-and-characterisation-endocrine-disrupting

Klammer H., Schlecht C., Wuttke W., and Jarry H. (2005): Multi-organic risk assessment of estrogenic properties of octyl-methoxycinnamate in vivo A 5-day sub-acute pharmacodynamic study with ovariectomized rats. Toxicology 215 (1-2), 90-96. DOI: 10.1016/j.tox.2005.06.026

Klammer H., Schlecht C., Wuttke W., Schmutzler C., Gotthardt I., Köhrle J., and Jarry H. (2007): Effects of a 5-day treatment with the UV-filter octyl-methoxycinnamate (OMC) on the function of the hypothalamo-pituitary-thyroid function in rats. Toxicology 238 (2-3), 192-199. DOI: 10.1016/j.tox.2007.06.088

Kunz P.Y. and Fent K. (2006): Multiple hormonal activities of UV filters and comparison of in vivo and in vitro estrogenic activity of ethyl-4-aminobenzoate in fish. Aquat Toxicol 79 (4), 305-324. DOI: 10.1016/j.aquatox.2006.06.016

Kunz P.Y., Galicia H.F., and Fent K. (2006): Comparison of in vitro and in vivo estrogenic activity of UV filters in fish. Toxicol Sci 90 (2), 349-361. DOI: 10.1093/toxsci/kfj082

Lab. Pharm. (1979): 1979004 3 Wochen-Toxizität von Neo-Heliopan E 1000 und Neo Heliopan AV an Sprague-Dawley-Ratten bei Verabreichung per Magensonde (orientierende Prüfung). Laboratorium für Pharmakologie und Toxikologie. Unpublished study report.

Lee I., Lee J., Jung D., Kim S., and Choi K. (2019): Two-generation exposure to 2-ethylhexyl 4-methoxycinnamate (EHMC) in Japanese medaka (Oryzias latipes) and its reproduction and endocrine related effects. Chemosphere 228, 478-484. DOI: 10.1016/j.chemosphere.2019.04.123

Ma R., Cotton B., Lichtensteiger W., and Schlumpf M. (2003): UV Filters with Antagonistic Action at Androgen Receptors in the MDA-kb2 Cell Transcriptional-Activation Assay. Toxicological Sciences 74 (1), 43-50. DOI: 10.1093/toxsci/kfg102 (last accessed 5/11/2020)

Mnif W., Pascussi J.-M., Pillon A., Escande A., Bartegi A., Nicolas J.-C., Cavaillès V., Duchesne M.-J., and Balaguer P. (2007): Estrogens and antiestrogens activate hPXR. Toxicology Letters 170 (1), 19-29. DOI: https://doi.org/10.1016/j.toxlet.2006.11.016

Morohoshi K., Yamamoto H., Kamata R., Shiraishi F., Koda T., and Morita M. (2005): Estrogenic activity of 37 components of commercial sunscreen lotions evaluated by in vitro assays. Toxicol In Vitro 19 (4), 457-469. DOI: 10.1016/j.tiv.2005.01.004

Nashev L.G., Schuster D., Laggner C., Sodha S., Langer T., Wolber G., and Odermatt A. (2010): The UV-filter benzophenone-1 inhibits 17beta-hydroxysteroid dehydrogenase type 3: Virtual screening as a strategy to identify potential endocrine disrupting chemicals. Biochem Pharmacol 79 (8), 1189-1199. DOI: 10.1016/j.bcp.2009.12.005

NTP (2020): NTP Technical Report on the Toxicology and Carcinogenesis Studies of 2-Hydroxy-4-methoxybenzophenone (CASRN 131-57-7) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and B6C3F1/N Mice: Technical Report 597 [Internet]. Research Triangle Park (NC): National Toxicology Program; 2020 May. Appendix F, Endocrine Disruptor Screening Panel Studies. https://www.ncbi.nlm.nih.gov/books/NBK563384/

NOTOX (2000a): 96-hour acute toxicity study in carp with 2-Ethylhexyl 4-methoxycinnamate (static). 284221. NOTOX B.V., 's-Hertogenbosch, The Netherlands. Unpublished study report.

NOTOX (2000b): Fresh water algal growth inhibition test with water accomodated fractions of 2-Ethylhexyl 4-methoxycinnamate. 284232. NOTOX B.V., 's-Hertogenbosch, The Netherlands. Unpublished study report.

NTP (2022): NTP Developmental and Reproductive Toxicity Reports. In: NTP Developmental and Reproductive Toxicity Technical Report on the Modified One-Generation Study of 2-Ethylhexyl p-Methoxycinnamate (CASRN 5466-77-3) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley(®) SD(®)) Rats with Prenatal, Reproductive Performance, and Subchronic Assessments in F(1) Offspring: DART Report 06. National Toxicology Program, Research Triangle Park (NC)

OECD (2012): Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption. https://www.oecd-ilibrary.org/docserver/9789264304741-en.pdf

O'Shaughnessy K.L., Thomas S.E., Spring S.R., Ford J.L., Ford R.L., and Gilbert M.E. (2019): A transient window of hypothyroidism alters neural progenitor cells and results in abnormal brain development. Sci Rep 9 (1), 4662. DOI: 10.1038/s41598-019-40249-7

Olker J.H., Korte J.J., Denny J.S., Hartig P.C., Cardon M.C., Knutsen C.N., Kent P.M., Christensen J.P., Degitz S.J., and Hornung M.W. (2019): Screening the ToxCast Phase 1, Phase 2, and e1k Chemical Libraries for Inhibitors of Iodothyronine Deiodinases. Toxicol Sci 168 (2), 430-442. DOI: 10.1093/toxsci/kfy302

Olker J.H., Korte J.J., Denny J.S., Haselman J.T., Hartig P.C., Cardon M.C., Hornung M.W., and Degitz S.J. (2021): In vitro screening for chemical inhibition of the iodide recycling enzyme, iodotyrosine deiodinase. Toxicol In Vitro 71, 105073. DOI: 10.1016/j.tiv.2020.105073

Paredes E, Perez S, Rodil R, Quintana JB, Beiras R (2014): Ecotoxicological evalua-tion of four UV filters using marine organisms from different trophic levels Isochrysis galbana, Mytilus galloprovincialis, Paracentrotus lividus, and Siriella armata. Chem-osphere 2014 Jun, 104, 44-50. DOI: 10.1016/j.chemosphere.2013.10.053

Paul-Friedman K., Martin M., Crofton K.M., Hsu C.W., Sakamuru S., Zhao J., Xia M., Huang R., Stavreva D.A., Soni V., Varticovski L., Raziuddin R., Hager G.L., and Houck K.A. (2019): Limited Chemical Structural Diversity Found to Modulate Thyroid Hormone Receptor in the Tox21 Chemical Library. Environ Health Perspect 127 (9), 97009. DOI: 10.1289/ehp5314

Perez S., Beiras R. (2010): The mysid Siriella armata as a model organism in marine ecotoxicology: comparative acute toxicity sensitivity with Daphnia magna. Ecotoxi-cology 2010, 19(1), 196-206. DOI: 10.1007/s10646-009-0405-3.

Phelan-Dickinson S.J., Palmer B.C., Chen Y., and DeLouise L.A. (2020): The UVR Filter Octinoxate Modulates Aryl Hydrocarbon Receptor Signaling in Keratinocytes via Inhibition of CYP1A1 and CYP1B1. Toxicol Sci 177 (1), 188-201. DOI: 10.1093/toxsci/kfaa091

Rehfeld A., Dissing S., and Skakkebæk N.E. (2016): Chemical UV Filters Mimic the Effect of Progesterone on Ca2+ Signaling in Human Sperm Cells. Endocrinology 157 (11), 4297-4308. DOI: 10.1210/en.2016-1473 (last accessed 5/11/2020)

Rehfeld A., Egeberg D.L., Almstrup K., Petersen J.H., Dissing S., and Skakkebaek N.E. (2018): EDC IMPACT: Chemical UV filters can affect human sperm function in a progesterone-like manner. Endocr Connect 7 (1), 16-25. DOI: 10.1530/ec-17-0156

Rodil R., Moeder M., Altenburger R., Schmitt-Jansen M., (2009): Photostability and phytotoxicity of selected sunscreen agents and their degradation mixtures in water. Anal Bioanal Chem. 2009, 395(5), 1513-24. DOI: 10.1007/s00216-009-3113-1

Schlumpf M., Cotton B., Conscience M., Haller V., Steinmann B., and Lichtensteiger W. (2001): In vitro and in vivo estrogenicity of UV screens. Environ Health Perspect 109 (3), 239-244. DOI: 10.1289/ehp.01109239

Schmutzler C., Bacinski A., Gotthardt I., Huhne K., Ambrugger P., Klammer H., Schlecht C., Hoang-Vu C., Grüters A., Wuttke W., Jarry H., and Köhrle J. (2007a): The ultraviolet filter benzophenone 2 interferes with the thyroid hormone axis in rats and is a potent in vitro inhibitor of human recombinant thyroid peroxidase. Endocrinology 148 (6), 2835-2844. DOI: 10.1210/en.2006-1280

Schmutzler C., Gotthardt I., Hofmann P.J., Radovic B., Kovacs G., Stemmler L., Nobis I., Bacinski A., Mentrup B., Ambrugger P., Grüters A., Malendowicz L.K., Christoffel J., Jarry H., Seidlovà-Wuttke

D., Wuttke W., and Köhrle J. (2007b): Endocrine disruptors and the thyroid gland--a combined in vitro and in vivo analysis of potential new biomarkers. Environ Health Perspect 115 Suppl 1 (Suppl 1), 77-83. DOI: 10.1289/ehp.9369

Schmutzler C., Hamann I., Hofmann P.J., Kovacs G., Stemmler L., Mentrup B., Schomburg L., Ambrugger P., Gruters A., Seidlova-Wuttke D., Jarry H., Wuttke W., and Kohrle J. (2004): Endocrine active compounds affect thyrotropin and thyroid hormone levels in serum as well as endpoints of thyroid hormone action in liver, heart and kidney. Toxicology 205 (1-2), 95-102. DOI: 10.1016/j.tox.2004.06.041

Schneider S., Deckardt K., Hellwig J., Küttler K., Mellert W., Schulte S., and van Ravenzwaay B. (2005): Octyl methoxycinnamate: two generation reproduction toxicity in Wistar rats by dietary administration. Food Chem Toxicol 43 (7), 1083-1092. DOI: 10.1016/j.fct.2005.02.013

Schreurs R., Lanser P., Seinen W., and van der Burg B. (2002): Estrogenic activity of UV filters determined by an in vitro reporter gene assay and an in vivo transgenic zebrafish assay. Arch Toxicol 76 (5-6), 257-261. DOI: 10.1007/s00204-002-0348-4

Schreurs R.H., Sonneveld E., Jansen J.H., Seinen W., and van der Burg B. (2005): Interaction of polycyclic musks and UV filters with the estrogen receptor (ER), androgen receptor (AR), and progesterone receptor (PR) in reporter gene bioassays. Toxicol Sci 83 (2), 264-272. DOI: 10.1093/toxsci/kfi035

Seidlova-Wuttke D., Christoffel J., Rimoldi G., Jarry H., and Wuttke W. (2006a): Comparison of effects of estradiol with those of octylmethoxycinnamate and 4-methylbenzylidene camphor on fat tissue, lipids and pituitary hormones. Toxicol Appl Pharmacol 214 (1), 1-7. DOI: 10.1016/j.taap.2005.11.002

