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

SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Benzotriazole

EC No 202-394-1

CAS RN 95-14-7

Evaluating Member State(s): Germany

Dated: December 2023

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Evaluating Member State Competent Authority

BAuA
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Year of evaluation in CoRAP: 2016

Before concluding the substance evaluation a Decision to request further information was issued on: 19 December 2017
Member State concluded the evaluation without any further need to ask more information from the registrants under Article 46(1) decision.

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

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

Benzotriazole ("the Substance", "BTA", EC No. 202-394-1, CAS RN 95-14-7) was originally selected for substance evaluation in order to clarify concerns about:

- endocrine disrupting properties in the environment
- exposure of the environment

During the evaluation, persistency and mobility in the environment were identified as additional concerns.

2. OVERVIEW OF OTHER PROCESSES/EU LEGISLATION

On 30 September 2014, ECHA issued a compliance check decision on BTA requesting the following information:[2]

- Active sludge respiration inhibition testing (Annex VIII, 9.1.4.)

ECHA issued a decision on testing proposals on BTA on 26 November 2018, requesting the following information:[3]

- Pre-natal developmental toxicity study in a first species (Annex IX, Section 8.7.2.)
- Simulation testing in surface water (Annex IX, Section 9.2.1.2.) including identification of the degradation products (Annex IX, Section 9.2.3.)
- Long-term toxicity on terrestrial invertebrates (Annex IX, Section 9.4.1., column 2)
- Effects on soil microorganisms (Annex IX, Section 9.4.2.)
- Long-term toxicity testing on plants (Annex IX, Section 9.4.3., column 2)

On 20 August 2021, the evaluating Member State Competent Authority (eMSCA) submitted a dossier for harmonised classification and labelling (CLH) of BTA to ECHA with respect to chronic aquatic toxicity.[4] The Risk Assessment Committee (RAC) adopted an opinion which supports the proposed classification on 15 September 2022.[5]

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.

The eMSCA has prepared a Regulatory Management Option Analysis (RMOA) for BTA based on its ED properties and other environmental toxicity which also expands on the Substance's potential for repro toxicity[6] in follow-up to the data generated under testing proposal evaluation.

[2] ECHA CCH decision on BTA from 30 September 2014: https://echa.europa.eu/documents/10162/ab67955b-a571-c40b-d624-2880669f5ee9
[3] ECHA TPE decision on BTA from 26 November 2018: https://echa.europa.eu/documents/10162/a1ec7f7a-fd44-abe7-b9ee-90e6e57df5ab
[4] ECHA section on CLH process for BTA: https://echa.europa.eu/de/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e1821c4f08
[5] RAC opinion on CLH proposal for BTA: https://www.echa.europa.eu/documents/10162/997bec2b-41ec-c54e-33fc-34abcca9edd6
[6] ECHA section on RMOA for BTA: https://echa.europa.eu/de/assessment-regulatory-needs/-/dislist/details/0b0236e188b8f0be

Table 1

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

4. FOLLOW-UP AT EU LEVEL

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

4.1.1. Harmonised Classification and Labelling

The eMSCA concludes that BTA meets the criteria for an endocrine disruptor (ED) for the environment according to the criteria of the World Health Organisation (WHO) and also the criteria for classification as ED ENV 1, EUH430 of the new hazard criteria of the revised CLP Regulation (EC) No 1272/2008 (EC, 2022).

Furthermore, due to fulfilling the criteria for being persistent/very persistent (P/vP) and mobile/very mobile (M/vM) and fulfilling the T criteria due to the ED ENV 1, the eMSCA also considers the criteria for PMT as fulfilled under the revised CLP regulation.

Additionally, due to fulfilling the criteria for very persistent and very mobile substance the vPvM criteria are fulfilled.

The assessment of the available mammalian data on BTA was performed only in the context of the evaluation of the ED concern for the environment. Based on the assessment, the eMSCA considers the Substance as eligible for classification as a reprotoxicant (see section 7.10.1.3).

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

The eMSCA considers that both the ED properties for the environment and the PMT/vPvM status on their own could already be sufficient to identify BTA as a substance of very high concern (SVHC) according to REACH Article 57(f). For these properties, this route would currently require an additional assessment of the "equivalent level of concern" to substances identified according to Article 57(a) through (e), i.e. substances with CMR (Cat. 1A or 1B) or PBT and vPvB properties.

The eMSCA scrutinised the potential regulatory options to reach an EU-wide understanding on the environmental ED properties and/or the PMT/vPvM properties via REACH and/or CLP in a Regulatory Management Option Analysis (RMOA).[7]

4.1.3. Restriction

The eMSCA considers environmental ED properties and PMT/vPvM properties as hazardous properties for which derivation of a safe threshold in the environment is very difficult, if possible at all. Hence, after reaching consensus on the environmental hazard properties at EU level, the eMSCA considers that a restriction could be a way to further limit emissions of BTA to the environment as much as possible but considers authorisation to be a more appropriate risk management measure.

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

BTA fulfils the criteria for classification as Aquatic Chronic 2, H411. The eMSCA has prepared and submitted a CLH dossier based on the available information for the Substance proposing the respective classification. Following submission, the Committee for Risk Assessment (RAC) has adapted an opinion[8] on the proposal on 15 September 2022, supporting the eMSCA's proposal.[9]

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.

[7] RMOA conclusion for BTA: https://echa.europa.eu/assessment-regulatory-needs/-/dislist/details/0b0236e188b8f0be

[8] https://www.echa.europa.eu/documents/10162/997bec2b-41ec-c54e-33fc-34abcca9edd6

[9] CLH proposal on BTA: https://echa.europa.eu/de/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e1821c4f08

Table 2

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
RMOA	2023 (published)	eMSCA
CLH dossier	2024	eMSCA
SVHC	Following the CLH dossier	eMSCA

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

Benzotriazole ("the Substance", "BTA", EC 202-394-1, CAS 95-14-7) was originally selected for substance evaluation in order to clarify concerns about:

- endocrine disrupting properties in the environment
- exposure of the environment

During the evaluation, persistency and mobility in the environment were identified as additional concerns.

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Endocrine disrupting properties in the environment	Concern confirmed. Based on the newly generated information in the substance evaluation process, BTA fulfils the WHO criteria for endocrine disruption in the environment and could be classified as environmental ED category 1 according to CLP.
Exposure of the environment	Concern confirmed. BTA is registered for use by consumers and in articles which may lead to wide dispersive emissions of the Substance in the environment.
Persistency	Concern confirmed. Based on available information, BTA fulfils the CLP criteria for vP (very persistent) according to CLP and REACH Annex XIII.
Mobility	Concern confirmed. Based on its log k_{oc} value of <2, BTA fulfils the CLP criteria for vM (very mobile).
PMT	Concern confirmed. Based on the above considerations on the respective criteria and on the eMSCAs consideration that the T criterion according to CLP is fulfilled due to the possible classification of BTA as ED ENV Cat. 1.
vPvM	Concern confirmed based on the above considerations on the respective criteria.

Additional endpoints evaluated	
Bioaccumulation	The eMSCA considers the bioaccumulation potential of BTA as low and thereby not fulfilling the criteria for B or vB according to CLP and REACH Annex XIII.
PBT	The eMSCA considers that the Substance is not PBT based on the low bioaccumulation potential of BTA.
vPvB	The eMSCA considers that the Substance is not vPvB based on the low bioaccumulation potential of BTA.
Reproductive toxicity	The eMSCA considers that the substance fulfils the criteria for classification as reproductive toxicant according to CLP. The available information does not suggest that the adverse effects on development observed in rodents are endocrine-mediated.

7.2. Procedure

The Substance was initially included in the CoRAP update 2014-2016. The substance evaluation was initiated following the publication of the CoRAP 2016-2018 on 22 March 2016. The evaluation covered all environmental endpoints (physico-chemical data, ecotoxicity data, exposure, fate and behaviour).

The eMSCA conducted a comprehensive and structured literature research to gather literature on the ecotoxicity to different organism groups, endocrine modes of action, endocrine effects, exposure, fate and monitoring studies using defined keywords and synonyms.

The concern with respect to the endocrine disrupting properties for the environment was evaluated considering the available *in vitro* and *in vivo* data. Additionally, the available mammalian data in the registration was assessed by the eMSCA to inform on the concern for endocrine disruption in the environment.

ECHA's Endocrine Disruptors Expert Group was consulted twice. The first consultation took place in September 2016 during the initial assessment period (ED EG meeting 8 in September 2016). During the second consultation, the results obtained from the requested FSDT study[10] (OECD TG 234) were presented and discussed at the ED EG meeting 22 in April 2022. The discussion and views of the experts were reflected in concluding the present substance evaluation.

7.3. Identity of the substance

Table 4

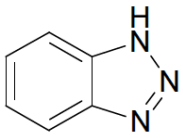
SUBSTANCE IDENTITY	
Public name:	Benzotriazole
IUPAC name:	1*H*-Benzotriazole
EC number:	202-394-1
CAS number:	95-14-7

[10] Decision 2017: c25f2924-ac95-d14c-4740-21273d31fddd (europa.eu)

Index number in Annex VI of the CLP Regulation:	-
Molecular formula:	$C_6H_5N_3$
Molecular weight range:	119.12 g/mol
Synonyms:	*1,2,3-1H-Benzotriazole* *1,2,3-Benzotriazole* *1,2,3-Triaza-1H-indene* *1,2,3-Triazaindene* *1,2-Aminoazophenylene* *1H-1,2,3-Benzotriazole* *1H-Benzo[d]-1,2,3-triazole* *2,3-Diazaindole* *Azimidobenzene* *Aziminobenzene* *Benzene azimide* *Benzisotriazole* *1,2,3-Benzotriazole* *1,2,3-Benztriazole* *Benztriazole* *BTA*

Type of substance: Mono-constituent

Structural formula:



7.4. Physico-chemical properties

Table 5

OVERVIEW OF PHYSICO-CHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20 °C and 101.3 kPa	*colourless or pale-coloured solid (powder)*
Melting point at 101.3 kPa	*100 °C*
Boiling point at 101.3 kPa	*204 °C*
Density	*1.36 g/cm³ (20 °C)*
Vapour pressure	*< 0.01 kPa (25 °C)*
Water solubility	*19.8 g/L (25 °C)*
Partition coefficient n-octanol/water (log K_{ow})	*1.34 (22.7 °C)*
Granulometry	*D_{10} = 849 [µm]* *D_{50} = 1256 [µm]* *D_{90} = 2133 [µm]* *mass median aerodynamic diameter (MMAD): 1256 [µm].*
Stability in organic solvents and identity of relevant degradation products	*N/A. The registration states the following:* *The stability of benzotriazole in usual organic solvents is not considered to be critical. There is no evidence of any decomposition in organic solvents e.g. ethanol, acetone, acetonitrile.*
Dissociation constant	*pK_a at 20 °C: 8.37*

7.5. Manufacture and uses

7.5.1. Quantities

Table 6

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

BTA is used as cooling agent and additive in lubricants, in washing and cleaning products, including disinfection products (as non-active substance), for metal surface treatment, industrial water treatment, and de-icing of roads and parking areas – in this order of relevance. There are further several uses by down-stream users. Consumer uses focus on dish wash products and a few functional fluids.