Seidlova-Wuttke D., Jarry H., Christoffel J., Rimoldi G., and Wuttke W. (2006b): Comparison of effects of estradiol (E2) with those of octylmethoxycinnamate (OMC) and 4-methylbenzylidene camphor (4MBC) — 2 filters of UV light – on several uterine, vaginal and bone parameters. Toxicology and Applied Pharmacology 210 (3), 246-254. DOI: https://doi.org/10.1016/j.taap.2005.05.006

Sieratowicz A, Kaiser D, Behr M, Oetken M, Oehlmann J. (2011): Acute and chronic toxicity of four frequently used UV filter substances for Desmodesmus subspicatus and Daphnia magna. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2011, 46(12), 1311-1319. DOI: 10.1080/10934529.2011.602936

Søgaard R., Holbech H., Nørum U., and Bjerregaard P. (2021): Investigation of the in vivo estrogenicity of the UV-filters 4-methylbenzylidene camphor and octyl methoxy cinnamate in rainbow trout (Oncorhynchus mykiss). Ecotoxicol Environ Saf 224, 112657. DOI: 10.1016/j.ecoenv.2021.112657

Song M., Song M.K., Choi H.S., and Ryu J.C. (2013): Monitoring of deiodinase deficiency based on transcriptomic responses in SH-SY5Y cells. Arch Toxicol 87 (6), 1103-1113. DOI: 10.1007/s00204-013-1018-4

Soto M.R.-F., G. (2014): Evaluation of the estrogenic effects of UV filters on the sergeant major damselfish, Abudefduf saxatilis. Ciencias Marinas 40 (3), 187-196. DOI: https://doi.org/10.7773/cm.v40i3.2390

Strajhar P., Tonoli D., Jeanneret F., Imhof R.M., Malagnino V., Patt M., Kratschmar D.V., Boccard J., Rudaz S., and Odermatt A. (2017): Steroid profiling in H295R cells to identify chemicals potentially disrupting the production of adrenal steroids. Toxicology 381, 51-63. DOI: 10.1016/j.tox.2017.02.010

Szwarcfarb B., Carbone S., Reynoso R., Bollero G., Ponzo O., Moguilevsky J., and Scacchi P. (2008): Octyl-methoxycinnamate (OMC), an ultraviolet (UV) filter, alters LHRH and amino acid neurotransmitters release from hypothalamus of immature rats. Exp Clin Endocrinol Diabetes 116 (2), 94-98. DOI: 10.1055/s-2007-1004589

Wang J., Richard A.M., Murr A.S., Buckalew A.R., Lougee R.R., Shobair M., Hallinger D.R., Laws S.C., and Stoker T.E. (2021): Expanded high-throughput screening and chemotype-enrichment

analysis of the phase II: e1k ToxCast library for human sodium-iodide symporter (NIS) inhibition. Arch Toxicol. DOI: 10.1007/s00204-021-03006-2

WHO/International Programme on Chemical Safety. (2002): Global assessment of the state of the science of endocrine disruptors (Damstra T, Barlow S, Bergman A, Kavlock R, Van Der Kraak G, eds.). http://www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en

Yin Q., Fischer L., Noethling C., and Schaefer W.R. (2015): In vitro-assessment of putative antiprogestin activities of phytochemicals and synthetic UV absorbers in human endometrial Ishikawa cells. Gynecol Endocrinol 31 (7), 578-581. DOI: 10.3109/09513590.2015.1047448

Zhou R., Lu G., Yan Z., Bao X., Zhang P., and Jiang R. (2019a): Bioaccumulation and biochemical effects of ethylhexyl methoxy cinnamate and its main transformation products in zebrafish. Aquat Toxicol 214, 105241. DOI: 10.1016/j.aquatox.2019.105241

Zhou R., Lu G., Yan Z., Jiang R., Shen J., and Bao X. (2019b): Parental transfer of ethylhexyl methoxy cinnamate and induced biochemical responses in zebrafish. Aquat Toxicol 206, 24-32. DOI: 10.1016/j.aquatox.2018.11.001

Zucchi S., Oggier D.M., and Fent K. (2011): Global gene expression profile induced by the UV-filter 2-ethyl-hexyl-4-trimethoxycinnamate (EHMC) in zebrafish (Danio rerio). Environ Pollut 159 (10), 3086-3096. DOI: 10.1016/j.envpol.2011.04.013

7.15. Abbreviations

%	Percentage
^{125}I	Iodide-125
4-HT	4-hydroxytamoxifen
α-NF	α-naphthoflavone
AC50	Half maximal activating concentration
AGD	Anogenital distance
AhR	Aryl hydrocarbon receptor
AMA	Amphibian Metamorphosis Assay
AR	Androgen receptor
Asp	Aspartate
BCF	Bioconcentration factor
BMD	Bone mineral densitiy
BPS	Balanopreputial separation
C3	Complement protein 3
CA	Competent authority
Ca2+	Calcium cation
CF	Conceptual Framework
CFSE	Carboxyfluorescein-succinimidyl ester
CHG	Choriogenin
CLP	Classification, labelling and packaging (of substances and mixtures)
cm	Centimetre
CoRAP	Community Rolling Action Plan
CPN	Chronic progressive nephropathy
CSR	Chemical Safety Report
CYP	Cytochrome p450 enzymes
d	Day
DDE	Diphenyldichloroethylene
DES	Diethylstilbestrol
DES-DP	Diethylstilbestrol dipropionate
DHT	Dihydrotestosterone
DIO	(Iodothyronine) Deiodinase(s)
DMEL	Derived Minimal Effect Level
DMSO	Dimethylsulfoxide
DNEL	Derived No Effect Level
DO	Dissolved oxygen
DSD	Dangerous Substances Directive
d.w.	Dry weight
E2	17β-oestradiol
EC_{50}	Half maximal effective concentration
ECx	Effect concentration
ECETOC TRA	European Centre for Ecotoxicology and Toxicology of Chemicals Targeted Risk Assessment
ECHA	European Chemicals Agency
ED	Endocrine Disruptor
EE2	17α-ethinyloestradiol
ELISA	Enzyme-linked Immunsorbant Assay
ELoC	Equivalent Level of Concern
eMSCA	evaluating Member State Competent Authority
EQS	Environmental Quality Standard
ER	Oestrogen receptor
ErCx	Effect concentration (growth endpoint)
EROD	Ethoxyresorufin-O-deethylase
ES	Exposure Scenario
EtOH	Ethanol
EPA	Environmental Protection Agency

EU	European Union
FDA	Food & Drug Administration
FICZ	6-formylindolo[3,2-b]carbazole
FITC-PSA	Fluorescein isothiocyanate-labelled pisum sativum lectin
FRTL-5	Fischer rat thyroid cell line
FT	Flutamide
FTC-238	Human follicular thyroid carcinoma cell line
g	Gramme
GABA	Gamma-amino butyric acid
GC	Gas chromatography
GC-1	TR agonist
GC/FID	Gas chromatography – Flame Ionisation Detection
GC/MS	Gas chromatography – mass spectrometry
GD	Gestational Day
GH3	Rat pituitary tumor cell line
GLP	Good laboratory practice
Glu	Glutamate
GnRH	Gonadotropin-releasing hormone
GR	Glucocorticoid receptor
h	Human
H1L1.1c2	Mouse hepatoma cell line
HDL-C	High-density lipoprotein cholesterol
HELA	Human cervical cancer cell line
HepG2	Hepatoma derived liver cell line
HEK293T	Human embryonic kidney cell line
hPa	Hectopascal
HPT	Hypothalamic-pituitary-thyroid
HSD	Hydroxysteroid dehydrogenase
IC50	Half maximal inhibitory concentration
ICI 182,780	Fulvestrant (an oestrogen receptor antagonist)
IGF1	Insulin-like growth factor
IL2	Interleukin 2
IPCS	International Programme on Chemical Safety
IPMC	Isopentyl p-methoxycinnamate
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database
IUPAC	International Union of Pure and Applied Chemistry
IYD	Iodotyrosine deiodinase
kg	Kilogram
kJ	Kilojoule
km	Kilometer
kPa	Kilopascal
Kow	Octanol-water partition coefficient
L	Liter
LBD	Ligand-binding domain
LC-MS	Liquid chromatography – mass spectrometry
LDL-C	Low-density lipoprotein cholesterol
LEV	Local Exhaust Ventillation
LH	Luteinising hormone
LHRH	Luteinising hormone-releasing hormone
LOEC	Lowest observed effect concentration
Log	Logarithmic value
LOD	Limit of detection
LOQ	Limit of quantitation
Luc	Luciferase
M	Molar
m	Metre(s)
MCF-7	Malignant human mammary epithelial cancer cell line
MDA-kb2	Huamn breast cancer-derived cell line

ME	Malic enzyme
Meas.	Measured
µg	Microgram
mg	Milligram
min	Minute
mL	Millilitre
mol	Mole
mRNA	messenger RNA
MS	Mass spectrometry
MSCA	Member State Competent Authority
m/z	Mass to charge ratio
ng	Nanogramm
NH-3	TR antagonist
Nil	Nilutamide
NIS	Na^+/I^--symporter
nm	Nanometre
NOEC	No observed effect concentration
Nom.	Nominal
NTP	(US) National Toxicology Programme
OC	Operational condition
OECD	Organisation for Economic Co-operation and Development
OMC	2-ethylhexyl (2*E*)-3-(4-methoxyphenyl)acrylate
OPPTS	Office of Prevention, Pesticides and Toxic Substances
ORG2058	16α-ethyl-21-hydroxy-19-norprogesterone
p	Statistical probability
Pa	Pascal
PBT	Persistent Bioaccumulative and Toxic
PC	Product category
PEC	Predicted Environmental Concentration
pg	Picogramme
pKa	Acid dissociation constant
ppb	Parts per billionppm Parts per million
PND	Post Natal Day
PNW	Post Natal Week
PR	Progesterone receptor
PROC	Process Category
PNW	Post Natal Week
PTU	6-propyl-2-thiouracil
PXR	Pregnane X receptor
QSAR	Quantitative structure-activity relationship
r2	Correlation coefficient
RAM	Radial arm maze (test)
RatLaps	C-terminal breakdown product of collagen1-a1
rt	Rainbow trout
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals (EU Regulation No. 1907/2006)
RCR	Risk Characterisation Ratio
RMM	Risk Management Measures
RPE	Respiratory Protective Equipment
rT3	Reverse T3
RSS	Robust Study Summary
RT-QPCR	Reverse transcription quantitative polymerase-chain reaction
RU487	Mifepristone
SH-SY5Y	Human neuroblastoma cell line
siRNA	Small interfering RNA
SS	Suspended solids
STAR	Steroidogenic acute regulatory protein
SR12813	PXR agonist
SULT1E1	(Oestrogen) sulfotransferase family 1E member 1

SVHC	Substance of Very High Concern
t	Tonne
T3	Triiodothyronine
T4	Thyroxine
TEDX	Endocrine Disruption Exchange
Terp1	Truncated oestrogen receptor product-1
TG	Test Guideline
TP	Testosterone propionate
TPO	Thyroidperoxidase
TR	Thyroid hormone receptor
TRIAC	Triiodothyroacetic acid (a thyroid hormone analogue)
TRE	Thyroid hormone-response element
TRH	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone
TSH-R	TSH receptor
U2-OS	Human bone osteosarcoma epithelial cell line
UK	United Kingdom
UV	Ultraviolet
VO	Vaginal opening
vPvB	Very Bioaccumulative and very Persistent
VTG	Vitellogenin
WAF	Water Accommodated Fraction
WHO	World Health Organisation
wt.	Weight
w.w.	Wet weight
WWTP	Wastewater Treatment Plant

Annex 1: *In vitro* and mammalian *in vivo* studies conducted with OMC

Table 17

In vitro assays (OECD level 2)			
Method	**Results**	**Remarks**	**Reference**
	Oestrogenic/anti-oestrogenic activity		
ER binding assay ELISA-based binding assay for hERα; with or without addition of S9 mix Vehicle: DMSO Concentrations employed: up to 37.5 µM Positive control: Diethylstilbestrol (DES)	With or without S9 mix, no binding of OMC to hERα IC50 of DES: 8.3 nM and 12.9 nM with and without S9 mix, respectively	2 (reliable with restrictions) Test material: **OMC** (CAS-No. 5466-77-3)	Morohoshi et al. (2005)
ER binding assay Radioactive binding assay (radio-labelled E2) using recombinant human ERα and ERβ, or a cytosolic extract of procine uteri Concentrations employed: up to 1 mM Positive control: E2	No binding of OMC to hERα, hERβ, or cytosolic extract of procine uteri up to 1 mM EC50 of E2: 7 nM (hERα), 2 nM (hERβ), 0.4 nM (procine uterus cytosol)	2 (reliable with restrictions) Test material: **OMC** (Eusolex 2292)	(Seidlova-Wuttke et al., 2006a)
ER binding assay Radioactive binding assay using a cytosolic extract of uteri from ovarectomised rats (Sprague Dawley) and radio-labelled E2 According to OPPTS 890.1250 Vehicle: DMSO Postive control: E2 and 19-norethindrone Negative control: Octyltriethoxysilane Three nonconcurrent runs	OMC: considered not interactive; IC50: 0.46 - 1.9 mM IC50 of 19-norethindrone: 2.2 – 2.7 µM IC50 of E2: 0.9 – 1.3 nM IC50 of Octyltriethoxysilane: 1.9 – 2.5 mM	2 (reliable with restrictions) Test material: **OMC** (CAS-No. 5466-77-3)	NTP (2020) Published in the appendix of carcinogenesis studies on 2-hydroxy-4-methoxy-benzophenone (CAS no. 131-57-7)
ER transactivation assay Recombinant yeast assay expressing hERα; induction of	No oestrogenic activity of OMC at hERα (With or without S9 mix)	2 (reliable with restrictions)	Morohoshi et al. (2005)

In vitro assays (OECD level 2)			
Method	**Results**	**Remarks**	**Reference**
	Oestrogenic/anti-oestrogenic activity		
beta-galatctosidase Concentrations employed: up to 10 µM Positive controls: E2 and DES for oestrogenic activity and 4-hydroxytamoxifen (4-HT) for anti-oestrogenic activity	EC x 10 (concentration inducing 10 fold signal above background) of E2: 0.124 nM and > 2 nM with or without S9 mix No anti-oestrogenic activity of OMC without S9 mix With S9 mix, suspected anti-oestrogenic activity of OMC (response curve below 50 % of the maximum levels); EC50 > 5 µM EC50 of 4-HT: 1.24 µM and 0.27 µM with and without S9 mix, respectively	Test material: **OMC** (CAS-No. 5466-77-3)	
ER transactivation assay Recombinant yeast assay expressing the hERα or rtERα (Kunz et al., 2006); induction of beta-galatctosidase Vehicle: ethanol Concentrations employed: 10 µM to 10 mM (oestrogenic assay) and 1 µM to 10 mM (anti-oestrogenic assay) Positive controls: E2 for oestrogenic activity and 4-HT for anti-oestrogenic activity	No oestrogenic activity of OMC at hERα and rtERα EC50 of E2: 0.29 nM (hERα) and 18 nM (rtERα) Anti-oestrogenic activity at hERa: IC50 4.3 mM Antioestrogenic activity not tested at rtERα IC50 of 4-HT (hERα): 0.49 µM	2 (reliable with restrictions) Test material: **OMC** (CAS-No. 5466-77-3)	Kunz and Fent (2006) Kunz et al. (2006)
ER transactivation assay HELN cells transfected with hERα and ERβ; Luciferase induction Vehicle: ethanol Concentrations employed: 0.1 µM to 10 µM Positive control: E2	OMC activated hERα at ≥ 3 µM (max. response about 40% of E2) and hERβ at 10 µM (response about 20% of E2) EC50 of E2: 25 pM (hERα) and 70 pM (hERβ)	2 (reliable with restrictions) Test material: **OMC** (CAS-No. 5466-77-3)	Gomez et al. (2005)
ER transactivation assay HEK243 cells stably transfected with hERα or hERβ; Luciferase induction Vehicle: ethanol Concentrations employed:	Oestrogenic activity at hERα; Luciferase induction at 100 µM (response about 40% of E2 activity); No EC50 calculated No oestrogenic activity at hERβ EC50 of E2: 2.1 pM (hERα) and	2 (reliable with restrictions) Test material: **OMC** (Eusolex 2292)	Schreurs et al. (2002) Schreurs et al. (2005)

In vitro assays (OECD level 2)			
Method	**Results**	**Remarks**	**Reference**
	Oestrogenic/anti-oestrogenic activity		
0.1 µM to 100 µM Positive control: E2 for oestrogenic activity; 4-HT and ICI 182,780 for anti-oestrogenic activity	83 pM (hERβ) No anti-oestrogenic activity but partial agonistic activity at hERα in anti-oestrogenic assay (at 10 µM and 100 µM; response 120% of E2 alone) IC50 of 4-HT: 74 pM (hERα) and 803 pM (hERβ) IC50 of ICI 182,780: 32 pM (hERα) and 239 pM (hERβ)		
ER transactivation assay HeLA-9903 cells stably transfected with hERα; Luciferase induction According to OPPTS 890.1300 Vehicle: DMSO Concentrations employed: 1 pM to 10 µM Positive controls: E2, EE2, and 17α-methyltestosterone; Negative control: corticosterone Two experiments	OMC: negative EC50 of E2: 33 - 46 pM EC50 of EE2: 1.6 – 2.6 nM ECH50 of 17α-methyltestosterone: 14 – 23 µM Corticosterone: negative	2 (reliable with restrictions) Test material: **OMC** (CAS-No. 5466-77-3)	NTP (2020) Published in the appendix of carcinogenesis studies on 2-hydroxy-4-methoxy-benzophenone (CAS no. 131-57-7)
Proliferation assay (E-screen) Proliferation of oestrogen sensitive MCF-7 cells, and pS2 protein secretion Vehicle: ethanol Concentrations employed: 0.1 µM to 50 µM Positive control: E2	Oestrogenic activity; EC50: 2.37 µM; maximal proliferative effect: 61.9% compared to E2 (100%) pS2 protein secretion increased by OMC (10 µM; not significant) EC50 of E2: 1.22 pM pS2 protein secretion increased by E2 (10 pM)	2 (reliable with restrictions) Test material: **OMC** (Eusolex 2292)	Schlumpf et al. (2001)
pS2 mRNA- expression in MCF-7 cells Vehicle: Ethanol Concentrations employed: 10 nM to 10 µM Positive control: E2	Oestrogenic activity of OMC (EC50: 1.04 µM) EC50 of E2: 4.8 pM	2 (reliable with restrictions) Test material: **OMC** (Eusolex 2292; CAS-No. 5466-77-3; >98% purity)	Heneweer et al. (2005)

In vitro assays (OECD level 2)			
Method	**Results**	**Remarks**	**Reference**
Oestrogenic/anti-oestrogenic activity			
Androgenic/anti-androgenic activity			
AR binding assay Radioactive binding assay using a cytosolic extract of vetral prostate from castrated rats (Sprague Dawley) and radio-labelled R1881 According to OPPTS 890.1150 Vehicle: DMSO Concentrations employed: 0.1 pM to 100 µM Positive controls: R1881 and dexamethasone Three independent runs	OMC: nonbinder (less than 20% displacement); IC50: 95 µM – 200 mM IC50 of dexamethasone: 25 – 38 µM IC50 of R1881: 0.1 – 1.3 nM	1 (reliable without restriction) Test material: **OMC** (CAS No. 5466-77-3)	NTP (2020) Published in the appendix of carcinogenesis studies on 2-hydroxy-4-methoxy-benzophenone (CAS no. 131-57-7)
AR transactivation assay Recombinant yeast expressing hAR; induction of beta-galatctosidase. Vehicle: ethanol Concentrations employed: 10 µM to 1 M (androgenic assay) and 0.1 µM to 100 mM (anti-androgenic assay) Positive controls: Dihydrotestosterone (DHT) for androgen activity and flutamide (FT) for anti-androgenic activity	Androgenic activity: EC50: 10 mM EC_{50} of DHT: 0.21 nM Anti-androgenic activity; IC_{50}: 312 µM IC_{50} of FT: 4.3 µM	2 (reliable with restrictions) key study experimental result Test material: **OMC** (CAS-No. 5466-77-3)	Kunz and Fent (2006)
AR transactivation assay (AR-CALUX) U2-OS cells stably transfected with hAR; induction of Luciferase. Vehicle: ethanol Concentrations employed: 0.1 µM to 10 µM Positive control: DHT for androgenic activity; FT and vinclozolin for anti-androgenic activity	No androgenic activity of OMC EC_{50} of DHT: 0.1 nM No anti-androgenic activity of OMC IC_{50} of FT: 0.5 µM IC_{50} of vinclozolin: 0.1 µM	2 (reliable with restrictions) Test material: **OMC** (Eusolex 2292)	Schreurs et al. (2005)

In vitro assays (OECD level 2)			
Method	**Results**	**Remarks**	**Reference**
	Oestrogenic/anti-oestrogenic activity		
AR transactivation assay HEK-293 cells transfected with human AR; induction of Lucife-rase. Vehicle: DMSO Concentrations employed: up to 20 µM Positive control: testosterone	OMC: No androgenic or anti-androgenic activity	2 (reliable with restrictions) Test material: **OMC**	Nashev et al. (2010)
AR transactivation assay hAR-mediated reporter-gene activiation (luciferase) in MDA-kb2 cells (endogenously expressing hAR and hGR) Vehicle: ethanol Concentrations employed: 1 pM to 10 µM Positve controls: different agonist (including DHT) and antagonist (including FT)	OMC: No androgenic or anti-androgenic activity EC_{50} of DHT: 0.136 nM IC_{50} of FT: 3.62 µM	2 (reliable with restrictions) Test material: **OMC** (CAS-No. 5466-77-3, purity 98%)	Ma et al. (2003)
AR transactivation assay hAR-mediated reporter-gene activiation (luciferase) in MDA-kb2 cells (endogenously expressing hAR and hGR) Vehicle: DMSO Concentrations employed: up to 1 mM Positive control: DHT Antagonists: Nilutamide (Nil) and dichloro-diphenyldichloroethylene (DDE)	OMC: no androgenic or antiandrogenic activity EC_{50} of DHT: not provided IC_{50} of DDE: 13 – 15 µM IC_{50} of Nil: 0.31 – 0.46 µM	2 (reliable with restrictions) Test material: **OMC** (CAS No. 5466-77-3)	NTP (2020) Published in the appendix of carcinogenesis studies on 2-hydroxy-4-methoxy-benzophenone (CAS no. 131-57-7)
	Gestagenic/anti-gestagenic activity		
PR transactivation assay (PR-CALUX) U2-OS cells stably transfected with hPR; induction of Luciferase. Vehicle: ethanol	No gestagenic activity of OMC EC50 of ORG2058: 30 pM Anti-gestagenic activity of OMC; IC50: 0.5 µM IC50 of RU486: 4.9 pM	2 (reliable with restrictions) Test material: **OMC** (Eusolex 2292)	Schreurs et al. (2005)