Table 7

USES	Use(s)
Uses as intermediate	Industrial use as an intermediate
Formulation	ERC 2: Formulation of preparations for all major uses
Uses at industrial sites	ERC 4: Processing aid, not becoming part of the article; use of lubricants and lubricant additives in various open processes, and as industrial cleaning product and metal working fluid (corrosion inhibitor and anti-scaling agent), as well as binding agent in industrial water treatment.
Uses by professional workers	ERC 8a: Wide dispersive indoor use of processing aids in open systems; professional uses as corrosion inhibitor and anti-scaling agent in various products, among them de-icing products (ERC 8d: outdoor use) and heat transfer fluids. ERC 9a, 9b: Wide dispersive indoor/outdoor use of processing aids in closed systems; professional uses in lubricants and lubricant additives, de-icing products and heat transfer fluids.
Consumer Uses	ERC 8a, 8d: Wide dispersive indoor/outdoor use of processing aids in open systems; consumer uses as corrosion inhibitor and anti-scaling agent in dish wash products and in lubricants and greases. ERC 9a, 9b: Wide dispersive indoor/outdoor use of processing aids in closed systems; consumer uses as corrosion inhibitor and anti-scaling agent in functional fluids, as well in lubricants and greases for vehicles and machinery.
Article service life	Subsequent service life is not relevant for these uses.

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

BTA is not listed in Annex VI CLP. However, a CLH proposal for long-term aquatic toxicity (Aquatic Chronic 2, H411) has been submitted by the eMSCA which is supported by RAC in its opinion from 2022 (cf. section 5.1).

7.6.2. Self-classification

In the registration(s):

Acute Tox. 4	H302
Eye Irrit. 2	H319
Aquatic Chronic 2	H411

Additional notified hazard classes in the C&L dossier:

STOT SE 3	H336 (Central nervous system)
Muta. 2	H341
Eye Dam. 1	H318
Flam. Sol. 1	H228
Skin Irrit. 2	H315
Acute Tox. 3	H301
Acute Tox. 4	H332
Acute Tox. 4	H312
Aquatic Chronic 3	H412
STOT SE 3	H335 (Respiratory irritation)

7.7. Environmental fate properties

7.7.1. Degradation

Several studies investigating the degradation of BTA are included in the registration dossier.

Study 1: Biodegradability
An OECD 301 D test (Rheinchemie, 1991a) using non-adapted activated sludge on the one hand and adapted activated sludge on the other hand showed respectively 0% degradation (oxygen demand) after 28 days of incubation at a concentration of 1 000 mg/L for both types of inocula.

Study 2: Biodegradability
In an OECD 301 B test (Procter & Gamble ETC, 1994a) with adapted activated sludge at a concentration of 10 mg/L, 0% degradation (CO_2 evolution) after 29 days of incubation was observed. Considering that a test on bacterial inhibition according to OECD TG 209 and ISO 8192 (Bayer AG, 1991) showed an EC50 >1 000 mg/L it can be expected no inhibitory effects at the test concentration occurred.

Study 3: Biodegradability
In an OECD 302 A test (Procter & Gamble, 1994) for inherent biodegradability using BTA at a test concentration of 20 mg/L and adapted sewage, 1% degradation (DOC removal) was observed after 30 days of incubation.

Study 4: Biodegradability

An OECD 302 B test (Rheinchemie, 1988) for inherent biodegradability with BTA using activated sludge with unknown adaptation showed 90% degradation (test material analysis) after 28 days of incubation in daylight and 83% degradation (test material analysis) after 28 days of incubation in darkness at a concentration of 2 mg/L. The reliability of this test on inherent biodegradation is considered to be limited as the adaption of the inoculum cannot be excluded. This is due to the fact that it was sampled from a sewage treatment plant, where a biological treatment step handles sewage from households and different chemical industry sites[11].

Study 5: Degradability in freshwater

In a water simulation study (Connect Chemicals GmbH, 2021a) of radiolabelled BTA, according to OECD TG 309, degradation was investigated in surface water without the addition of suspended sediment. The test was performed as flow-through system on an orbital shaker as a pelagic surface water test. For that, 250 mL surface water was filled in 0.5 L Erlenmeyer flasks. Volatile residues were quantified with NaOH traps. The water was sampled from Biggesee, DE. After collection, the surface water was cooled at 8 °C for 8 days prior to the test.

BTA with a radiolabelled chemical purity of 99.9% was dissolved in acetonitrile to prepare a stock solution, followed by dilution to the final test concentrations of 10 µg/L and 50 µg/L, which were added to the test system respectively. Duplicate samples were incubated for day 0, 7, 14, 21, 28, 42 and 62 days, respectively at 12 ± 2 °C in darkness. At the respective sampling days, radioactivity in surface water and NaOH were quantified by means of liquid scintillation counting. Prior to radio-HPLC analysis the water was extracted by solid phase extraction.

As reference substance, radiolabelled benzoic acid was used. After 7 days of incubation >50% of applied radioactivity (AR) of ^{14}C-benzoic acid evolved as $^{14}CO_2$. At the end of the study (day 62), the mineralisation was >70% AR in surface water. Based on the results it is concluded that the surface water used in the test contained an active microbial population.

The total recoveries of samples applied with BTA ranged between 90 and 110% AR for all individual samples. According to OECD TG 309, recovery should be between 90% and 110% for labelled substances. Thus, the study is regarded as valid.

In the samples incubated with BTA neglectable amounts (<1% AR at day 62) of $^{14}CO_2$ were detected. Analysis of the solid phase extraction (SPE) extracts shows that 94.4 – 98.6% AR at the beginning (day 0) and 92.9 – 96.1% AR at the end of incubation (day 62) in the 50 µg/L samples were attributed to the parent substance BTA. No transformation products were detected in the surface water during the study. The kinetic assessment for the measured concentrations resulted in a DT_{50} of 1050 days (10 µg/L) and 658 days (50 µg/L). For both assessments, the single first order (SFO) model was applied as best fit. No ultimate or primary degradation of ^{14}C-BTA took place in the surface water test system and thus BTA has to be evaluated as very persistent in surface water.

Study 6: Degradability in freshwater

In another study by Hofman-Caris and and Claßen, according to OECD TG 309, the degradation of unlabelled BTA was investigated in surface water without the addition of suspended sediment (Hofman-Caris and Claßen, 2020). The test was performed using stationary biometer test systems using 300 mL of surface water. After collection, the surface water was cooled at 13 °C for 7 days prior to the test.

[11] https://www.wupperverband.de/projekte/kooperationen/gemeinschaftsklaerwerk-in-leverkusen (accessed 03.05.2023).

BTA with a purity of 99.9% was dissolved in water resulting in a concentration of 5.47 mg/L and was added in a concentration of 1 µg/L to the test system. Duplicate samples were incubated for 0, 7, 15, 30, 45, and 60 days, respectively at 13 ± 1 °C in darkness. As reference substance, unlabelled aniline was used. The application rate of aniline was 1.0 µg/L. Besides the reference substance, the microbial activity of the water was also investigated by determining the amount of adenosine triphosphate (ATP). Degradation was evaluated based on the concentration of reference substance and BTA detected in the water phase using GC-MS/MS for aniline and LC-MS/MS for BTA. The amount of ATP in the surface water without the addition of BTA at day 0 was 86 ± 1.2 pg/mL, after autoclaving, the ATP concentration was reduced (<1 ± 0.44 pg/mL), indicating that the ATP assay is able to reflect the microbial activity of the surface water. In the presence of BTA, the ATP concentration was slightly higher (110 ± 3.1 pg/mL) to those measured in surface water without any addition after 2 days, meaning that BTA has no negative effect on the microbial population. The concentration of the reference substance aniline decreased over the course of the study. After 15 days of incubation, the concentration was 0.418 ± 0.1 µg/L. At the end of the study (day 60), the aniline concentration amounted to < 0.01 µg/L in the surface water. Degradation of aniline based on the concentration observed amounted to 52% and >98% after 15 and 30 days of incubation. Based on the results it is concluded that the surface water used in the test contained an active microbial population.

Recovery of the BTA applied to the test ranged between 105 - 120%. According to OECD TG 309, initial recovery should be between 70% and 110% for non-labelled substances. Thus, the study is regarded as valid.

The BTA concentration remained stable over the course of time and ranged between a minimum value of 1.05 ± 0.01 µg/L at day 7 and a maximum value of 1.20 ± 0.0 µg/L at the end of the study (Figure 1).

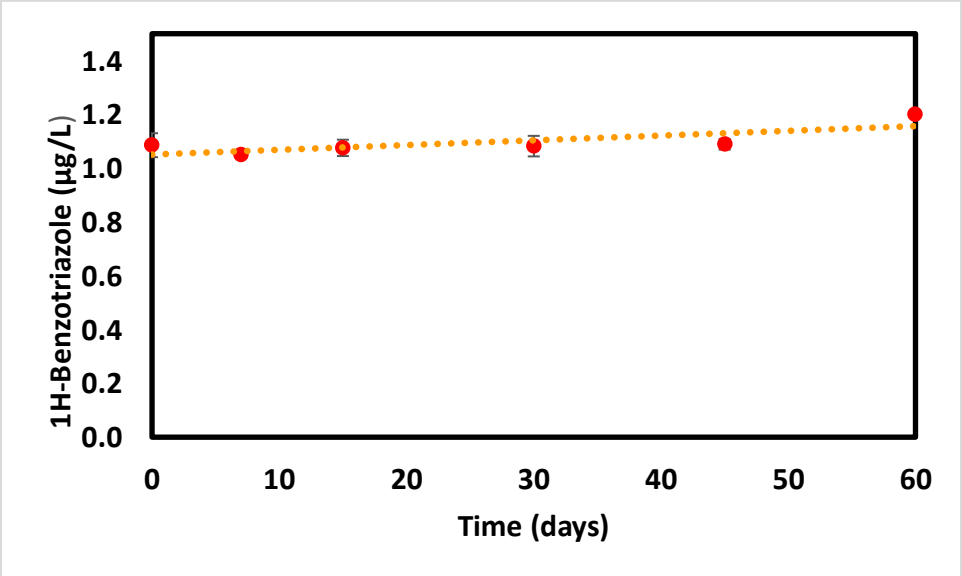


Figure 1: Concentration of BTA (µg/L) in a surface water test system over the course of time (days). Data were shown as mean value of two individual bottles for each sampling point with error bars indicating standard deviation.

As unlabelled BTA was used in this study, ultimate degradation of BTA could not be determined. Nevertheless, as no decline in test concentration was detected during the study, no ultimate or primary degradation of BTA took place in the test system and thus BTA has to be evaluated as very persistent in surface water. The kinetic assessment for the measured concentrations resulted in a DT_{50} of >10,000 days where the SFO model was applied due to best visual fit and smallest Chi^2 error (3.4%).

Study 7: Degradability in soil
Breedveld and colleagues investigated the aerobic and anaerobic degradation of BTA in the terrestrial compartment (Breedveld et al., 2002). Therefore, a series of batch reactors were inocculated with microorganisms from the area of the abandoned airport Gardermoen, Norway and airport Fornebu, Norway. BTA (1 000 µg/L) as substrate as well as other substances for achieving necessary oxygen consumption (benzoate or glycol) were added. As control (aerobic conditions) $CuSO_4$ was used. After a five-month period no degradation of the test substance was observed under anaerobic conditions. Decreasing concentrations under aerobic conditions and in the abiotic control showed that biotic and abiotic degradation of BTA in soil took place in presence of oxygen. However, as the adaption of the inoculum cannot be excluded, no conclusion on biodegradability of BTA in soils can be drawn.

Summary
The degradation of BTA has been tested within different test systems and media, which all indicated an incomplete degradation. The degradation half-life of BTA in surface water exceeds the threshold of 60 days for very persistent substances. Thus, the eMSCA concludes that BTA is very persistent in the environment according to Regulation 1907/2006 (REACH), as well as the revised version of Regulation 1272/2008 (CLP) (EC, 2022).