In vitro assays (OECD level 2)			
Method	**Results**	**Remarks**	**Reference**
Oestrogenic/anti-oestrogenic activity			
Concentrations employed: 1 nM to 10 µM Positive control: ORG2058 for gestagenic activity; RU486 for anti-gestagenic activity			
PR-dependent mRNA expression of SULT1E1 in human endometrial epithelial adenocarcinoma Ishikawa cells (expressing endogenous PR and ERa) Readout: SULT1E1 mRNA expression (PR-dependent) Concentrations employed: 0.1 nM to 100 µM of OMC in the presence of progesterone (0.1 µM) Positive controls: a range of different anti-gestagens	No anti-gestagenic effect of OMC on progesterone-induced SULT1E1 mRNA	2 (reliable with restrictions) Test material: **OMC** (CAS-No. 5466-77-3)	Yin et al. (2015)
Interaction with human sperm *in vitro* Intracellular Ca2+ (Fluo4 staining); acrosome reaction (FITC-PSA staining); sperm penetration (into a viscous medium via nitrocellulose); Sperm hyperactivity (computer-assisted semen analysis); sperm viability (propidium iodide staining) Vehicle: DMSO Concentration employed: 1 µM to 1 mM for Ca2+ influx 10 µM for testing acrosome reaction, penetration, hyperactivity, and viability	OMC activated the CatSper Ca2+ channel and increased Ca2+ influx (EC50: 7.3 µM) OMC did not induce/influence acrosome reaction, sperm penetration, proportion of hyperactivated sperm, or sperm viability	2 (reliable with restrictions) Test material: **OMC** (CAS-No. 5466-77-3)	(Rehfeld et al., 2016; Rehfeld et al., 2018)
Steroidogenesis			
Steroid production and gene expression of steroidogenic enzymes H295R cells; based on OECD	OMC induced the production of aldosterone, corticosterone, cortisol, and 11β-hydroxy-androstenedione, and decreased	2 (reliable with restrictions) Test material:	Strajhar et al. (2017)

In vitro assays (OECD level 2)			
Method	**Results**	**Remarks**	**Reference**
Oestrogenic/anti-oestrogenic activity			
TG 456 Measurement of steroid profile by LC-MS, and mRNA quantification of steroidogenic enzymes Vehicle: DMSO Concentrations employed: 0.1 µM to 10 µM for steroid profiling Positive control: 0.3 µM Torcetrapib (inducer of corticosteroid-production)	11β-dehydro-androsteron sulfate at the highest concentration tested (10 µM) OMC increased the expression of HSD3β2, CYP21A2, CYP11B1, CYP11B2, and decreased the expression of STAR and CYP17A1 The profile of OMC resembled that of Torcetrapib	**OMC** (CAS-No. 5466-77-3)	
Steroidogenesis assay H295R cells OECD TG 456 Measurement of testosterone and E2 by HPLC/MS-MS Concentrations employed: 1 nM- 100 µM Reference compounds: forskolin and prochloraz	OMC: no effects on testosterone and E2 production	2 (reliable with restrictions) Test material: **OMC** (CAS No. 5466-77-3)	NTP (2020) Published in the appendix of carcinogenesis studies on 2-hydroxy-4-methoxy-benzophenone (CAS no. 131-57-7)
Aromatase inhibition using recombinant hCYP19 (aromatase) and P450 reductase Supersomes™ (Gentest™) Concentrations employed: 0.1 pM – 1 mM Measurement of conversion of [1β-^{3}H]-androstenedione to estrone by liquid scintillation counting Positive control (inhibitor): 4-hydroxyandrostenedione Three independent runs	OMC: no inhibition of aromatase activity 4-hydroxyandrostenedione inhibited aromatase activity in a concentration-dependent manner	2 (reliable with restrictions) Test material: **OMC** (CAS No. 5466-77-3)	NTP (2020) Published in the appendix of carcinogenesis studies on 2-hydroxy-4-methoxy-benzophenone (CAS no. 131-57-7)
Activity of steroidogenic enzymes Enzyme-activity of human 17β-	OMC: no inhibition of 17β-HSD1, 2, 3, 5	2 (reliable with restrictions) Test material:	Nashev et al. (2010)

In vitro assays (OECD level 2)			
Method	**Results**	**Remarks**	**Reference**
Oestrogenic/anti-oestrogenic activity			
HSD1, 2, 3, and 5 in transfected HEK-293 cells Measurement of conversion of androstenedione to testosterone (17β-HSD3, 5); oestrone to E2 (17β-HSD1); E2 to oestrone (17β-HSD2)		**OMC**	
Thyroid activity			
TRα transactivation assay HepG2 cells transfected with hTRα; induction of Luciferase. Vehicle: ethanol Concentrations employed: 10 pM to 10 μM Positive controls: T3, TRIAC, GC-1 (agonists); NH-3 (antagonist)	OMC: Slight agonistic activity at hTRα (LOEC: 1 μM; max. induction 1.5 fold) T3; LOEC: 0.1 nM; max. induction 122 fold) OMC: No antagonistic activity NH-3; NOEC: 50 nM; max change 0.1 fold	2 (reliable with restrictions) Test material: **OMC** (purity ≥ 98%)	(Hofmann et al., 2009)
TR transactivation assay Luciferase induction in rat GH3 cells (GH3-TRE-Luc) endogenously expressing TRα and TRβ Vehicle: DMSO Concentrations employed: 1 nM to 200 μM Positive control: T3	No agonistic activity of OMC AC50 of T3: 1.1 nM Borderline antagonistic activity of OMC (AC50 of CAS nos. 5466-77-3 and 83834-59-7: 58.7 μM and 107 μM, respectively); only highest concentration above baseline; less than 50% efficiency; no cytotoxicity	2 (reliable with restrictions) Test material: **OMC** (CAS nos. 5466-77-3 and 83834-59-7)	Paul-Friedman et al. (2019)
TPO inhibition Source of TPO: cell lysate of FTC-133 thyroid carcinoma cells stably transfected with hTPO; guaiacol oxidation assay Vehicle: DMSO Concentrations employed: 1 nM to 100 μM Positive control: genistein	OMC: No inhibition of TPO activity IC50 of genistein: 50 μM	2 (reliable with restrictions) Test material: **OMC**	Schmutzler et al. (2004)
TPO inhibition Source of TPO: cell lysate of	OMC: No inhibition of TPO activity	2 (reliable with restrictions)	Schmutzler et al. (2007a)

In vitro assays (OECD level 2)			
Method	**Results**	**Remarks**	**Reference**
	Oestrogenic/anti-oestrogenic activity		
FTC-238 thyroid carcinoma cells stably transfected with hTPO; guaiacol oxidation assay Vehicle: DMSO Concentrations employed: 1 nM to 600 µM Positive control: genistein	IC50 of genistein: 61.1 µM	Test material: **OMC**	
Gene expression in human neuroblastoma cells (SH-SY5Y) Identification of genes differentially regulated by T3 treatment and DIO knockdown (siRNA) by microarray (SurePrint G3 Human GE 8 9 60 K array) Analysis of expression of candidate genes after treatment of SH-SY5Y cells with test chemicals (RT-QPCR) Vehicle: DMSO Concentration employed: 50 µM	OMC (50 µM) differentially affected expression of genes regulated by T3 treatment and DIO knockdown OMC (50 µM) downregulated DIO3 expression by 69% compared to controls	2 (reliable with restrictions) Test material: **OMC**	Song et al. (2013)
DIO inhibition DIO inhibition assays Source of DIO 1, 2, or 3: Cell lysate of adenovirally expressed human enzymes in HEK293 cells Sandell-Kolthoff reaction to measure DIO-liberated iodide Substrates for DIO1, 2, 3 were reverse triiodothyronine (rT3), thyroxine (T4), and triiodothyronine (T3), respectively Vehicle: DMSO Concentrations employed: Initial screening with 200 µM;	OMC: negative; no inhibition of DIO 1,2,3 activity at 200 µM Positive controls: PTU (IC50 of 5.4 µM for DIO1) Xanthohumol (IC50 of 0.8 µM and 0.3 µM for DIO 2 and DIO 3, respectively)	2 (reliable with restrictions) Test material: **OMC** (CAS-No. 83834-59-7)	Olker et al. (2019)

In vitro assays (OECD level 2)			
Method	**Results**	**Remarks**	**Reference**
Oestrogenic/anti-oestrogenic activity			
since negative; no concentration range tested Positive controls: PTU (for DIO1); Xanthohumol (for DIO 2 and DIO 3)			
IYD inhibition Source of IYD: cell lysate of baculovirally expressed human enzyme in SF21 cells Sandell-Kolthoff reaction to measure IYD-liberated iodide with monoiodotyrosine as substrate Vehicle: DMSO Concentrations employed: 32 nM to 200 µM Positive control: 3-Nitro-L-tyrosine	OMC: 5% inhibition at 200 µM (max. concentration); considered negative, no IC50 could be determined Positive control: 3-Nitro-L-tyrosine (IC50: 40 nM)	2 (reliable with restrictions) Test material: **OMC** (CAS-No. 83834-59-7)	Olker et al. (2021)
NIS inhibition I- uptake in rat FRTL-5 cells endogenously expressing NIS; measurement of uptake of 125I	OMC: inhibition of I- uptake at 0.1 and 1 µM	Reliability 3 Test material: **OMC** Assay decribed in Schmutzler et al. (2007a) Data reported in a review paper. No details reported	Schmutzler et al. (2007b)
NIS inhibition HEK293T-EPA cells stably expressing hNIS; measurement of uptake of ^{125}I Concentrations employed: Initial screening with 100 µM; since negative, no concentration range tested Several control chemicals including NaClO4	OMC: Negative; no inhibition at 100 µM AC_{50} of $NaClO_4$: 0.58 µM	2 (reliable with restrictions) Test material: **OMC** (CAS-No. 83834-59-7)	Wang et al. (2021)

In vitro assays (OECD level 2)			
Method	**Results**	**Remarks**	**Reference**
Oestrogenic/anti-oestrogenic activity			
Splenocyte proliferation Concentration employed: 10 to 200 µg/mL Co-treatment with T4 (10 µM) Splenocytes from 3 weeks old male swiss mice Anti-CD3 stimulated splenocyte proliferation (CFSE-based commercial assay) IL2 concentration in medium	OMC (10 to 200 µg/mL), inhibited anti-CD3 induced splenocyte proliferation OMC reduced IL2 secretion at 100 µg/mL Inhibition of splenocyte proliferation and IL2-secretion could be reversed by T4 (10 µM)	Test material: **OMC**	Ferraris et al. (2019)
Other in vitro studies			
Pregnane X receptor (PXR) transactivation assay hPXR LBD in stably transfected HELA cells; Luciferase induction Vehicle: DMSO Concentrations employed: 100 nM to 10 µM Positve control: SR12813	OMC: no activity at PXR	2 (reliable with restrictions) Test material: **OMC** (Eusolex 2292; CAS-No. 5466-77-3)	Mnif et al. (2007)
In vitro hypothalamic release of luteinising hormone releasing-horonone (LH-RH), aspartate (Asp), glutamate (Glu), and gamma amino butyric acid (GABA) Hypothalamic fragments from prepubertal (15 days old) and peripubertal (30 days old) male and female rats (Wistar) Vehicle: Ethanol Concentration employed: 0.263 µM	Decreased hypothalamic release of LH-RH (males and females, 15 days old and 30 days old) Decreased relase of Glu (15 days and 30 days females) Increased release of GABA (15 days and 30 days males)	2 (reliable with restrictions) Test material: **OMC** (Eusolex 2292)	Szwarcfarb et al. (2008)
In vitro hypothalamic release of gonadotropin releasing-horonone (GnRH aka LH-RH), Asp, Glu, and GABA	Decreased hypothalamic release of GnRH in males (N, C+TP) and females (N, C+E2) Decreased relaese of Glu and	2 (reliable with restrictions) Test material: **OMC** (Eusolex	Carbone et al. (2010)

In vitro assays (OECD level 2)			
Method	**Results**	**Remarks**	**Reference**
	Oestrogenic/anti-oestrogenic activity		
Hypothalamic fragments from adult male and female (dioeostrous) rats (Wistar) either uncastrated (N) or castrated (C) and with or without supplementation with testosterone propionate (TP) (1 mg/kg, males; C+TP) or E2 (20 µg/kg, females; C+E2) Vehicle: Ethanol Concentration employed: 0.263 µM	increased release of GABA in males (N, C+TP) Decreased release of Glu and Asp in females (N, C+E2)	2292)	
Interaction with AhR and CYP1A1 and CYP1B1 AhR transactivation in H1L1.1c2 cells endogenously expressing mouse AhR; luciferase induction Cell based EROD assay (H1L1.1c2 cells) Expression of CYP1A1 and CYP1B1 mRNA in HaCaTs cells Cell-free CYP1A1 and CYP1B1 inhibition assays (commercially available P450-Glo Assay 1A1 and 1B1 systems) Concentrations employed: 10 µM for reporter gene and EROD assay; 10 nM to 25 µM for CYP inhibition assays Positive control: 6-formylindolo[3,2-b]carbazole (FICZ),α-naphthoflavone (α-NF)	OMC (10 µM) potentiated FICZ (10 nM)-induced and UV-radiation-induced AhR activation in H1L1.1c2 cells OMC reduced FICZ-induced EROD activity in H1L1.1c2 cells OMC potentiated FICZ-induced mRNA-expression of CYP1A1 and CYP1B1 in HaCaTs cells; knock-down of AhR (by siRNA) completely abolished the effects of FICZ and OMC OMC potentiated UV-radiation induced CYP1B1 mRNA-expression in HaCaTs cells OMC inhibited the activity of CYP1A1 (IC50: 1 µM) and CYP1B1 (IC50: 0.6 µM) in cell-free inhibition assays; α-NF: 33.4 nM (CYP1A1), and 2.35 nM (CYP1B1)	2 (reliable with restrictions) Test material: **OMC** OMC also potentiated FICZ-induced mRNA-expression of CYP1A1 and CYP1B1 in the skin of mice treated *in vivo* (not decribed here)	Phelan-Dickinson et al. (2020)

Table 18

Mechanistic in vivo studies (OECD level 3)			
Method	**Results**	**Remarks**	**Reference**
Uterotophic assay Immature female rats (Wistar)	OMC: No increase in uterine weight, no histological findings DES-DP: 4.7 to 4.9 fold increase in	2 (reliable with restrictions) Test material:	BASF-SE (2001)

Mechanistic in vivo studies (OECD level 3)			
Method	**Results**	**Remarks**	**Reference**
Sub-cutaneous administration; 0, 200 and 1000 mg/kg bw/d (N=10/dose) Vehicle: olive oil Exposure: 3 days (once a day) Positive control: diethylstilbestrol dipropionate (DES-DP; 5 µg/kg bw/d)	absolute and relative uterus weight	**OMC** (Uvinul MC 80; EC-No: 226-775-7; purity >99%)	
Uterotophic assay Immature female rats (Long-Evans) Oral administration corresponding to 0, 268, 522, 1035, 1518, 2667 mg/kg bw/d (N = 5 to 10/dose) Vehicle: ethanol Exposure: 4 days (PND 21 - 24) Positive control: EE2 (0, 0.085, 0.342, 0.780, 0.856, 1648, 8631 µg/kg bw/d)	OMC significantly increased uterine weight at ≥ 1035 mg/kg bw/d Max. response at 2667 mg/kg bw/d: 1.7 fold vs control Max. response of EE2 at 8631 µg/kg bw/d: 3.45 fold No effects of OMC and EE2 on body weight	2 (reliable with restrictions) Test material: **OMC** (Eusolex 2292)	Schlumpf et al. (2001)
Uterotrophic assay Ovarectomised female rats (Sprague Dawley) According to OPPTS 890.1600 Oral: gavage 0, 320, and 1000 mg kg bw/d (N=8/dose) Vehicle: corn oil Exposure: 3 days (daily) Positive control: EE2 (0.1 mg/kg bw/d)	OMC: No effect on uterus weight; no effect on body weight; EE2: Increased wet (2.8-fold) and dry uterus weight (2.3-fold; lower terminal body weight (-6.4% vs control)	2 (reliable with restrictions) Test material: **OMC** (CAS No. 5466-77-3)	NTP (2020) Published in the appendix of carcinogenesis studies on 2-hydroxy-4-methoxy-benzophenone (CAS no. 131-57-7)
Hershberger assay Male castrated rats (Wistar) OECD TG 441 GLP Oral: gavage 0, 300, 1000 mg/kg bw/d (n=6/dose) Vehicle: corn oil	TP alone increased testosterone and decreased LH levels Absolute weight of ventral prostate decreased (fixed ≥ 300 mg/kg bw/d; fresh: 1000 mg/kg bw/d); relative weight not changed Weight of other accessory sex organs was not significantly reduced	1 (reliable without restriction) Test material: **OMC** (Uvinul MC 80; EC-No: 226-775-7; purity 99.9%)	BASF-SE (2003)

Mechanistic in vivo studies (OECD level 3)			
Method	**Results**	**Remarks**	**Reference**
Exposure: 10 days (daily) Vehicle control, 0, 300, or 1000 mg /kg bw/d OMC + TP (0.4 mg/kg bw/day)	No histological effects on accessory sex glands No effects of OMC on testosterone, DHT, and LH Decreased body weigth at 300 mg/kg bw/d (-2.3%) and 1000 mg/kg bw/d (-5.5%); not significant Increased absolute and relative liver weights (1000 mg/kg bw/d)		
Hershberger assay Male castrated rats (Sprague Dawley) According to OPPTS 890.1600 Oral: gavage (N=6-8/dose) Vehicle: corn oil 0, 320, 1000 mg/kg bw/d (agonist mode) 0, 100, 320, and 1000 mg/kg bw/d (antagonist mode) Exposure: 10 days (daily) Positive control: 0.4 mg/kg bw/d TP (agonist via subcutaneous injection) and 3 mg/kg bw/d Flu (antagonist)	OMC: No androgenic or anti-androgenic effects according to reproductive organ weights; no effect on body weight	2 (reliable with restrictions) Test material: **OMC** (CAS no. 5466-77-3)	NTP (2020) Published in the appendix of carcinogenesis studies on 2-hydroxy-4-methoxy-benzophenone (CAS no. 131-57-7)
Subchronic *in vivo* study Based on OECD TG 407 with deviations GLP Study scope does not cover full OECD TG 407; focus on hormone measurements Rat (Wistar, 13 females/dose) 35-day study Oral: diet Vehicle: none 0, and 1000 mg/kg bw/d Blood sampling from day 28 onwards when females were in pro-oestrus	Decreased food consumption (-5.5%) and body weight (-4.3%) Increased T4 (+19%)	Test material: **OMC** (EC-No. 226-775-7) Purity: 99.9% Gross pathology, no organ weights (except ovary), no histology T3, T4, TSH, prolactin, follicle-stimulating hormone, luteinizing hormone, E2, progesterone	BASF-SE (2004)
Subchronic *in vivo* study Ovar-ectomised female rats	T4 increased in soy-free diet group (high dose), and decreasd in soy-	2 (reliable with restrictions)	Schmutzler et al. (2004)

Mechanistic in vivo studies (OECD level 3)			
Method	**Results**	**Remarks**	**Reference**
(Sprague–Dawley, n=8-11 per group) Oral: diet 0, 2.5, or 12.5 g/kg feed prepared in soy-free diet, or 0, and 12.5 g/kg feed prepared in soy-containing diet Corresponding to approximately 0, 54, or 285 mg/animal/d (soy-free diet) or 266 mg/animal/d (soy-fed) Positive control: E2 (34.2 mg/kg) corresponding to 0.64 mg/animal/d (soy-free diet) or 0.58 mg/animal/d (soy-fed) Investigations: plasma T3, T4, TSH; Malic enzyme (ME) activity in liver, heart, kidney DIO1 activity in liver	fed group (high dose) TSH increased in soy-fed group (high dose) ME activity in liver increased in soy-fed group (high dose); ME activity in kidney increased in soy-free group (both doses) DIO1 activity in liver decreased in soy-free (both doses) and soy-fed group (high dose) E2: Increased T3, T4 and TSH in soy-fed group ME activity increased in liver (soy-free and soy-fed group) and kidney (soy-fed group) DIO1 activity in liver decreased (soy-free and soy-fed groups)	Test material: **OMC**	
Short-term *in vivo* study Ovar-ectomised female rats (Sprague–Dawley) Oral: gavage Vehicle: olive oil 0, 10, 33, 100, 333, 1000 mg/kg bw/d (N=12/group) Exposure: 5 days Positive control: E2 (0.6 mg/kg bw/d) Investigations: Uterus weight, plasma leptin, cholesterol, triglycerides, HDL-C, LDL-C, glucose Gene expression in uterus (ERa, ERb, C3), liver (IGF1), and pituitary (TERP1)	No effects on body weight Increased (relative) uterus wet weight at 1000 mg/kg bw/d Decreased cholesterol triglycerides, and LDL-C at 1000 mg/kg bw/d Decreased mRNA-expression of ERβ and C3 (uterus), IGF1 (liver), and TERP1 (pituitary) at 1000 mg/kg bw/d; ERa mRNA (uterus) not changed E2: No significant effects on body weight at termination Decreased leptin, cholesterol, LDL-C, and HDL-C Increased uterus weight Expression of C3 increased and ERβ decreased (uterus); Increased expression of TERP1 (pituitary) and decreased expression of IGF1 (liver)	2 (reliable with restrictions) Test material: **OMC** (Eusolex 2292)	Klammer et al. (2005)
Short-term *in vivo* study Ovar-ectomised female rats (Sprague–Dawley) Oral: gavage 0, 10, 33, 100, 333, 1000 mg/kg bw/d (N=12/group) Vehicle: olive oil Exposure: 5 days	No effects of OMC on body weight, liver, spleen, and kidney weight; no macroscopical effects Decreased T3 (1000 mg/kg bw/d), T4 (≥ 333 mg/kg bw/d), and TSH (≥ 333 mg/kg bw/d)	2 (reliable with restrictions) Test material: **OMC** (Eusolex 2292)	Klammer et al. (2007)

Mechanistic in vivo studies (OECD level 3)			
Method	**Results**	**Remarks**	**Reference**
Positive control: E2 (0.6 mg/kg bw/d) All doses: T3, T4, TSH measurents; TPO activity (thyroid), DIO1 activity (liver); prepro thyrotropin releasing-hormone (preproTRH) mRNA expression (mediobasal hypothalamus) High dose: protein expression of TSHR and NIS (thyroid)	Hepatic DIO1 activity decreased (≥ 333 mg/kg bw/d) NIS protein expression (thyroid) unchanged; TSHR protein (thyroid) expression increased (1000 mg/kg bw/d; only highest dose tested) TPO activity (*in vivo* in the thyroid) not changed preproTRH mRNA (hypothalamus) not changed E2: Slight decrease in body weight, no significant effect on any other parameters		
Subchronic *in vivo* study Ovar-ectomised female rats (Sprague–Dawley); n=11 per dose Oral: diet Doses corresponding to 0, 52, and 280 mg/animal/d Vehicle: none Exposure: 12 weeks Positive control: E2 (0.445 mg/animal/d) Investigations: Body weight gain, size of paratibial fat depot, uterus weight, serum LH, TSH, T4, T3, leptin, cholesterol, HDL-C, LDL-C, triglycerides	Decreased body weight gain at both doses Trend for decreased size of paratibial fat depot at the high dose Slight increase of uterus weight at the high dose Increased LH at the high dose Decreased leptin, cholesterol, HDL-C, and triglyceride levels at the high dose Decreased LDL-C at both doses Decreased T4 at the low dose E2: Reduced body weight gain, and paratibial fat Increased uterus weight Decreased LH Decreased leptin, cholesterol, HDL-C, LDL-C	2 (reliable with restrictions) Test material: **OMC** (Eusolex 2292)	Seidlova-Wuttke et al. (2006a)
Subchronic *in vivo* study Ovar-ectomised female rats (Sprague–Dawley), n=11 per dose Oral: diet Doses corresponding to 0, 58, and 275 mg/animal/d Vehicle: none Exposure: 6 weeks Positive control: E2 (0.6	No effect on uterus weight; thickness of epithelium increased and of endometrium decreased (both doses), myometrium increased (low dose) ERa expression in uterus increased (low dose); PR and IGF1 increased (both doses)	2 (reliable with restrictions) Test material: **OMC** (Eusolex 2292)	Seidlova-Wuttke et al. (2006b)