7.7.2. Volatility

According to the registration dossier, the Substance has a calculated Henry`s Law constant of 0 Pa· m³/mol by using water solubility and vapour pressure of the substance according to equation R.16-4 (ECHA, 2016). Therefore, BTA is judged to be of low volatility and to remain in the water phase after its release into the environment.

7.7.3. Mobility

In the registration dossier four studies are available that investigated the sorption behaviour of BTA, two QSAR based studies and two experimental studies.

Study 1: Model-based
The QSAR estimation of the sorption behaviour according to Schüürmann and colleagues indicated a partitioning coefficient between organic carbon and water ($\log K_{OC}$) of 1.69 for BTA (Schüürmann et al., 2006).

Study 2: Model-based
Based on a QSAR using the programme KOCWIN v2.00 the $\log K_{OC}$ = 1.72 was calculated (MCI method). Based on an equation provided in the ECB Technical Guidance Document from the year 2000 on how to calculate the $\log K_{OC}$ from the partitioning coefficient between octanol and water ($\log K_{OW}$), the calculated $\log K_{OC}$ is 1.80.

Study 3: Experimental
Hart and colleagues performed a study that investigated the adsorption and partioning behavior of three different benzotriazoles – including BTA - in four top soils according to OECD TG 106 (Hart et al., 2004). The compositions of the soils – seemingly obtained from regions in a rather temperate climate zone - were as following:

Soil 1: 1.72% Organic Carbon, pH 7.4, 16% Clay, 36% Sand, 48% silt
Soil 2: 0.99% Organic Carbon, pH 7.5, 16% Clay, 34% Sand, 50% silt
Soil 3: 0.33% Organic Carbon, pH 6.8, 4% Clay, 88% Sand, 8% silt
Soil 4: 0.27% Organic Carbon, pH 7.6, 14% Clay, 50% Sand, 36% silt

The test was performed with a soil-solution ratio of 1:1 and the test item was applied in triplicates at 10, 20, 50, 100, 200 and 500 mg/L. Samples were shaken for 12 h to reach equilibrium and left standing for 24 h to allow for sedimentation. Adsorption was followed by HPLC analysis of aliquots of the solution which were previously centrifuged. Considering that the pH within the investigated soils ranges from 7.6 to 6.8 while the pK_a of BTA amounts to 8.39 it has to be assumed that it is present in the soils mostly in its neutral form – likely 10 - 15% are anionic in soil 1, 2 and 4 (calculated via the Henderson-Hasselbalch Equation).

The linear partition coefficient (K_d) was obtained by fitting sorption data over the equilibrium concentration range of 0 to 100 mg/L. Furthermore, the Freundlich partitioning coefficient (K_f) was calculated, which assumes a non-linear partitioning behavior (e.g. decreasing K_d with increasing substance concentrations). K_d was normalized according to the organic carbon (OC) content to allow for a comparability between the different soils, thereby assuming that OC acts as the main sorbent. The results are summarized in Table 8.

The stated $log\ K_{OC}$ values range between 1.50 and 1.90. However, according to OECD TG 106, soils with <0.3% OC may disturb the correlation between organic content and adsorption. In line with this recommendation, soil 4 with an OC content of 0.27% should not be further regarded in the assessment of mobility of BTA. Following this assumption, the $log\ K_{OC}$ values that should be considered range from 1.66 to 1.90, which indicates that the compound has a very high mobility. These $log\ K_{OC}$ values are also reported in the REACH registration dossier.

The study did not strictly follow the guidance for selection of soils given in table 1 of the OCED TG 106. Compared to (experimental) study 4, the pH values of the considered soils are slightly higher, which may impact the obtained K_{OC} values by reducing them. Therefore, while being relevant, the results of this study might be more conservative than those achieved in experimental study 4.

Table 8

Organic carbon content and partition coefficients determined for study by Hart et al. (2004)				
	Soil 1	Soil 2	Soil 3	Soil 4
Linear partitioning parameters				
K_d	0.782	0.621	0.262	0.086
R^2	0.962	0.993	0.923	0.869
Organic Carbon Normalization				
C org [%]	1.72	0.99	0.33	0.27
Log K_{oc}	1.66	1.80	1.90	1.50
Freundlich parameters				
K_f [$(kg^{-1}*L)^{1/n}$]	3.35	2.02	0.97	0.23
n	0.60	0.66	0.69	0.78
R^2	0.9970	0.9644	0.9956	0.9973

Study 4: Experimental

In a study by Breedveld et al. (2003) the adsorption of BTA to organic carbon was investigated according to the requirements of the test guideline OECD TG 106. Composition of the soils were as follows:

Soil 1: medium sand 0.2% Organic Carbon, pH 7.1-9
Soil 2: Fe-rich sand, 1.2% Organic Carbon, pH 4.5-4.6
Soil 3: Fine sand, 1.7% Organic Carbon, pH 5.1-5.7
Soil 4: Clay soil, 1.6% Organic Carbon, pH 6.1-6.2
Soil 5: Compost, 26.5% Organic Carbon, pH 7.0-7.1
Soil 6: Peat, 47.4% Organic Carbon, pH 3.0

The test was performed with a soil-solution ratio of 1:4.2 and the substance was applied at 0.1, 10 and 100 mg/L. No information on the use of replicates were provided. Samples were shaken for 24 h to reach equilibrium, left for sedimentation for 30 minutes and centrifuged. This was followed by HPLC analysis of aliquots of the solution. The obtained sorption data were analysed under the use of the K_f only (Table 9).

As only K_f was analysed, no data for K_d and $log\ K_{OC}$ were provided. However, within the registration it was estimated that the highest $log\ K_{OC}$ within the study amounts to 1.4 (suggested for soil 6 with the highest organic carbon content and a pH of 3). While the $log\ K_{foc}$ values obtained here cannot simply be compared towards the K_d mobility criteria, they allowed the authors of the study to conclude that there is almost no sorption and a high mobility of BTA in mineral soils with relatively little OC. For soils with very high amounts of OC, like compost, which is not a natural soil, and peat a higher sorption was observed. Accordingly, the authors concluded that the sorption of BTA is mostly determined by the OC content of the soil.

The soils used within study 4 do not strictly follow the characteristics of soils recommended in Table 1 of the OECD TG 106 and their origin is unclear (they could have been sampled or artificially formed). In regard to the mass of soil to volume of aqueous solution ratio, it can be stated that it is within possible ranges from OECD TG 106. While the origin of the used soils is not completely clear the results of this study should still be considered relevant for the assessment of the mobility of BTA.

Table 9

Organic carbon content and partition coefficients determined for study by Breedveld et al. (2003)						
	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5	Soil 6
Freundlich parameters						
K_f [$(kg^{-1}*L)^{1/n}$]	0.86	1.96	3.18	3.65	32.5	56.3
n	0.93	1.22	1.35	1.12	1.15	1.05

Summary
All the before mentioned information, including modelled and experimental $log\ K_{OC}$ values for soils of different properties, indicate a $log\ K_{OC}$ that is <2 for BTA. Considering the mobility (M) criteria (M: $log\ K_{OC}$ <3); and the "very mobile" (vM) cirtieria (vM: $log\ K_{OC}$ <2) within the CLP regulation it has to be assumed that BTA has a low tendency to adsorb to and be retained by organic matter in soil and sediment, thus causing it to be very mobile within the aquatic environment.

Considering the persistency of the substance and its mobility in the aquatic environment, following assumptions on the behaviour of BTA can be made: 1) The substance is not likely to be efficiently removed by adsorption to organic materials in sewage treatment plants or in drinking water production, and 2) the substance is able to spread throughout all water bodies and finally also reach the groundwater. Monitoring data for BTA in surface waters, marine water, ground water, tap water and wastewater treatment plants is available in section 7.12.2.

In accordance to the data presented here the eMSCA concludes that BTA is very mobile (vM) according to the new hazard criteria introduced into Regulation 1272/2008 (CLP) (EC, 2022) and can be expected to contaminate the aqueous environment.

7.7.4. Bioaccumulation

Screening of the bioaccumulation potential of BTA is based on the measured log K_{ow} of 1.34 (at 22.7 °C, pH 5.7) based on an OECD TG 107 study. Strictly speaking, this value needs to be accompanied by the pH in the measurement for dissociating substances – the pKa of BTA is reported to be 8.37 (at 20 °C). The log K_{ow} is not a good descriptor of bioaccumulation potential for ionisable substances at environmental pH. Therefore, the following conclusion for the bioaccumulation potential with regard to the screening criterion of log K_{ow} < 4.5 does not apply.

Nevertheless, the eMSCA considers the bioaccumulation potential of BTA as low and does not consider further testing on bioaccumulation in aquatic species as warranted.

7.8. Environmental hazard assessment

Partly the following tables for the aquatic compartment were copied from the CLH dossier[12] for BTA.

7.8.1. Aquatic compartment (including sediment)

7.8.1.1. *Fish toxicity*

7.8.1.1.1. Fish acute toxicity

Table 10

Summary of relevant information on acute fish toxicity					
Species	***Test substance***	***Results [mg/L]***	***Test method and experimental conditions***	***reliability***	***Ref.***
Brachydanio rerio (new name: *Danio rerio)*	BTA CAS 95-14-7	96h-LC_{50} = 180	OECD 203; semi-static; conc.: 32 – 56 – 100 – 180 – 320 mg/L; 10 fishes (1 replicate); length: 2.7 ± 0.2 cm; temp.: 24.3 – 25.4 °C; pH 7.2-8.2	1	Registration dossier: (Procter & Gamble ETC, 1993)

[12] https://echa.europa.eu/de/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e1821c4f08

Summary of relevant information on acute fish toxicity					
Species	***Species***	***Species***	***Species***	***Species***	***Species***
Brachydanio rerio (new name: *Danio rerio)*	BTA CAS 95-14-7	96h-LC_{50} > 100	Verfahrensvorschlag (F.1.1) "Letale Wirkung beim Zebrabärbling Brachydario rerio" (LC0, LC50, LC100; 48-96 Stunden) des Umweltbundesamtes, Stand 01.06.83; static; conc.: 100 mg/L (Limit-test); 10 fishes (1 replicate); length: 30 ± 5 mm; temp.: 23 ± 2 °C; pH 6.4 – 7.0	2 – deficiencies in reporting	Registration dossier: (Rheinchemie, 1985)
Oncorhynchus mykiss	Sodium 1*H*-benzotriazolide CAS 15217-42-2 (3DT199)	96h-LC_{50} = 14.84	According to USEPA, "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms," Fourth Edition, EPA/600/4-90/027; conc.: 6.25 – 12.5 – 25 – 50 – 100 mg/L; static, no analysis; temp.: 12.5 – 12.7 °C, pH 6.85 – 8.89; 16 h light per day; age of fish: 15 – 30 days, number of fish and replicates not specified	4 – Reporting deficiencies: replicates and number of fish unknown	Registration dossier: (Nalco Company, 2007)
Lepomis macrochirus (Bluegill sunfish)	Sodium 1*H*-benzotriazolide CAS 15217-42-2 (73199)	96h-LC_{50} = 75.85	According to EPA/600/4-90/027, conc.: 125 – 250 – 500 - 1000 - 2000 mg/L, no analysis, static, 3 replicates with 10 fish each, temp. 21.4 - 21.6 °C., pH 7.38 – 8.33, 16 h light per day	2	Registration dossier: (Nalco Chemical Company, 2003b)
Fathead minnow	Sodium 1*H*-benzotriazolide CAS 15217-42-2 (73199)	96h-LC_{50} = 67.24	According to EPA/600/4-90/027, conc.: 62.5 – 125 – 250 - 500 - 1000 mg/L, no analysis, static, 3 replicates with 10 fish each, age of fish < 5 day, temp.: 25.3 - 25.4 °C, pH 8.11 – 8.34, 16 h light per day	2	Registration dossier: (Nalco Chemical Company, 2003a)
Menidia beryllina (Silversides)	Sodium 1*H*-benzotriazolide CAS 15217-42-2 (73199)	96h-LC_{50} = 30.75	According to EPA/600/4-90/027, conc.: 6.25 – 12.5 – 25 – 50 – 100 – 200 mg/L, no analysis, salt water, 3 replicates with 10 fish each, temp. 25.3 – 25.5 °C, pH 7.68 – 8.99, 16 h light per day	2	Registration dossier: (Nalco Chemical Company, 2003c)
Menidia beryllina (Silversides)	Sodium 1*H*-benzotriazolide CAS 15217-42-2 (3DT199)	96h-LC_{50} = 39.36	According to EPA/600/4-90/027, conc.: 62.5 – 125 – 250 - 500 – 1000 mg/L, no analysis, saltwater, 3 replicates with 10 fish each, temp. 24.7 - 25.3 °C, pH 7.40 - 8.01, 16 h light per day	2	Registration dossier: (Nalco Chemical Company, 2005)