Mechanistic in vivo studies (OECD level 3)			
Method	**Results**	**Remarks**	**Reference**
mg/animal/d) Investigations: Uterine and vaginal histology, and gene expression of ERα, ERβ, PR, and insulin-like growth factor 1 (IGF1) Bone mineral densitiy (BMD), serum osteocalcin, and C-terminal breakdown product of collagen1-a1 (RatLaps)	Thickness of vaginal epithelium increased (both doses) Increased expression of PR (low dose) and IGF1 (both doses) in vagina Decreased osteocalcin (high dose), decreased RatLaps (low dose) E2: Increased uterus weight, increased thickness of epithelium, endometrium and myometrium Decreased expression of ERβ, increased expression of PR and IGF1 in uterus Increased thickness of vaginal epithelium, decreased expression of ERβ, increased expression of PR and IGF1 (not significant) Increased BMD, decreased osteocalcin		

Table 19

Repeated-dose toxicity studies (OECD level 4)			
Method	**Results**	**Remarks**	**Reference**
Subchronic repeated-dose toxicity study OECD TG 408 GLP Rats (Füllinsdorf Albino SPF, 12/sex/dose) 90-day study Oral: diet Vehicle: none 0, 200, 450, and 1000 mg/kg bw/d	Increased absolute and relative Kidney weight (males) at 1000 mg/kg bw/d; reversible Increased GLDH; reversible Decreased liver glycogen content and slight shrinkage of hepatocytes (5 of 12 animals) at 1000 mg/kg bw/d; reversible Slight increase of iron positive material phagocytized by Kupffer cells at ≥ 450 mg/kg bw/d; reversible	Test material: **OMC** (EC-No. 226-775-7) Purity: 99% Half of the animals in the control and high dose were used to perform an recovery experiment (n=6/sex) for 5 weeks after 90-day treatment	Hoffmann-La Roche (1984)
Subchronic repeated-dose toxicity study Similar to OECD TG 410 Rats (Sprague-Dawley, 5/sex/dose with intact skin or abraded skin, repectively)	Dermal irritation most marked in high dose group (erythema and oedema, desquamation of the application site); mostly regressed till the end of treatment No evidence for systemic toxicity	Test material: **OMC** (EC-No. 226-775-7) Purity: 98%	Hazleton (1980a)

28-day study Dermal: occlusive, 6 hours contact period/day Vehicle: none Doses: corresponding to: 0, 500, 1500, 5000 mg/kg bw/d			
Subchronic repeated-dose toxicity study EPA OPPTS 870.3200 Rabbits (New Zealand White) 21-day study Dermal: occlusive, 6 hours contact period/day Vehicle: none Doses: corresponding to: 0, 500, 1500, 5000 mg/kg bw/d	Clinical signs (lethargy, hunched posture, alopecia etc.) in the high dose Dermal irritation in all dose groups 3 high dose animals died or were killed in extremis Lower food consumption and body weight in high dose Increased neutrophils, decreased lymphocytes Decreased ALP, increased urea nitrogen (high dose) At high dose: Low incidence of macroscopically observable focal liver necrosis, depleted liver glycogen and immature testes	Test material: **OMC** (EC-No. 226-775-7) Purity: 99.7% Histology: liver kidneys brain (3 levels) pituitary heart thyroids adrenals testes ovaries treated and untreated skin	Hazleton (1980b)

Table 20

Reproductive toxicity studies (OECD level 4 and 5)			
Method	**Results**	**Remarks**	**Reference**
Prenatal developmental toxicity study Equivalent to OCED TG 414 GLP Rat (Füllinsdorf albino) oral: gavage Vehicle: 5% Carboxymethylcellulose, 0.5% Benzyl-EtOH, 0.4% TWEEN 80, 0.9% NaCl 0, 250, 500, and 1000 mg/kg bw/d Exposure: From day 7 through day 16 of gestation	Slight reduction of maternal body weight gain at 1000 mg/kg bw/d Increased incidence of skeletal variations in fetuses at 1000 mg/kg bw/d	Test material: **OMC** (EC-No. 226-775-7)	Hoffmann-La Roche (1983a)
Prenatal developmental toxicity study Similar to OECD TG 414	Slight decrease in maternal body weight gain at the high dose Slightly increased frequency of constipated and anorectic dams	Test material: **OMC** (EC-No. 226-775-7) Low number of	Hoffmann-La Roche (1983b)

Reproductive toxicity studies (OECD level 4 and 5)			
Method	**Results**	**Remarks**	**Reference**
Rabbit (Swiss) oral: gavage Vehicle: 5% Carboxymethylcellulose, 0.5% Benzyl-EtOH, 0.4% TWEEN 80, 0.9% NaCl 0, 80, 200, 500 mg/kg bw/day Exposure: GD7-20 (once daily); termination on GD30	at the high dose Slightly decreased body weight of foetuses at the high dose No teratogenic effects	pregnant females in the high dose group (13 from 20) and 3 animals died due to application accidents	
2-generation reproductive toxicity study OECD TG 416 GLP Rats (Wistar) Oral: diet 0, 150, 450 ,1000 mg/kg bw/d	Reduced food consumption and body weight in P0 (at termination -5% in females and -16% in males vs controls), and P1 animals (at 1000 mg/kg bw/d (at termination -4% in females and -14% in males vs controls) Increased liver weight (females) and hepatic cytoplasmatic eosinophilia in P0 and P1 animals at the high dose Reduced ovary weight in P0 (-17% and -14%, absolute and relative, respectively vs controls) and P1 females (-18% and -14%, absolute and relative, respectively vs controls) at the high dose; no histological findings Reduced implantation sites in the mid and high dose in P0 females (12, 12.3, 10.9, 9.2 at 0, 150, 450 ,1000 mg/kg bw/d, respectively), and P1 females (12.4, 11.3, 10.7, 10.3 at 0, 150, 450 ,1000 mg/kg bw/d, respectively) Reduced number of F1 pups/Litter in the high dose (11, 11.9, 10.9, 9.2 at 0, 150, 450 ,1000 mg/kg bw/d, respectively) Delayed vaginal opening (VO) in F1 females at the mid and high dose (31.3, 32.5, 33.6, 33.8 days at 0, 150, 450, 1000 mg/kg bw/d, respectively); slightly higher body weight at VO at 450, mg/kg bw/d, no significant difference at 1000 mg/kg bw/d Delayed balanopreputial separation (BPS) in F1 males at	Test material: **OMC** (EC-No. 226-775-7) Historical control data for spermatids/g: range 517-727 spermatids/g with a mean of 625 (n=19) Effects of OMC on spermatid count considered incidental by the study authors Historical control data for implantation sites (12 studies, 2000-2002): range 10.2-11.5 implants per dam Due to high number of implantations in controls compared to historical controls, effect at high dose is considered by the study authors as not treatment related	(BASF-SE, 2007); Schneider et al. (2005)

Reproductive toxicity studies (OECD level 4 and 5)			
Method	**Results**	**Remarks**	**Reference**
	the high dose (43.6, 43.7, 43.4, 45.4 days at 0, 150, 450, 1000 mg/kg bw/d, respectively); lower body weight (not significant) at BPS Decrease (-8% vs controls) of total spermatids/g cauda epdidymides at the high dose (700 spermatids/g vs 763 spermatids/g in controls) in P0 males; no effects in F1 males		
Developmental toxicity study Rats (Wistar) Oral: gavage Vehicle: Corn oil 0, 500, 750 or 1000 mg/kg bw/d Gestation day (GD) 7 to postnatal day (PND) 17 Investigations: AGD, preputial separation, vaginal patency and nipple retention T4 (dams, male and female pups), Testosterone (male pups), Progesterone (dams and female pups) and E2 (dams and female pups) Sperm motility and sperm count Histopathology focussed on the thyroid and reproductive organs and any organs showing significant weight changes Gene expression in adrenal, testis, and prostate Motor activity and habituation, Radial arm maze (RAM), hearing test and acoustic startle response	Lower maternal body weight gain in all dose groups Lower pup body weigth in all dose groups; Lower body weight of males and females at PND 16 at 1000 mg/kg bw/d Increased liver weight at ≥ 750 mg/kg bw/d on PND16 in F1 males and females (no histology findings) Decreased testis weight at ≥ 750 mg/kg bw/d on PND16 in F1 males Decreased lumen formation in seminiferous tubules (1000 mg/kg bw/d) at PND16 in F1 males Decreased prostate weight on PND16 and in adult F1 males at 1000 mg/kg bw/d; decreased presence of large fluid-filled acini Increased thyroid weight at ≥ 750 mg/kg bw/d on PND 16 (both sexes combined); recovery at PND 28 No changes in thyroid histology T4 decreased in dams at GD15 and at PND15 (-70%, -97% and -100% at 500, 750 and 1000 mg/kg bw/d, respectively at PND15); recovery at PND 28 T4 decreased in F1 male pups (PND16) by -14%, -34% and -35% at 500, 750 and 1000 mg/kg bw/d, respectively. Testosterone decreased in F1 male pups (PND16) by -54%, -60% and -70% at 500, 750 and 1000	2 (reliable with restrictions) Test material: **OMC** (CAS-No. 5466-77-3, purity 98%)	Axelstad et al. (2011)

Reproductive toxicity studies (OECD level 4 and 5)			
Method	**Results**	**Remarks**	**Reference**
	mg/kg bw/d, respectively Progesterone in F1 females (PND28 only) decreased by -41%, -66% and -57% at 500, 750 and 1000 mg/kg bw/d, respectively E2 decreased in F1 females at PND28 at 500 and 750 mg/kg bw/d only Sperm/g cauda epididymides decreased by -25%, -15% and -25% at 500, 750 and 1000 mg/kg bw/d respectively Motor activity decreased in F1 females at post-natal week (PNW) 9 (at ≥ 750 mg/kg bw/d) and at PNW17 (1000 mg/kg bw/day) Decreased number of errors in RAM in F1 males at PNW22-25 (500 and 750 mg/kg bw/d)		
Developmental toxicity study Rats (Sprague Dawley) Oral: gavage Vehicle: Corn oil 0, 500, or 750/375 mg/kg bw/d Study performed in 3 blocks separated by two weeks High dose of 750 mg/kg bw/d was reduced after 15 days of dosing to 375 mg/kg bw/d due to excessive maternal toxicity (about -50% body weight gain between GD7 and GD21) A group receiving 375 mg/kg bw/d was introduced in block 2 of the study Investigations: 3-6 dams per group were used for Caesarian section T3, T4, TSH (dams, male and female pups) Organ weights: thyroid, liver,	Reduced maternal body weight gain at GD7 to PND1 at both doses (375 and 500 mg/kg bw/d) Increased post-implantation loss at 500 mg/kg bw/ (10.2 vs 5.09 in controls) Increased perinatal losses at 500 mg/kg bw/d (17.9 vs 6.24 in controls) Decreased birthweight at 375 and 500 mg/kg bw/d, and decreased pup body weight at 500 mg/kg bw/d T4 increased in dams at GD15 and decreased at PND22 (375 and 500 mg/kg bw/d) T4 decreased in male pups at PND16 (375 mg/kg bw/d) and increased in males and females at PND22 (500 mg/kg bw/d) T3 decreased in dams at GD15 (500 mg/kg bw/d) and PND22 (375 and 500 mg/kg bw/d) T3 decreased in female pups at PND 17 and PND22 (500 mg/kg bw/d)	2 (reliable with restrictions) Test material: **OMC** (CAS-No. 5466-77-3, purity 98%) Complex dosing scheme due to dose reduction: 4 litters received 750 mg/kg bw/d during gestation and 375 mg/kg bw/d during lactation, 12 litters received 500 mg/kg bw/d during both gestation and lactation (4 of them got 750 mg/kg bw/d from GD 7-9) and 10 litters received 375 mg/kg bw/d during gestation and lactation (6 of them got 500 mg/kg bw/d from GD 7-9)	Axelstad et al. (2019)

Reproductive toxicity studies (OECD level 4 and 5)			
Method	**Results**	**Remarks**	**Reference**
brain Brain heterotopias by immunohistochemistry (NeuN staining) in F1 males at PND16 Gene expression analysis of TH-regulated gens in F1 male brains at PND16 Exposure to PTU and DE-71 (a commercial mixture of polybrominated diphenyl ethers) was part of this study	TSH increased in dams at GD15 and trend for decrease at PND 22 (all doses, sign. at 500 mg/kg bw/d on GD15) TSH increase in female pups at PND 17 (375 mg/kg bw/d) Pup brain weight (males at PND22) decreased at 375 and 500 mg/kg bw/d Heterotopia volume not significantly affected (decrease rather than increase) Significant and dose-related decrease of expression of Pvalb, Hopx Coll11a2, Klf9, Gjb6 in brain; expression profile resembles that of PTU treatment; expression other genes not affected in a similar was as by PTU **PTU**: clearly decreased T4 and increased TSH levels in dams and pups, and significantly increased heterotopia volume; Changes in TH-regulates genes in the brain		
Developmental toxicity study Mice (Swiss Webster) Oral: gavage Vehicle: corn oil 0, 250, 500, and 1000 mg/kg bw/d Exposure of lactating dams from PND1 to PND22 Positive control : PTU (4 mg/kg bw/d) Investigations: developmental parameters (eruption of the incisors, hair growth and opening of eyes Termination of dams and pups on PND23 Organ weight: thymus and spleen Cell count and flow cytometry analysis (spleen, thymus; high	Reduced weight gain and delayed eye opening of pups at 1000 mg/kg bw/d Decreased T4 in dams and pups at 1000 mg/kg bw/d Increased relative spleen weight and increased splenocyte number at 1000 mg/kg bw/d; increased number of B- and T-lymphocytes (not significant) Increased polymorphonuclear cells in blood at 1000 mg/kg bw/d PTU: decreased T4 in dams and pups Increased relative spleen weight and increased number of splenocytesincreased number of B- and T-lymphocytes (not significant for T-lymphocytes) Increased number of leucocytes, mononuclear cells, and polymorphonuclear cells	2 (reliable with restrictions) Test material: **OMC**	Ferraris et al. (2019)

Reproductive toxicity studies (OECD level 4 and 5)			
Method	**Results**	**Remarks**	**Reference**
dose and controls) Blood sampling for hormone analysis and leucocyte count (high dose and controls)			
Dose-range finder study for modified one-generation study (see below) In compliance with FDA GLP regulations Rat (Sprague Dawley) Oral diet 0, 2250, 5000, 10000, and 20000 ppm P0 time-mated females: 26 per group exposed from GD6 till LD14	Lower body weight in dams (-20% and -22%, and up to -37% vs control in the high dose on GD 21, LD1, and LD1-14 respectively); transient body weight loss of dams during GD6-9 in the high dose Lower pup birth weight at the high dose (-39% vs control) Lower postnatal weight (after PND14: -15% at 5000 ppm, -16 till -76% at 10000 and 20000 ppm) Increased postnatal pup mortality at the high dose (no live pups after LD14)	2 (reliable with restrictions) Test material: **OMC** (CAS no. 5466-77-3) Test item intake at 0, 2250, 5000, 10000, and 20000 ppm during GD6-21: 161, 365, 714, and 1841 mg /kg bw body weight/d, respectively Test item intake at 0, 2250, 5000, 10000, and 20000 ppm during LD1-14: 410, 925, and 1615 mg/kg bw/d, respectively For the subsequent modified one-gerenation study following doses were chosen: 0, 1000, 3000, and 6000 ppm	NTP (2022)
Developmental and reproductive toxicity study Modified one-generation study In compliance with FDA GLP regulations Rat (Sprague Dawley) Oral diet 0, 1000, 3000, and 6000 ppm P0 time-mated females: 26 per group exposed from GD6 till LD28 F1 and F2 standardised to 10	Decreased F1 and F2 preweaning body weights (males: -5% and -13%, females: -5% and -15% in the mid and high dose respectively at PND 28); mitigation of body weight effect in females (-7% in hight dose at PND 91) Delayed vaginal opening (VO) in F1 females in mid and high dose (34.1, 35.0, 35.8, and 36.8 days at 0, 1000, 3000, and 6000 ppm, respectively); body weight at day of attainment was unchanged Delayed BPS in F1 males in the high dose (44.9, 45.4, 45.3, and	2 (reliable with restrictions) Test material: **OMC** (CAS no. 5466-77-3) Reproductive performance cohort: Dams sacrificed on LD 28, sires within one week; Determination of fertility and fecundity, organ weights and histology; epididymal	NTP (2022)

Reproductive toxicity studies (OECD level 4 and 5)			
Method	**Results**	**Remarks**	**Reference**
pups (5/sex/group) per litter At weaning F1 animals were assigned to the following cohorts: - Reproductive performance cohort (up to 2/sex/Litter, when available); mating to produce F2 (F2 maintained till PND 28) - Prenatal cohort (1/sex/Litter); mating to produce F2 (caesarean section; investigation of foetal abnormalities) - Subchronic cohort (1/sex from 10 litters) - Biological sampling cohort (1/sex/Litter) Sexually mature F1 animals in the prenatal (14–15 weeks; 1 male and 1 female/Litter) and reproductive performance cohorts (17–18 weeks; 2 males and 2 females/Litter) were mated	48,4 days at 0, 1000, 3000, and 6000 ppm, respectively); body weight at day of attainment was unchanged; Rather than being a specific effect on pubertal development, delayed BPS and VO is considered as a consequence of significantly lower body weights at weaning compared to controls (at 1000, 3000, and 6000 ppm: -4.26%, -7.46, and -12.9% in males; -5.81%, -10.5%, and -14.7% in females, respectively) Longer time in oestrus and shorter time in dioestrus in F1 females (sign. at all dose-groups); length of oestrus cycle unchanged Sign. decreased ventral prostate weight in high dose F1 males of the subchronic cohort (absolute: -26.3%; relative -23.5% vs ctrl) No effect observed in other cohorts Sign. increased weight of seminal vesicles with coagulating gland in F1 males of the reproductive performance cohort (absolute: +9.0% and +11.3% in the mid and high dose, respectively; relative: +13.8% and +20.4% in the mid and high dose, respectively) No effect observed in other cohorts	sperm motility, number, and density; gross necropsy of F2 animals on PND 28 including determination of AGD (PND 1) and nipple retention (PND 13) Prenatal cohort: Dams sacrificed on GD 21 and male sires after mating; Determination of fertility and fecundity, organ weights, hematology and clinical chemistry, investigation of F2 foetuses on GD 21 for morphological abnormalities Subchronic cohort: F1 males and females sacrificed on PND 110–112 and PND 111–113, respectively; hematology, clinical chemistry, micronucleus determination; organ weights and histology Biological sampling cohort: Internal dose assessment not performed because test substance was not stable under sampling and storage conditions Test item intake	

Reproductive toxicity studies (OECD level 4 and 5)			
Method	**Results**	**Remarks**	**Reference**
		at 0, 1,000, 3,000, and 6,000 ppm in P0 females during gestation, and P0 and F1 animals postweaning: 0, 70-87, 207-263, and 491-528 mg/kg/day, respectively Test item intake at 0, 1,000, 3,000, and 6,000 ppm in P0 females during gestation: 0, 161, 475, and 920 mg/kg bw/d, respectively	

Annex 2: In vivo environmental studies conducted with OMC

Table 21

Ecotoxicological studies with and without endocrine relevant endpoints			
Method	**Results**	**Remarks**	**Reference**
Pimephales promelas Adult male and female fish (based to OECD 229, but shorter duration) Exposure 14 d Semi-static (two-thirds renewal of test medium every 48 hours) Solvent (1:1 dilution of ethanol: 20% Cremophor RH40) Temperature: 25 +- 1°C Photoperiod 16:8 light/dark Four replicates with two male and four female fish per replicate Measured concentrations: 5.4, 37.5, 245, 394 µg/L OMC (30, 300, 1000, 3000 µg/L nominal)	Plasma VTG sign. increased in males at 244.5 µg/L OMC Gene expression in the liver: ERα sign. down-regulated at 394 µg/L OMC in females (dose dependently) AR sign. down-regulated at 37.5, 244.5 and 394 µg/L OMC in females 3β-HSD sign. down-regulated in males (at 37.5, 245, 394 µg/L) and females (at 245, 394 µg/L) No sign. effects on vtg1 gene expression Histology: Males: Sign. decreased proportion of spermatocytes → a secondary criteria for histopathological change due to endocrine activity according to OECD TG 123 Increased proportion of spermatides (not sign.) Females: reduced proportion of primary oocytes (not sign.); significant increase in vitellogenic oocytes in the ovary No effects on survival and growth	Reliability: 2	Christen et al. (2011)
Oryzias latipes Adult males Length 2,5-3,5 cm Exposure 7 days 5 fish per treatment, Semi-static (media changed every day), Solvent: Ethanol (0.1 %) Nominal concentrations: 0.034, 0.34, 3.4, 34 mM OMC (9.87, 98.7, 987, 9877 mg/L), no analytics Pos. control: E2 (3.7, 37, 185 nM)	Plasma VTG level dose-dependently increased but not statistically sign. Gene expression in the liver: ERα sign. and dose dependently upregulated at all concentrations VTG1 and VTG2 sign. upregulated at all concentrations, dose-dependend (aside from VTG2 at the lowest conc.) CHG-L gene sign. upregulated at all concentrations, CHG-H at the three higher concentrations, dose dependency existed No effect on ERβ and AR gene expression	Reliability: 2-3 (only 5 fish per treatment used, however results on gene expression reliable)	Inui et al. (2003)
Danio rerio Adult males Exposure 14 days Whole genom microarray 12 fish/ vessel, four replicates, Semi-static, complete water exchange every 48 h, Temperature 27 ± 1°C, photoperiod 16:8 h light/dark,	Gene expression: VTG1 sign. down-regulated in all tissues except the liver (there upregulated) at 2.2 and 890 µg/L ERα sign. upregulated at 2.2 and 890 µg/L in whole fish ERß sign. upregulated at 890 µg/L in whole fish and liver 17ß-HSD3 sign. down-regulated at 2.2 and 890 µg/L in whole fish AR sign. down-regulated at 2.2 and	Reliability: 2	Zucchi et al. (2011)