7.8.1.1.2. Long-term toxicity to fish

A Fish Sexual Development Test (FSDT) according to OECD TG 234 with exposure duration of 63 days was conducted as a requirement from the SEV decision on BTA (Connect Chemicals GmbH, 2021b). The tested fish species was *Danio rerio*. The NOEC at 35 dpf for post-hatch survival was 1.0 mg/L and the LOEC 3.2 mg/L. Percentages of post-hatch survival were 95.8, 92.5, 90.0, 94.2, 84.2, 83.3 % in control and at 0.1, 0.32, 1.0, 3.2, 10 mg/L (nominal), respectively.
Length was significantly decreased at the lowest and highest concentration. The differences were smaller than 5 % and considered not biologically relevant. This study is evaluated in detail in section 7.10.1.2.

A fish early life stage test (FELS) with *O.latipes* (Shin et al., 2022) is available with a setup based on OECD TG 210. The exposure duration was 42 days and the concentrations were 0.04, 0.4, 4 and 40 mg/L (nominal); 0.041, 0.406, 4.028, and 40.538 mg/L (measured). Up to 6-7 dph a semi-static design using six-well plates was applied, with water removal two times a week. After the sac-fry stage, the exposure was performed using a flow-through system with replacement of water about three times a day. Four replicates with 20 fish embryos per replicate were exposed. There were no effects on hatching. The NOEC of 4 mg/L (LOEC 40 mg/L) after 30 dph was based on significantly increased mortality and abnormal appearance and sign. decreased weight and length, also spine curvature occurred. At 40 mg/L (13 dpf) a significantly failed or reduced inflation of sac-frys swim-bladder was seen. At 40 mg/L histological effects on the swim bladder were observed: tumours, inflammation, vacuolation in the gas glands from swim bladders (no statistics). Histological effects on the kidneys: glomerular vasodilation, Bowman capsule expansion, tubular atrophy (sign. increased) and liver: plasma abnormalities and atrophy (increased, but not significantly).

The test by (Liang et al., 2017) was conducted to examine hepatotoxicity in adult male rare minnows after 28 and 42 days in two tests. The nominal tested concentrations were 50, 500 und 5000 µg/L. They were not analytical verified. Information about mortality and growth were not given. After 28 days effects on histopathology of the liver were seen at 5000 µg/L, the effects were not specified. After 42 days the histopathological effects hypertrophy of the hepatocytes, nuclei pyknosis, and increases in cellular vacuolization were seen at 50 to 5000 µg/L. The hypertrophy of the hepatocytes was more prevalent at 5000 µg/L than at 50 or 500 µg/L.

Error! Reference source not found. summarises the relevant information on chronic fish toxicity.

Table 11

Fish – chronic toxicity					
Species	**Substance**	**Results [mg/L]**	**Test method and experimental conditions**	**Reliability**	**Ref.**
Danio rerio	BTA CAS 95-14-7	35d-NOEC= 1.07, LOEC 3.2 mg/L (post hatch survival), Length: sign. decreased at 0.1 and 10 mg/L, but differences less than 5 %, considered not relevant. This study is evaluated in detail in section 7.10.1.2.	OECD TG 234, exposure duration: 63d; flow-through, conc.: nominal: 0.10 0.32, 1.00, 3.20, 10.0 mg/L, measured: 0.104, 0.331, 1.07, 3.34, 11.0 mg/L; no vehicle; 30 fertilized eggs per replicate, 4 replicates; 27 ± 2 °C; pH: 6.24 – 7.24; 87-117% diss. Oxygen; 12h light per day; 1000 lumen	1	Registration dossier: (Connect Chemicals GmbH, 2021b)

Fish – chronic toxicity					
Species	**Substance**	**Results [mg/L]**	**Test method and experimental conditions**	**Reliability**	**Ref.**
Oryzias latipes	BTA CAS 95-14-7	No effects on hatching NOEC 4 mg/L, LOEC 40 mg/L: At 40 mg/l (30 dph) sign. increased mortality and abnormal appearance, sign. decreased weight and length; spine curvature occurred At 40 mg/L (13 dpf) significantly failed or reduced inflation of sac-fry swim-bladder Histology: - Swim bladder: at 40 mg/L: tumours, inflammation, vacuolation in the gas glands from swim bladders (no statistics); - Kidneys: glomerular vasodilation, Bowman capsule expansion, tubular atrophy (sign. increased); - Liver: plasma abnormalities and atrophy (increased, but not significantly)	Based on OECD TG 210, Exposure duration 42 days (up to 30 dph); Conc.: 0.04, 0.4, 4, and 40 mg/L (nom.); 0.041, 0.406, 4.028, and 40.538 mg/L (meas.), Up to 6 – 7 dph (sac-fry stage) semi-static with water renewed two times per week. After this flow-through system with replacement of water about three times a day. 20 fertilised eggs per replicate (4 replicates), Temp.: 24 – 26.4 °C, pH 7.74 – 8.16	2	(Shin et al., 2022)
Gobiocypris rarus (rare minnow)	BTA CAS 95-14-7	No information on mortality or growth were given After 28 days: effects on histopathology of liver at 5000 µg/L, not at 50 and 500 µg/L After 42 days: effects on histopathology of liver at 50, 500 and 5000 µg/L: Hypertrophy of the hepatocytes, nuclei pyknosis, and increases in cellular vacuolization. Hypertrophy of the hepatocytes was more prevalent at 5000 µg/L than at 50 or 500 µg/L.	Two tests with adult male rare minnows: 1. Exposure 28 days 2. Exposure 42 days Conc: 50 - 500 - 5000 µg/L (nominal), no analysis conducted. 20 fish per group and three replicates, no solvent was used.	2	(Liang et al., 2017)

7.8.1.2. *Aquatic invertebrates*

7.8.1.2.1. Short-term toxicity to aquatic invertebrates

The most sensitive result occurred in a short-term toxicity test with *Daphnia galeata* (Seeland et al., 2012) with a 48 h-EC_{50} of 15.8 mg/L. *Daphnia magna* is less sensitive with a 48 h-EC_{50} of 91 mg/L (Rheinchemie, 1991c).

Table 12

Short-term toxicity to aquatic invertebrates					
Species	**substance**	**Results [mg/L]**	**Test method and experimental conditions**	**reliability**	**Ref.**
Daphnia magna	BTA, CAS 95-14-7	48h-EC_{50} = 137	OECD 202; static; conc.: 32 – 56 – 100 – 180 – 320 – 560 – 1000 mg/L; 4 replicates with 5 daphnids each; 19.9 – 20.3 °C, pH 7.0 – 8.1; 16 h light/d	1	Registration dossier: (Procter & Gamble ETC, 1994c)
Daphnia magna	BTA, CAS 95-14-7	48h-EC_{50} = 91	"Bestimmung der Schwimmunfähigkeit beim Wasserfloh – Daphnia magna - (EC_0, EC_{50}, EC_{100}; 24 Stunden; statisches System) Verfahrensvorschlag: Umweltbundesamt Berlin, Stand Mai 1984"; static; nominal: 63 – 88 – 125 – 177 – 250 – 354 and 500 mg/L; 10 replicates with 1 daphnid each; 21 ± 0.5 °C; pH 7.6-7.7;	2	Registration dossier: (Rheinchemie, 1991c)
Daphnia magna	BTA, CAS 95-14-7	48h-EC_{50} = 107	OECD 202; static; conc.: 3.0 – 4.5 – 6.7 – 10.1 – 15.1 – 22.8 – 34.2 – 51.3 – 76.9 – 115 mg/L; 4 replicates with 5 daphnids each; age: < 24h; 20°C; 16h light per day; no feeding	1	Registration dossier: (Seeland et al., 2012)
Daphnia galeata	BTA, CAS 95-14-7	48h-EC_{50} = 15.8	OECD 202; static; conc.: 0.75 - 1.5 - 3.1 - 6.25 - 12.5 - 25.0 mg/L; 4 replicates with 5 daphnids each; age: < 24h; 20°C; 16h light per day; no feeding	1	Registration dossier: (Seeland et al., 2012)
Daphnia magna	Sodium 1H-benzotriazolide, CAS 15217-42-2	48h-LC_{50} = 195.57	According to EPA/600/4-90/027, conc.: 62.5 – 125 – 250 - 500 – 1000 mg/L, static, 4 replicates with 5 daphnids each, temp. 19.4 - 19.5 °C, pH 8.03 - 8.34, 16 h light per day, no analytical monitoring	2	Registration dossier: (Nalco Chemical Company, 2003a)
Americamysis bahia (previous name: *Mysidopsis bahia*)	Sodium 1H-benzotriazolide, CAS 15217-42-2	96h-LC50 = 113.57 mg/L	According to EPA/600/4-90/027, conc.: 100 – 200 – 400 - 800 – 1600 mg/L, saltwater, 3 replicates with 10 organisms each, temp. 25.2 - 25.5 °C, pH 7.57 - 8.51, 16 h light per day, no analytical monitoring	2	Registration dossier: (Nalco Chemical Company, 2003c)
Americamysis bahia (previous name: *Mysidopsis bahia*)	Sodium 1H-benzotriazolide, CAS 15217-42-2	96h-LC50 = 141.04 mg/L	According to EPA/600/4-90/027, conc.: 62.5 – 125 – 250 - 500 – 1000 mg/L, saltwater, 3 replicates with 10 organisms each, temp. 24.7 - 25.3 °C, pH 7.64 - 8.01, 16 h light per day, no analytical monitoring	2	Registration dossier: (Nalco Chemical Company, 2005)

7.8.1.2.2. Long-term toxicity to aquatic invertebrates

The most sensitive organism was again *Daphnia galeata* with a 21d-EC10 of 0.97 mg/L. *Daphnia magna* was less sensitive with a 21d-EC10 of 12.8 mg/L (Seeland et al., 2012).

A test with the marine scallop *Chlamys nobilis* is available. It is assessed in section 7.10.1.2.