Solvent: DMSO 0.01% Measured concentrations: 2.2 and 890 µg/L OMC (3 and 3000 µg/L nominal)	890 µg/L in liver CYP19b sign. upregulated at 890 µg/L but not at 2.2 µg/L in brain		
Danio rerio Two tests with adults, age: 4 (5) months 1. exposure 14 days, concentrations: 10, 100 µg/L (nom), to detect transformation products 2. exposure 21 days, measured concentrations: 0.87, 8.5, 79.5 µg/L (3, 29.3, 273.7 nmol/L measured), (1, 10, 100 µg/L nom), examination of effects Three replicates with 6 fish each, Exposure media replaced once a day, Solvent: DMSO (0.01 %), Temperature: 28 ± 1°C, Photoperiod 16:8 h light/dark, pH 7.4 Test media was aerated (24 h)	E2 and VTG levels sign. decreased at all conc. in visceral mass after 21 d Testosterone sign. increased at all conc. in visceral mass after 21 d Gene expression (visceral mass) after 21 d: VTG1, CYP19a1, ER, PR and 17β-HSD1 down-regulated at all concentrations AR and 17β-HSD3 up-regulated at all conc. No effects were seen on CYP17a1 Oxidative stress: Catalase and superoxide dismutase increased at all conc., other indicators (malondialdehyde, glutathione, glutathione reductase) increased at higher conc., effects increased with increasing exposure time Transformation product with highest cumulative concentration: 3,5-dichloro-2-hydroxyacetophenone (in the 14-day test)	Reliability: 2 OMC purity > 98 %	Zhou et al. (2019a)
Danio rerio F0: Zebrafish embryos exposed for 4 months until sexual maturation, fish were paired at 120 dpf. Embryo age at test begin: 2 hpf F1: two groups with and without continued exposure for 5 dpf F0 and F1: 50 embryos per beaker, 3 replicates for treatments and controls Exposure solution replaced twice a day, Solvent: DMSO (0.01 %), Temperature: 28 ± 1°C, pH 7.4 Photoperiod 16:8 h light/dark Test media was aerated (24 h) Concentrations: 1, 10, 100 µg/L (nom)	F0 (40 dpf): VTG and E2 levels sign. decreased in the visceral mass at all concentrations Aromatase activity not decreased Gene expression: VTG1 sign. down-regulated at all concentrations, no effect on VTG2 ER, PGR, CYP19a, CYP19b, 17β-HSD1, 17β-HSD3 sign. down-regulated at all concentrations AR sign. upregulated at 10 and 100 µg/L F0 and F1: Hatching rates 3 dpf: F0 and F1 (without continued exposure): sign. decreased at 10 and 100 µg/L F1 (with continued exposure): sign. decreased at 1, 10 and 100 µg/L, (in controls: 80.7 to 84.7 %) Malformation 5dpf: F0: sign. increased at 100 µg/L, F1: at 10 and 100 µg/L with continued exposure sign. increased, without continued exposure no effect on malformation Survival 5 dpf: sign. decreased at 100 µg/L in the F0 generation, no effects in F1 Body weight 40 dpf: F0: sign. decreased at 100 µg/L, no effect on lenght,	Reliability: 2	

	(F1: no data on growth) Possibly indication for neurotoxicity: acetylcholinesterase activity sign. increased at all concentrations in the brain of F0 parents (120 d) and in F1 (5 dpf, homogenate, with and without further exposure) Content of OMC in fish (F0) and eggs (F1): Fish: Control: 2.11 ng/g ww (incomplete cleaning of instruments or mistake in operation) 1 µg/L: 102.2 ng/g ww 10 µg/L: 925.7 ng/g ww 100 µg/L: 6514.9 ng/g ww F1 eggs from exposed parents: 1 µg/L: 22.2 ng/g ww 10 µg/L: 146.1 ng/g ww 100 µg/L: 1184.5 ng/g ww → parental transfer of OMC to eggs		
Oryzias latipes 2-generation study, following OECD TG 234, with modifications Parents exposed from 0 dpf until 154 dpf 20 eggs per beaker and 4 replicates Fish paired at 106 dpf, 2:2 male:female ratio, 6 mating chambers per treatment, the eggs produced were recorded every day during the 49 d mating period At day 120 all eggs of one treatment were pooled and randomly distributed to beakers with 20 eggs per replicate (4 replicates except conc. 5 mg/L: only 3 replicates, because not enough eggs) F1 generation exposed to the same concentrations as parents until 38 dpf Concentrations: 50, 158, 500, 1580, 5000 µg/L OMC Solvent DMSO (0.01%)	Effect on reproduction: number of eggs per brood per day sign. decreased at 50 µg/L and higher, during mating period 106 to 154 dpf no sign. effects on E2 in plasma Gene expression: No sign. effects on gene expression of ERα, ARα and VTG1 at all concentrations ARα gene down-regulated (not sign.) at 38 dpf in F0 and F1, except at 50 µg/L in the F0 generation A down-regulation trend on 3β-HSD and 17β-HSD3 genes was observed (but no significant differences) Thyroid effects: T3 content decreased at 500 to 5000 µg/L in F1 (38 dpf) A decreasing trend was seen for both T3 and T4 levels (F1, whole-body, 38 dpf), however the T4 levels were rather fluctuating Gene transcription: DIO2 gene down-regulated from 158 to 5000 µg/L in F0 and F1	Reliability: 2	Lee et al. (2019)
Danio rerio Concentrations: 1, 3, 10, 30 µM 1. Test: male adults, exposure 21 d, conc.: 1, 3, 10, 30 µM 2. Test: larvae, exposure from 4 hpf to 5 dpf, conc.: 1, 3, 10, 30 µM	T3/ T4 – levels: Male adults (plasma): No sign. effect (but for T3 decreasing trend) Larvae (whole-body content): T3 sign. decreased at 3 µM (871 µg/L) and higher T4 sign. decreased at 1 µM (290 µg/L) and higher Gene expression (thyroid system):	Reliability: 2	Chu et al. (2021)

	Adult fish, liver: TRß down-regulated at 1 µM (290 µg/L) and higher DIO1 down-regulated at 3 µM (871 µg/L) and higher, Dio2 down-regulated at 10 µM and higher		
Danio rerio FSDT, OECD 234 Duration 60 dph Embryo age at test begin: ca. 4hpf Four replicates with 30 embryos each Flow-through: 6.5 volume exchanges per day 25.8- 27.1°C pH 6.7 – 7.6 Photoperiod 16:8 h light/dark Limit test: 50 µg/L (nominal), 46.9 µg/L (measured)	At 46.9 µg/L (measured): - No effect on hatching and survival - Sex ratio: Female: Male: undiff. fish 55.1 : 34.7 : 10.2 In the control: Female: Male: undiff. fish 51.5 : 44.4 : 4 Less males and more undifferentiated fish, but not significantly changed. - Significantly decreased length in males and females (not in undifferentiated fish) - Significantly decreased body weight in females and males; undifferentiated fish: mean weight 41% of mean control weight, but very high standard deviation in control, not sign. - VTG: no effects - Histopathology of gonads: At 46.9 µg/L: decreased mean ovarian stage score: 0.3 in control: 0.9 Intersex: - in the control 3 (6.8 %) males with testicular oocytes (minimal), none in OMC treated males; - one female fish with ovarian spermatogenesis was seen in the treatment and control each Intersex-like finding: - two control males showed a gonadal duct with a female phenotype (attachment of the testis to the dorsal coelomic mesothelium at two sites, not as normal at one site, forming a intervening space), it was not stated that this also appeared in the treatment Germ cell degeneration and oocyte atresia appeared in control and treatment, in control minimal, in treatment mostly minimal, in a few cases mild	Reliability: 1 Remark: intersex in control (3 males) Purity 99.8 %	(Fort Environmental Laboratories (2020a))

	- Histopathology kidney: No exposure related effects - Histopathology liver: Hepatocytes, karyomegaly (mostly minimal) in males and females in treatment, none in control; single cell necrosis in males and females in treatment, and some in control (mostly minimal), some cases of oval cell proliferation in treated females (6%)		
Abudefduf saxatilis (a tropical marine species), Sergeant major damselfish Intraperitoneal injection Doses: 5, 25 and 50 µg OMC/g fish (nominal) Fish monitored for 96 hours prior to analysis of expression of the VTG gene expression in liver samples	VTG (liver): no effects (96 h) some effects were noted with the dose of E2 used	Reliability: 2 Relevance for environment questionable because of exposure over injections	Soto and Rodriguez-Fuentes (2014)
Danio rerio Semi-static Investigation of oestrogenic response in transgenic zebrafish (*Danio rerio*) Fish exposed for 96 hours to one test concentration of 10 µM OMC (≡ 2.9 mg OMC/L)	No oestrogenic activity in transgenic zebrafish reported at nominal 10 µM OMC	Reliability: 2	Schreurs et al. (2002)
Pimephales promelas Juvenile fathead minnow Semi-static exposure for 14 days Solvent: ethanol (0.01%) Concentrations: 10, 100, 500, 1000, 5000 µg/L OMC (n) (In vitro test recombinant yeast assay see above)	No effect on VTG after 14 days up to 5000 µg/L (n) no effect on growth up to 5000 µg/L	Reliability: 2	Kunz et al. (2006)
Oncorhynchus mykiss Sexually immature juvenile rainbow trout, weighing 92 +- 20 g; Duration of experiment: 14 d Intraperitoneal injections of OMC at days 0, 3, 6, 10: Volume approx. 225 µL per fish was adjusted to the weight of individual fish; Doses: 9, 31, 76, 202 mg OMC/kg injection dissolved in	VTG in plasma: no effect up to 202 mg/kg*injection (one fish at 76 mg/kg group and two fish at 202 mg/kg increased their VTG concentrations) 1 of 10 fish died at 9 mg OMC/injection, 2 of 10 fish died at 31, 76 and 202 mg/injection each Pos. control E2: sign. VTG increase	Reliability: 2 Relevance for environment questionable due to exposure over injections	Søgaard et al. (2021)

peanut oil, 10 fish per group, no replicates Pos. control: 930 µg E2/kg injection			
Oncorhynchus mykiss Juveniles Duration 6 weeks, Water in aquaria changed daily 20 fish per vessel Feeding: 6.9, 96, 395.6 µg/kg Feeding twice a day, Animales were weighed in the beginning to determine feed dose, check every second week.	No ED endpoints examined - No effect on mortality, feed intake, behaviour, growth - Haematology: No effect on erythrocytes Leukocyte count decreased at 395.6 µg/kg - Biochemical examination: statistical sign.changed were: glucose, lactate, total protein, albumin, cholesterol, triacylglycerols, ammonia → authors stated that the metabolism was disrupted at 96 and 395.6 µg/kg - Oxidative stress: significant increase of GPx activity in the liver (crucial role in the detoxification of pollutants) - Histopathology: Damage of hepatic parenchyma increased with increasing concentration, no effects on spleen, kidney, skin and gills	Reliability 2	Cahova et al. (2021)
Xenopus laevis AMA, OECD TG 231 Three concentrations: 5.97, 18.1, 44.2 µg/L (measured) Age of the organisms at test initiation: NF stage 51 Exposure: 21 d 20 tadpoles per test tanks Four replicates per concentration and water control	- Median developmental stage, snout-vent length, hind limb development and body weight were not statistically different from control on SD day 7 and 21 - Histopathology: No treatment-related findings in the thyroids of tadpoles exposed to OMC. - No thyroid effects and no other signs of toxicity were seen.	Reliability 1,	(Fort Environmental Laboratories Inc., 2020b)