Table 13

Long-term toxicity to aquatic invertebrates					
Species	***substance***	***Results [mg/L]***	***Test method and experimental conditions***	***reliability***	***Ref.***
Daphnia magna	*BTA CAS 95-14-7*	21d-NOEC= 25.9	"Daphnia Reproduction Test" of OECD Guideline 202, Part II (Draft 7/1993); semi-static; conc.: nominal: 0.7 – 2.2 – 7.0 – 22.1 and 70.0 mg/L; 20 ± 2 °C	2	Registration dossier: (Rheinchemie, 1995)
Daphnia magna	*BTA CAS 95-14-7*	21d-EC_{10}> 12.8	OECD 211; semi-static; conc.: 0.4 – 0.8 – 1.6 – 3.2 – 6.4 – 12.8 mg/L; 10 replicates with 1 daphnid each; age < 24h; 20°C; 16h light per day; feeding: 0.2 mg C/ (daphnid and day)	1	Registration dossier: (Seeland et al., 2012)
Daphnia galeata	*BTA CAS 95-14-7*	21d-EC_{10}= 0.97	OECD 211; semi-static; conc.: 0.4 – 0.8 – 1.6 – 3.2 – 6.4 – 12.8 mg/L; 10 replicates with 1 daphnid each; age < 24h; 20°C; 16h light per day; feeding: 0.2 mg C/ (daphnid and day)	1	Registration dossier: (Seeland et al., 2012)
Chlamys nobilis marine scallop	BTA CAS 95-14-7	No information about mortality, other endpoints see section 7.10.1.2	Semi-static; conc.: 0.01, 0.1, 1 mg/L; 12 males and females in each vessel, 3 replicates per treatment, duration: 60	2	(He et al., 2019)

7.8.1.3. *Algae and aquatic plants*

Table 14

Toxicity to algae					
Species	***substance***	***Results [mg/L]***	***Test method and experimental conditions***	***reliability***	***Ref.***
Desmodesmus subspicatus (previous name: *Scenedesmus subspicatus*)	BTA CAS 95-14-7	72h-E_rC_{50} =not reported 72h-E_rC_{10} = 1.18	OECD TG 201; static; concentrations: 0.3 – 0.6 – 1.2 – 2.5 - 5.0 mg/L; temp.: 23 ± 1 °C; photoperiod 24 h with 6 500 to 10 000 lux; start cell number: $5*10^4$ cells/mL; 5 control replicates and 3 for the test substance;	1	Registration dossier: (Seeland et al., 2012)

Toxicity to algae					
Species	***substance***	***Results [mg/L]***	***Test method and experimental conditions***	***reliability***	***Ref.***
Desmodesmus subspicatus (previous name: *Scenedesmus subspicatus*)	BTA CAS 95-14-7	72h-E_rC_{50} =231 72h-E_rC_{10} = 58	DIN 38412-9; static; conc.: nominal: 1 - 3.2 – 10 – 32 – 100 - 320 and 1 000 mg/L	4 – Reporting deficiencies: replicates unknown	(Rheinchemie, 1991b)
Pseudokirchnerella subcapitata (reported as *Selenastrum capricornutum)*	BTA CAS 95-14-7	72h-E_rC_{50}= 75 72h-E_rC_{10}= 10.5 72h-NOEC= 10	OECD TG 201; static; conc.: 6.4 – 20 – 36 – 64 – 112 – 200 – 640 mg/L; 23 ± 1 °C; pH 8.0 – 8.4; 4 control replicates; 2 replicates per test concentration	2 – reduced replicates + some deficiencies in reporting	Registration dossier: (Procter & Gamble ETC, 1994b)
Lemna minor	BTA CAS 95-14-7	7d-EC_{10}= 3.94	OECD TG 221; static; conc.: 1.0 – 2.5 – 5.0 – 10.0 – 20.0 mg/L; 12 healthy fonds in each glass beaker (250 mL, 10.5 cm Ø); 6 control replicates and 3 for the test substance	1	Registration dossier: (Seeland et al., 2012)
Pseudokirchnerella subcapitata (reported as Raphidocelis subcapitata, previous name: Selenastrum capricornutum)	Sodium 1*H*-benzotriazolide CAS 15217-42-2	72h-ErC50 = 150 72h-NOErC 32 mg/L	OECD TG 201; static; conc.: 10 - 32 - 100 - 320 - 1000 mg, test conc. verified at 0 and 96 h (97% to 103% of nominal), temp.: 24 +-1 °C, pH 7.5 +- 0.1, photoperiod 24 h with approx. 7000 lux; 6 control replicates and 3 for the test substance; Validity criteria fulfilled	1	Registration dossier: (Nalco Limited, 2013)

7.8.1.4. *Sediment organisms*

Not assessed

7.8.1.5. *Other aquatic organisms*

Not assessed

7.8.2. Terrestrial compartment

Not assessed

7.8.3. Conclusions for classification and labelling

The eMSCA concluded that the available information on aquatic toxicity is sufficient to classify the Substance as Aquatic Chronic 2 (H411), based on the 21d-EC_{10} of 0.97 mg/L in *Daphnia galeata*. A CLH dossier for BTA has been prepared and submitted to ECHA which has been supported by RAC in its opinion from 2022.

The new ecotoxicological tests obtained with the structurally related substance sodium 1H-benzotriazolide (i.e. the sodium salt of BTA) do not change the result, since after the emission of the sodium salt into the environment, it will dissociate into the sodium cation and the negatively charged benzotriazolide anion. Owing to the pKa for sodium 1*H*-benzotriazolide (pKa = 8.5, 20 °C), the anion will be protonated under environmental conditions, thereby forming the uncharged BTA.

7.9. Human Health hazard assessment

Human health hazard assessment was outside the scope of the SEv. However, available mammalian data were analysed to support the assessment of environmental ED properties (see 7.10.1.3).

7.10. Assessment of endocrine disrupting (ED) properties

7.10.1. Endocrine disruption – Environment

7.10.1.1. *In vitro tests on endocrine activity*

Three *in vitro* studies (transcriptional activation assays using recombinant yeast) are available with the test substance BTA. Oestrogenic, anti-oestrogenic, androgenic and anti-androgenic effects were examined (Harris et al., 2007; Fent et al., 2014; Seeland et al., 2012).

Harris et al. used the yeast oestrogen screen (YES) test to examine oestrogenic and anti-oestrogenic activity of BTA (Harris et al., 2007). The standard assay procedure using yeast cells expressing the human oestrogen receptor alpha (ERα) described by Routledge and Sumpter (Routledge, 1996) was followed. In the YES test, yeast cells were exposed to several concentrations of BTA (serial diluted from 100 mg/L to 50 µg/L) or the positive control E2 at 2.72 µg/L to 1.33 ng/L.

For the anti-oestrogenic activity, additional E2 (68 ng/L, submaximal concentration) was added to the different concentrations of BTA. An inhibitory action (anti-oestrogenic effect) of BTA would antagonise the oestrogenic activity of estradiol (E2). 4-OH-tamoxifen served as the positive control in this assay (serial dilution from 4 mg/L to 2 µg/L).

Results:

In the YES test, no estrogenic activity was observed at 0.1 and 1 mg/L. The positive control (E2) showed a high activity at 0.1 to 1 µg/L.

In the anti-oestrogen assay, there was a decrease in E2-induced response, however, only at concentrations of 10 and 100 mg/L. At 100 mg/L BTA, the same decrease of oestrogen induced activity was seen as for 4 mg/L OH-tamoxifen. Hence, this result suggests that BTA may have anti-oestrogenic activity. However, due to the lack of statistical evaluation and information on cytotoxicity, no definitive conclusion can be drawn.

Fent et al. performed a yeast oestrogen screen (YES) assay (yeast cells expressing human oestrogen receptor α (ERα)) and a yeast androgen screen (YAS) assay (yeast

cells expressing the androgen receptor) (Fent et al., 2014). The YES assay was carried out as described by Routledge and Sumpter, (Routledge, 1996), and the YAS assay as described by Sohoni and Sumpter (Sohoni, 1998).

Testing was conducted in duplicate (YES) and triplicate (YAS). Each experiment was carried out two to three times. Ethanol was used as solvent. The yeast cells were serially diluted and exposed to concentrations of 8.39 mM to 1 nM BTA. E2, dihydrotestosterone (DHT), and flutamide (FLU) were used as standards for the appropriate assays and serially diluted from 0.499 nM to 0.244 pM (E2), 50 nM to 24.4 pM (DHT) and 0.112 mM to 54.4 nM (FLU).

DHT was added to the YAS antagonistic assay to produce an agonistic submaximal response of 65% at the concentration of 2 nM.

Results:

BTA showed no oestrogenic or androgenic activities. However, anti-androgenic activity was seen from > 100 to 10000 µM (> 11.9 to 1190 mg/L). The positive control Flutamide showed anti-androgenic activity between > 1 to 100 µM. Regarding cytotoxicity, the authors stated: *"The tested concentrations were below the cytotoxic concentration, thus demonstrating an anti-androgenic activity. An exception was BTA at the highest concentrations showing toxicity to the yeast cells."* It is not clear from the study whether the anti-androgenic effects appeared in the range of cytotoxic concentrations. In a personal communication the eMSCA was informed that cytotoxicity appeared only at the highest concentration of BTA tested. Hence, antiandrogenic activity of BTA in the YAS assay probably appeared starting at > 100 µM. However, there was no statistical evaluation provided in the publication by Fent et al. Therefore, no definitive conclusion can be drawn.

Seeland et al. (Seeland et al., 2012) conducted a yeast oestrogen assay (YES) according to Routledge (1996) with some modifications specified by Wagner and Oehlmann (2009), (Wagner and Oehlmann, 2009). In this assay, BTA was examined in a concentration range from 5 ng/L to 50 µg/L with a spacing factor of 10.

Results:

Oestrogenic activity of BTA was not seen in the YES assay. At concentrations above 50 µg/L, cell mortality could be observed in the wells.

Summary of *in vitro* tests

No receptor mediated oestrogenic activity was observed for the Substance in three yeast oestrogen assays (YES) (Harris et al. 2007, Fent et al. 2014, Seeland et al. 2012). No receptor-mediated androgenic activity was seen by (Fent et al., 2014). In the anti-oestrogen screen (YES) (Harris et al. 2007), BTA showed anti-oestrogenic activity at concentrations of 100 mg/L and higher, however, no definitive conclusion can be drawn due to the lack of statistical evaluation and information on cytotoxicity. In the anti-androgen screen (YAS) by Fent et al. (2014) an anti-androgenic effect was observed. However, it is not really clear from the study, whether the effect appeared in the range of cytotoxic concentrations. The authors also reported cytotoxicity effects at the highest concentrations but did not specify the concentrations at which significant cytotoxicity was observed. Personal communication showed that cytotoxicity appeared only at the highest or two highest concentrations of BTA. Therefore antiandrogenic activity in the YAS assay probably existed. However, there was no statistical evaluation and no information on the cytotoxicity assay used. Therefore the result is not definitely conclusive and might be influenced by cytotoxic effects in the yeast cells.

Seeland et al. (2012) observed cell mortality in a YES assay at concentrations higher than 0.05 mg/L BTA.

Table 15

Transcriptional activation assay using recombinant yeast (yeast oestrogen screen (YES); yeast androgen screen (YAS))					
Species	**Reference**	**Cell type**	**Test conditions**	**Results**	**Comment**
Human	(Harris et al., 2007)	Recombinant yeast expressing human ER (hERα) Tested for both oestrogenic and anti-oestrogenic activity	Yeast cells were exposed to concentrations of BTA (serial diluted from 100 mg/L to 50 µg/L) or E2 (2.72 µg/L to 1.33 ng/L). For the anti-oestrogen screen, additional E2 was added at 6.8E-8 g/L. The positive control in this assay was 4-OH-tamoxifen (serial diluted from 4 mg/L to 2 µg/L)	Oestrogen screen: no agonistic activity Anti-oestrogen screen: same effect like 4-OH-Tamoxifen (oestrogen receptor antagonist) at higher concentrations Effects were in the range 10 to 100 mg/L BTA (read from diagram), however no clear conclusion is possible (no statistical calculations and possible cytotoxicity), hence not conclusive.	No information about cytotoxicity given.
Human	(Fent et al., 2014)	Recombinant yeast assay expressing hERα (yeast oestrogen screen, YES) OR androgen receptor (yeast androgen screen, YAS)	Yeast cells were exposed to concentrations of BTA 8.39 mM to 1nM. Chemicals tested in duplicate (YES) and triplicate (YAS). Each experiment was carried out two to three times. Solvent control: ethanol DHT (Dihydrotestosterone) was added to the YAS antagonistic assay at 2 nM to produce agonistic response	YES-agonist assay: no oestrogenic activity YAS-agonist assay: no androgen agonistic activity YAS-antagonist assay: anti-androgenic activity at > 100 µM (read from graph); effect appeared at lower than cytotoxic concentrations No statistical evaluation, hence not conclusive.	At the highest concentration of BTA, cytotoxic effects were observed
Human	(Seeland et al., 2012)	Recombinant yeast oestrogen screen	Yeast cells were exposed to concentrations of BTA in a range between 0.5E-5 to 0.05 mg/L	Oestrogen screen: No effects	Cell mortality was seen at concentrations > 0.05 mg/L

7.10.1.2. *In vivo tests on endocrine activity and adversity*

***In vivo* studies with fish species**
Five *in vivo* tests are available with four different fish species, which are summarized in the following:

- *Gobiocypris rarus* (rare minnow), with sexually mature fish (Liang et al. 2014)
- *Pimephales promelas* (fathead minnow), with sexually mature fish (Harris et al. 2007)
- *Oryzias melastigma* (marine medaka), age 3 months (Tangtian et al. 2012)
- *Danio rerio* (zebrafish), with eleuthero embryos (Fent et al. 2014)
- *Danio rerio* (zebrafish), FSDT, OECD TG 234 (Connect Chemicals GmbH, 2021b), requested during substance evaluation.

Study summary: Fent et al. 2014

Eleuthero-embryos of *Danio rerio* were exposed to BTA starting at 2-4 hpf for 6 days (Fent et al., 2014). Each dose group consisted of five replicates of 80 fertilised eggs. The nominal concentrations used were 10, 100, 1000 µg/L corresponding to 8, 97 and 1197 µg/L measured concentrations. The water was replaced every 24 h. Analytical determination of concentrations was done three times during exposure study, at the beginning of exposure (0 h), before and after water renewal at 24 h.

After exposure, embryos were sampled for gene expression analysis by real-time reverse transcription PCR. Genes belonging to different pathways including hormonal receptors, steroidogenesis, phase II metabolism, oxidative stress, apoptosis and DNA damage response were examined in order to identify unknown modes of action.

Embryos were inspected daily for mortality and hatching was recorded.

The Klimisch score 2 was assigned to the study.

Results:

No effects on mortality, time to hatch or hatching rate were observed.

Expressional changes of target genes: VTG mRNA was slightly up-regulated, but not statistically significantly (factor < 10). No other changes relevant for an endocrine mode of action were observed.

Only two phase II enzymes showed a significant down-regulation (gstp1 by a factor slightly above 10 – and ugt1a by a factor <10).

ARNT2 (aryl hydrocarbon receptor nuclear translocator 2) belonging to the AHR pathway was significantly upregulated (by a factor of approximately 10).

Study summary: Liang et al., 2014

Liang et al. (Liang et al., 2014) conducted a test on BTA with sexually mature rare minnows (*Gobiocypris rarus*). The exposure duration was 28 days. All tested fish were about 10 months old and the offspring of one male-female pair, hence there was no genetic diversity in the fish used for the test. The nominal concentrations used were 50, 500 and 5000 µg/L. They were not analytically monitored. 40 fish per concentration or control were exposed, divided in two replicates of 20 fish. The sex ratio was 1:1 in each treatment or control. After 28 days of exposure to BTA, length and weight of the fish were measured and histopathology of gonads and liver was conducted. Plasma estradiol (E2) and 11-ketotestosterone (11-KT) were measured using ELISA kit. The gene expression levels of hormones, ER- and androgen receptors (AR) were determined using

real-time PCR. The values given were normalised to control. Hence, a value >1 means up-regulation of the gene, a value <1 means down-regulation compared to the control.

The test is assessed with Klimisch 2.

Results:

- No effects on mortality, length and weight of the rare minnows.

- *General histopathology*: The authors stated that "Compared to the control fish, there were no obvious histological changes in the livers of the rare minnow following exposure to 0.05 or 0.5 mg/L_1 BT for 28 d (not shown)." At 5000 µg/L cytotoxic effects in the liver occurred (observations: hypertrophy of the hepatocytes and nuclei pyknosis; additionally, an increase in cellular vacuolisation was observed in both female and male fish).

- *Gonadal histopathology:*
  Females at 5000 µg/L: The ovaries were degenerated or undeveloped. According to OECD Guidance Document 123 (OECD, 2010) the ovaries were in stage 1, as there was a predominance of cortical alveolus stage and perinucleolar oocytes.
  The ovaries of control fish and at the lower concentrations were mature and oocytes developed into vitellogenic oocytes.
  Males at 5000 µg/L: The testis showed disorganisation of the lobular structure and an abnormal predominance of spermatozoa (mature sperm) and scarce intermediate germ cell stages were seen. The study authors concluded that the spermatogenesis was stimulated by BTA. In the control and at 50 and 500 µg/L, no histopathological effects were seen although different germ cell cysts during the spermatogenic process existed.

- *Plasma steroid hormone levels:*
  In females, plasma E2 was significantly decreased at 5000 µg/L (no dose-response, because E2 was increased at 500 µg/L, but not significantly).
  In males, the E2 level was significantly increased at 5000 µg/L (dose-response existed). The level of 11-KT was not altered in either males or females.

- *Transcriptional levels of the HPG axis-related genes (VTG and others). Effects were statistically significant (p< 0.05) unless stated otherwise.*

  Females:

  - Up-regulation in the brain:
    - gonadotropin-releasing hormone (GnRH2) (2.29-; 3.92-; 5.66-fold at 50, 500, 5000 µg/L respectively)
    - luteinising hormone ß (LHß) (3.7-, 3.7-, 4.3-fold at 50, 500, 5000 µg/L respectively).
  - Up-regulated in the gonad:
    - ERß2 (4.3-; 6.26-fold at 50 and 500 µg/L respectively, 1.22-fold not sign. at 5000 µg/L);
    - CYP19A (18.75-; 17.51-; 11.8-fold at 50, 500, 5000 µg/L, resp.);
    - CYP11A (5.33-; 6.58-; 6.2-fold at 50, 500, 5000 µg/L, resp.);
    - steroidogenic acute regulatory protein StAR (26.03-; 25.47-; 14.15-fold at 50, 500, 5000 µg/L, resp.);
    - Follicle stimulating hormone receptor FSHR (4.68-, 5.11-; 4.02-fold at 50, 500, 5000 µg/L, resp.);
    - luteinising hormone receptor LHR (4.08-; 2.12-; 2.84-fold at 50, 500, 5000 µg/L, resp.);
  - Up-regulated in the liver:
    - ERα (11.6-, 6.9-, and 30.7-fold at 50, 500, 5000 µg/L, resp.);
    - VTG (1.8-, 4.4-, and 28.6-fold at 50, 500, 5000 µg/L, resp.)

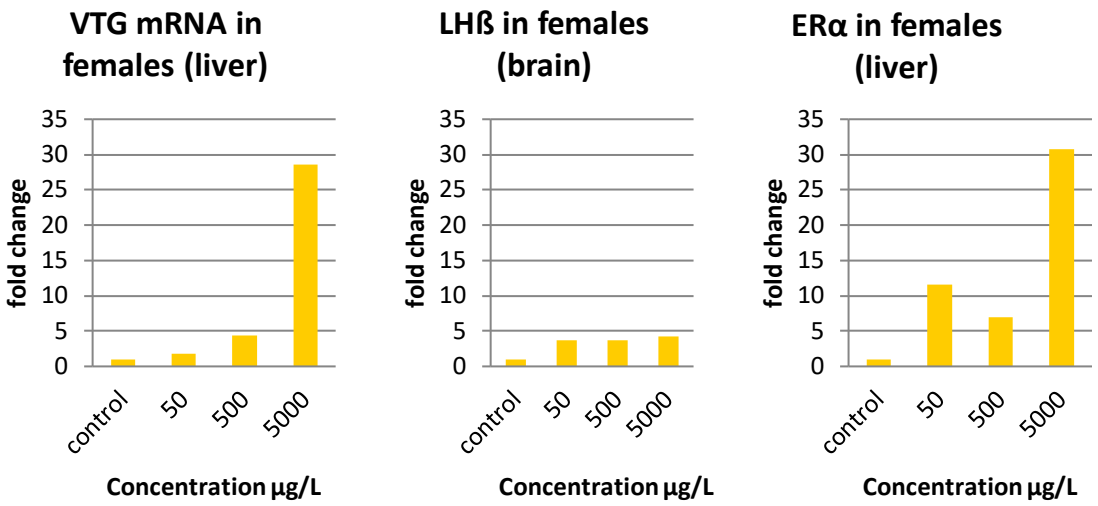


Figure 2: Gene expression in female rare minnow

– *Transcriptional levels of the HPG axis-related genes (VTG and others). Effects were statistically significant (p< 0.05) unless stated otherwise.*

Males:

- Up-regulated in the liver: VTG mRNA very pronounced at 50, 500 and 5000 µg/L;
- Up-regulated in the brain: LHß mRNA very pronounced at 50, 500 and 5000 µg/L;
- The effects on VTG mRNA and LHß were more pronounced in males than in females, and also more up-regulated than other gene expressions in males.

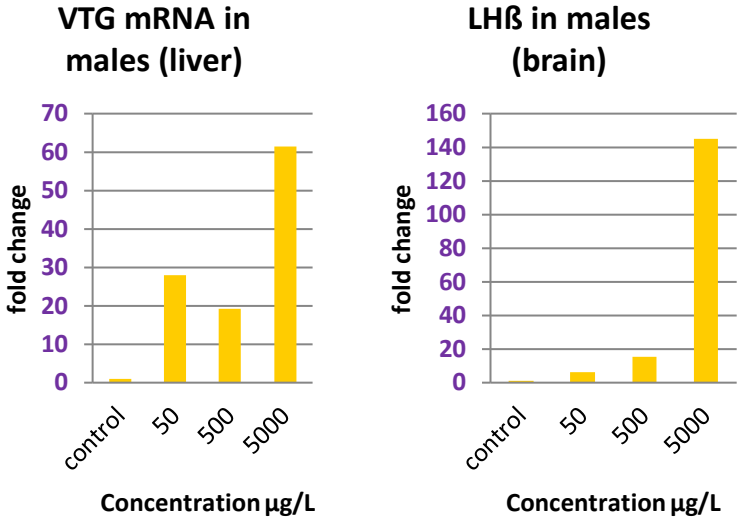


Figure 3: Gene expression in male rare minnow

The study authors assume different modes of action for males and females, because VTG mRNA was up-regulated in males and females, but E2 was elevated in males only (at 5000 µg/L) and decreased in females (at 5000 µg/L). ERα was up-regulated in the liver of females but not in males.

The authors concluded that in females BTA may act directly on the liver through ERs, to stimulate VTG expression whereas in males increased transcription of VTG might be caused by increased plasma E2 levels or resulted from the oestrogenic effect of BTA.

Furthermore, the authors wrote, that "*… in male rare minnows, an increase of LHß with a decrease of FSHR and LHR* [both down-regulated at 5000 µg/L] *was observed in this study. This might play some roles in the promotion of E2 and the stimulation of spermatogenesis."*

Study summary: (Liang et al., 2017)

Liang et al. (2017) conducted a test on BTA with adult male rare minnows (*Gobiocypris rarus*). The exposure duration was 28 days in a first experiment and 42 days in a second experiment. The nominal concentrations were 50, 500 and 5000 µg/L in both tests. The test concentrations were not measured. There were 20 fish per group and three replicates. No solvent was used. After 28 d (first test) and 42 d (second test) exposure the liver was used for analysis of histopathology. This study was conducted to examine hepatotoxicity and not endocrine endpoints. The test is described in section 7.8.1.1.2.

The study was assessed with Klimisch 2.

Results:

No information on mortality or growth were given.

After 28 days exposure effects on the histopathology of the liver at 5000 µg/L only were seen. The effects after 28 days were not specified in this study, but in Liang et al. 2014 after 28 days exposure at 5000 µg/L the same histopathological effects were seen as in Liang et al. 2017 after 42 days at 50 to 5000 µg/L.

After 42 days exposure there were seen histopathological effects at the concentrations 50, 500 and 5000 µg/L: "*Hypertrophy of the hepatocytes, nuclei pyknosis, and increases in cellular vacuolization were observed in all three treatment groups. Additionally, in the highest BT treatment group, the hypertrophy of the hepatocytes was even more prevalent than the other BT-treated groups*."

Study summary: Harris et al., 2007

Harris et al. (Harris et al., 2007) exposed sexually mature *Pimephales promelas* for 14 days at the concentrations 10, 100 and 1000 µg/L. The measured concentrations were in the range of 80 to 86 % of nominal. Males and females were exposed separately. Eight fish were deployed in each tank. The aim of the present study was to assess the potential for BTA to inhibit oestrogenic activity (anti-oestrogenic effect). Thus, in addition to the different BTA concentrations 17ß-estradiol (E2) (100 ng/L) was added to the tank water in which the males were exposed. Dimethylformamide (DMF) (67 µl/L) was used as solvent for E2. The males had two control groups: the negative solvent control group with 67 µl/L DMF and the positive control group with 100 ng/L E2 (inclusive the solvent DMF). The female group received water without E2 and there was no solvent in control and treatments.

After 14 days, plasma VTG was measured using ELISA. At the end of exposure, length and weight, as well as liver and gonad weights were recorded. Blood samples were taken in order to determine VTG via ELISA. Concentrations of BTA in the tanks were measured three times during the study: Once prior to introduction of the fish, and after one and two weeks of exposure.

The study was assessed with Klimisch 2.

Results:

The mean measured values for BTA in the tanks were between 80 and 86% of nominal values. The mean E2 concentrations in the tanks with male fish were measured to be 74, 70, 57, 67 ng/L.

Plasma VTG:

Males: No observable differences regarding plasma VTG were found between the E2 control group and the male fish treated with E2 plus BTA. However, it is to note that a substance should be particularly a potent anti-oestrogen to antagonise the effects of 100 ng/L E2 in vivo in fish, like performed in this co-exposure setup.

Females: No differences were found between the control group and the exposed female fish. The VTG value in female fish from the water control and the exposed fish were in the range of about 100,000 ng/mL (approx. 100 µg/mL). No significant elevation or decrease of VTG was observed.

Study summary: (Tangtian et al., 2012)

Tangtian et al. (Tangtian et al., 2012) exposed marine medaka (*Oryzias melastigma)* for 35 days in seawater. The male and female fish were three months old. Three concentrations (0.01, 0.1 and 1 mg/L) were used and one control (without replicate). A solvent was not used. The number of exposed fish was not given, however there were at the beginning of the test at least 10 fish per treatment (see section 2.2. of the publication). The fish were sampled after 4 and 35 days. The liver, gills, intestine and gonads were examined (n=5 on both time points). Quantitative real-time PCR (qPCR) was used to analyse VTG gene expression (and CYP1A1 and CYP19a mRNA gene expression). Data were evaluated by the two-way statistical test analysis of variance (two-way ANOVA) at $p < 0.05$ to identify significant differences, followed by Tukey's test for comparison of means.

The study is rated with Klimisch 3 due to the lack of replicates (and the unknown number of fish) and therefore inappropriate statistic, the quantitative values are only supporting. The study was otherwise well conducted.

Results:

No information on mortality or growth was given.

VTG gene expression:

The test by Tangtian et al. (2012) with marine medaka showed a significantly increased VTG gene expression in males at 10 µg/L in liver (after 4 and 35 d), and in gills and intestine after 35 days also at 10 µg/L. In females, VTG gene expression was significantly up-regulated at 10 µg/L after 4 and 35 days in the liver and intestine, but not in the gills. The elevations at 1000 µg/L on day 35 in liver and intestine were very pronounced. The fold change of VTG gene expression was normalized to 18S rRNA.

Table 16

VTG gene expression in marine medaka (expressed as x-fold increase)								
	Control		10 µg/L		100 µg/L		1000 µg/L	
Males								
	day 4	**day 35**	**day 4**	**day 35**	**day 4**	**day 35**	**day 4**	**day 35**
liver	1.5 [a]	1.5 [a]	2.45 [b]	3.88 [b]	4.19 [b]	9.36 [c]	7.26 [c]	29.29 [d]
intestine	2.0 [a]	1.5 [a]	2.77 [a.b]	5.33 [c.d]	4.52 [b.c]	8.67 [d]	7.22 [c.d]	31.38 [e]

Females								
	day 4	**day 35**	**day 4**	**day 35**	**day 4**	**day 35**	**day 4**	**day 35**
liver	1.4 [a]	2.4 [a,b]	2.91 [b]	22.8 [d,e]	7.43 [c]	47.86 [e]	18.18 [d]	94.87 [f]
intestine	2.0 [a]	2.0 [a]	4.36 [b]	10.12 [c]	8.62 [c]	28.5 [d]	13.61 [c]	78.1 [e]

Means (in µg/L) that differed significantly ($p < 0.05$) either from controls or among the treatments are indicated by different letters.

The values for the control (both days 4 and 35 for liver and intestine) were approximated because they were taken from the graph of the publication.

CYP19a and CYP1A1 gene expression:

CYP19a: Females: the CYP19a gene expression in ovaries was significantly up-regulated after 35 days exposure at 1.0 mg/L. No information about males.

CYP1A1: Gene expression of CYP1A1 was significantly down-regulated in males (liver and intestine) and females (liver) at all concentrations. It is not clear, if this was endocrine mediated.

Study summary FSDT, OECD TG 234 in zebrafish (Danio rerio), (Connect Chemicals GmbH, 2021b)

The test was conducted according to OECD TG 234. It was requested during the substance evaluation and included in the registration in 2021.

The nominal test concentrations were 0.1, 0.32, 1, 3.2, 10 mg/L, the mean measured concentrations were 0.1, 0.33, 1.07, 3.34, 11 mg/L. 120 eggs per concentration were exposed divided in four replicates, using a flow-through design. The testing duration was 63 d, starting with fertilized eggs. Test media was kept close to pH 7.

The concentration of the metabolites 4-hydroxy-1H-benzotriazole and 5-hydroxy-1H-benzotriazole at day 63 were measured in fish tissue (at 1 and 10 mg/L) and in test media.

The study was assessed with Klimisch 1.

Results:

Effects after 4 dpf:

There was no effect on hatching (100 % hatched larvae).

Effects after 35 dpf:

Post-hatch survival (35 dpf): NOEC 1.0 mg/L and LOEC 3.2 mg/. The percentages of post-hatch survival were 95.8, 92.5, 90.0, 94.2, 84.2, 83.3 % in control and at 0.1, 0.32, 1.0, 3.2, 10 mg/L (nom.), respectively. At the concentrations where significant effects on sex ratio of males and undifferentiated fish appeared, the post-hatch survival was ≥ 90 %.

The length was significantly decreased at the lowest and highest concentration, differences were smaller than 5 % and considered not biologically relevant.

Effects after 63 dpf:

Survival: Unexpected mortality occurred three days before test termination in two vessels of the control and of the first treatment (0.1 mg/L). In total, 21 fish in controls and 20 fish at 0.1 mg/L were found dead. The remaining fish did not show any sign of disease. Due to this mortality, the survival rate in controls was 78.3 % at test termination. Survival at 0.1 mg/L was 75.8 % and at 1.0 mg/L 94.2 %. There was no statistically determination of survival, due to this increased mortality three days before test termination.

Length and weight were increased in males, females and undifferentiated fish (no statistical determination as not considered adverse by the study authors).

Table 17:

Growth (63 dpf)						
Nominal conc.	**Control**	**0.1 mg/L**	**0.32 mg/L**	**1 mg/L**	**3.2 mg/L**	**10 mg/L**
Measured conc.	**Control**	**0.1 mg/L**	**0.33 mg/L**	**1.07 mg/L**	**3.34 mg/L**	**11.0 mg/L**
Weight [g] Females	0.242	0.244	0.266	0.244	0.249	0.277
SD	0.023	0.016	0.030	0.027	0.023	0.007
Weight [g] Males	0.201	0.177	0.210	0.208	0.231	0.233
SD	0.034	0.032	0.027	0.018	0.029	0.014
Weight [g] Undiff.	0.207	0.216	0.220	0.219	0.242	0.234
SD	0.011	0.010	0.012	0.015	0.012	0.016
Length [cm] Females	2.9	2.9	2.9	2.9	2.9	3.0
SD	0.1	0.0	0.1	0.1	0.1	0.0
Length [cm] Males	2.7	2.6	2.7	2.8	2.9	2.8
SD	0.2	0.1	0.2	0.1	0.1	0.1
Length [cm] Undiff.	2.7	2.8	2.8	2.8	2.9	2.8
SD	0.0	0.0	0.1	0.0	0.0	0.1

Histological sex ratio males to females to undifferentiated fish (see Table below):

Males (including males in transition phase): There were significantly less males at 0.1 mg/L than in control. In general, in all treatments the number of males was decreased (26.1% in controls, 6.7 to 18.9% in treatments).

For females there were no significant effects in all treatment groups compared to the control.

Undifferentiated fish (consists of fish with protogynic gonads and juvenile fish): The ratio of undifferentiated fish was statistically significantly increased at 0.1 and 1 mg/L, and in general there were in all treatments more undifferentiated fish (26.1% in control versus 49.8 to 64.2% in treatments).

Table 18

Histological sex ratio at 63 dpf: Ratio of female to male to undifferentiated fish (in %)						
Nominal	**Control**	**0.1 mg/L**	**0.32 mg/L**	**1 mg/L**	**3.2 mg/L**	**10 mg/L**
Measured	Control	0.1 mg/L	0.33 mg/L	1.07 mg/L	3.34 mg/L	11.0 mg/L
Female	**47.8**	**29.1**	**29.4**	**20.4**	**26.8**	**32.6**
SD	7.3	21.4	17.4	13.5	12.7	7.5
Male (including transition phase)	**26.1**	**6.7***	**16.8**	**15.9**	**18.9**	**17.6**
SD	7.4	5.1	7.5	6.5	9.9	9.7
Undifferentiated (protogynic gonads and juveniles)	**26.1**	**64.2***	**53.8**	**63.8***	**54.3**	**49.8**
SD	6.5	25.3	22.4	17.8	16.6	11.1

* Statistically significantly different; Dunnett's t-test; two-sided; p<0.05

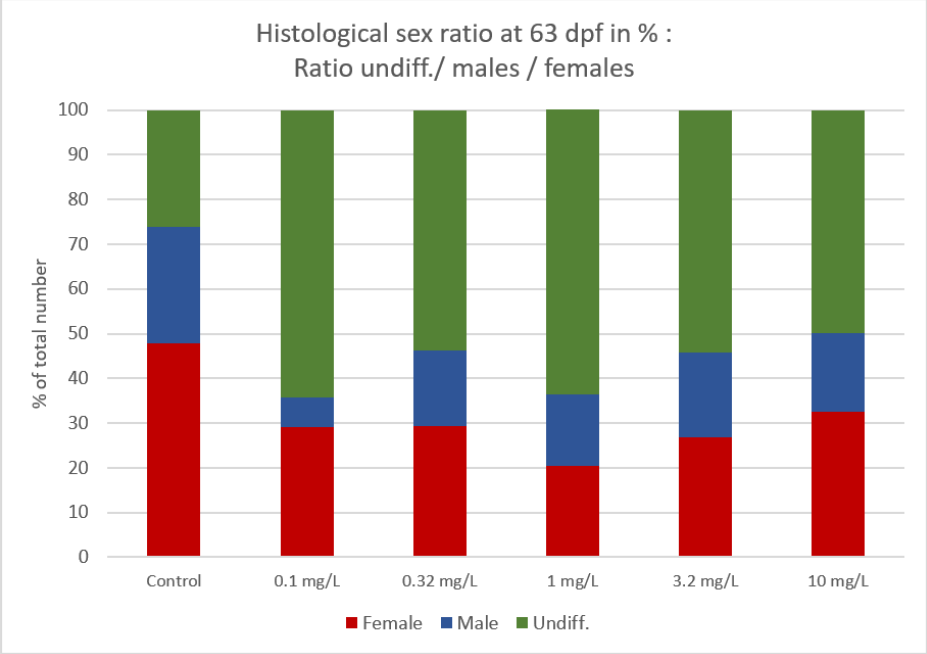


Figure 4: Histological sex ratio undiff./ males / females

Detailed histological analysis (Table 19 and Figure 5):

Males (fully developed) and males in transition phase: The detailed histological analysis shows that in the control the number of males consists of normal males and males in transition phase (see explanation below) in almost equal parts. The number of normal males in the treatments are fluctuating around the control value. However, in the treatment at 0.1 mg/L the part of normal males is smaller and males in transition phase do not exist at all, resulting in a significant decrease at 0.1 mg/L of males (normal males and males in transition phase, see above Table 18). In the treatments 0.1 to 3.2 mg/L the number of males in transition phase is significantly decreased compared to control (12.5 % in control versus 0 to 3.4 % at the concentrations 0.1 to 3.2 mg/L, at 10 mg/L: 4.5 %). Statistical evaluation showed that there were significant less males in transition phase at 0.1 to 10 mg/L by statistical calculation using Cochran Armitage and at 0.1 to 3.2 mg/L by calculation using CPFISH (see Annex I – statistical calculation (FSDT)). These values show that BTA impairs the development of males in an early stage of gonadal development.

Females (the same values as in comparison of ratios female to male to undifferentiated fish, see above): no effects.

Protogynic gonads: The presence of protogynic gonads is an indicator for undeveloped females, the ovaries are in 'stage 0' (oogonia to perinucleolar oocytes). The ratio of protogynic gonads is increased (no statistics) in all treatments compared to the control (22.6 % in control versus 48.7 to 62.1 % in treatments). At 0.1 and 1 mg/L the number of fish with protogynic gonads were highest (61.2 and 62.1 %, respectively). As a result of this, the part of undifferentiated fish (see Table 18 above) consisting mainly of protogynic gonads, was significantly increased at 0.1 and 1 mg/L. The FSDT study report states that the histopathological examination of fish gonads shows a treatment related shift towards non-developed females.

Explanation of Transition phase and Protogynic gonad: The study report states that *"most of the undifferentiated gonads were at a stage of protogynic gonad (stage 0 ovaries), with only undeveloped ovaries with oogonia to perinucleolar oocytes. Few of these protogynic gonads already show degenerated oocytes, and might enter into male development."* This stage (if protogynic gonads already show degenerated oocytes) is described as 'transition phase'. However, for this stage of early transition, a definitive development into males cannot be predicted unequivocally.

Juveniles: The number of juvenile fish, that have even less developed gonads than stage 0 (undeveloped germ cells are visible), was decreased in all treatments compared to control (3.6 % in control versus 1.1 to 3.1 % in treatments).

For the detailed histological analysis no statistical calculation was made by the study authors. The statistical analysis for males in transition phase was conducted by the eMSCA (see ANNEX I).

Table 19

Detailed histological analysis at 63 d (in %), with standard deviation (SD)						
Nominal	**Control**	**0.1 mg/L**	**0.32 mg/L**	**1 mg/L**	**3.2 mg/L**	**10 mg/L**
Measured	Control	0.1 mg/L	0.33 mg/L	1.07 mg/L	3.34 mg/L	11.0 mg/L
Female	**47.8**	**29.1**	**29.4**	**20.4**	**26.8**	**32.6**
SD	7.3	21.4	17.4	13.5	12.7	7.5
Male (without transition phase)	**13.6**	**6.7**	**14.1**	**12.4**	**17.9**	**13.2**
SD	11.0	5.1	5.2	8.2	8.1	4.5
Male (transition phase)	**12.5**	**0.0*+**	**2.7*+**	**3.5*+**	**1.0*+**	**4.5***
SD	6.3	0.0	5.4	2.8	2.0	6.4
Protogynic gonad	**22.6**	**61.2**	**52.0**	**62.1**	**53.3**	**48.7**
SD	3.2	26.7	20.9	16.0	14.6	9.6
Juvenile	**3.6**	**3.1**	**1.8**	**1.7**	**1.1**	**1.1**
SD	5.1	2.2	2.1	3.4	2.1	2.1

* Statistical sign. different from control calculated using Cochran Armitage, $p<0.05$
+ Statistical sign. different from control calculated using CPFISH, $p<0.05$

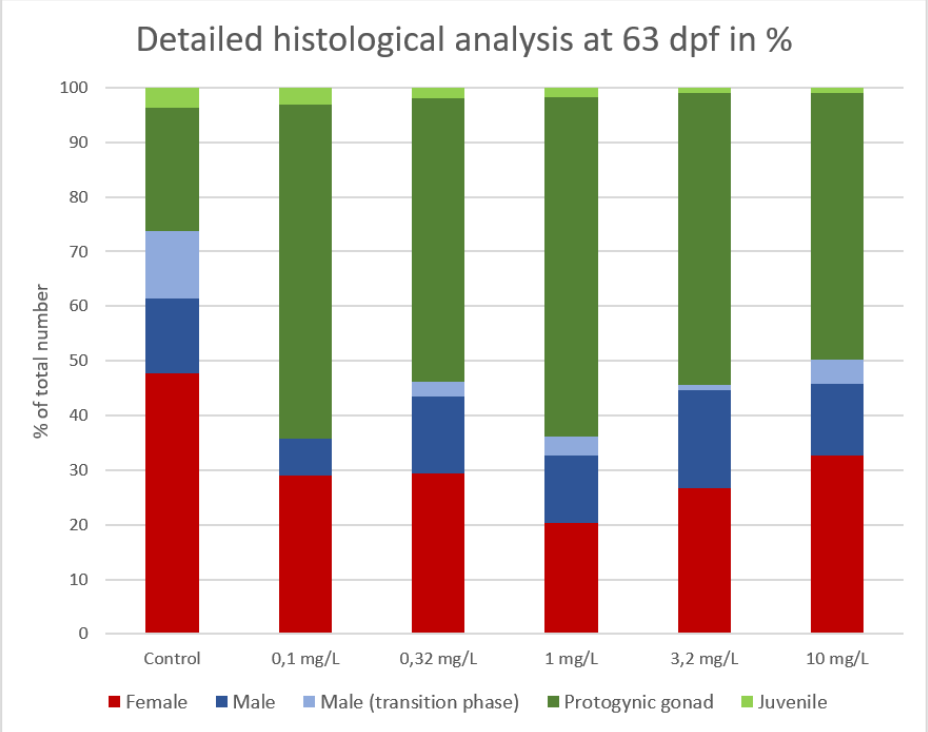


Figure 5: Detailed histological analysis

Vitellogenin

There are no significant changes of the VTG values for females, males and undifferentiated fish. Generally, the standard deviation is high (see Table 20).

Table 20

VTG content (mean values, VTG/total protein, ng/µg)						
Nominal Measured	**Control** Control	**0.1 mg/L** 0.1 mg/L	**0.32 mg/L** 0.33 mg/L	**1 mg/L** 1.07 mg/L	**3.2 mg/L** 3.34 mg/L	**10 mg/L** 11.0 mg/L
Females	73.4	59.3	46.4	9.9	112	44.4
SD	57.6	83.5	69.7	12.2	86.8	42
Males	0.06	0.02	0.04	0.03	0.05	0
SD	0.07	0.02	0.04	0.02	0.07	0
Undiff.	0.18	0.07	0.15	0.45	0.05	0.13
SD	0.32	0.02	0.09	0.75	0.03	0.08

Histopathological analysis of liver at 63 dpf:

There were some histopathological effects on the liver: significant increase at 0.1, 3.2 and 10 mg/L for single cell necrosis; at 3.2 and 10 mg/L for hydropic degeneration; at 0.1 mg/L for bile duct hyperplasia. There were no significant effects on hyalinised hepatocytes, vacuolation, infiltration, karyomegaly and multinucleated cells.

Metabolites:

The concentration of the BTA metabolites 4-hydroxy-1H-benzotriazole(4-OH-BTA) and 5-hydroxy-1H-benzotriazole (5-OH-BTA) in the fish tissue was measured at day 63. The measured concentrations were < LOQ at the treatments 1 and 10 mg/L, however the

LOQs were rather high: 100 µg/kg for 4-OH-BTA and 50 µg/kg for 5-OH-BTA in fish samples.

In test media the LOQs for both 4-OH-BTA and 5-OH-BTA were 0.1 µg/L. In the control and at nominal 0.1 mg/L BTA the metabolite concentrations were < LOQ. There were temporarily in the test vessels at nominal 0.32 mg/L BTA concentrations of 4-OH-BTA > LOQ measured: roughly 0.1 to 0.3 µg/L. At nominal 1 to 10 mg/L BTA the concentration of 4-OH-BTA were in the beginning > 0.1 to 2 µg/L, increasing from day 28 at nominal 10 mg/L BTA up to 10 µg/L 4-OH-BTA on the days 35 and 42. Afterwards the concentrations were slowly decreasing. The level of 5-OH-BTA remained in the range of 0.1 to 1 µg/L at the nominal concentrations 1 to 10 mg/L BTA and temporarily also at nominal 0.32 mg/L BTA.

Systemic toxicity in comparison with other studies:

It is noted that length and growth of undifferentiated fish are increasing, as in males and females, which indicates absence of systemic toxicity in the FSDT (Connect Chemicals GmbH, 2021b). Some histopathological effects in the liver were seen (see above). There was not a large extent of effects on survival. The survival in treatment groups was between 83.3 and 92.5% at 35 dpf. The NOEC for post-hatch survival after 35 dpf was 1 mg/L, the LOEC 3.2 mg/L. The LOEC was at higher concentrations than where the significant effects on decreased ratio of males and increased ratio of undifferentiated fish were seen. The significant effects on ratio of males in transition phase began at the lowest concentrations. Due to unexpected mortality in the control and at 0.1 mg/L the survival after 63 dpf was between 75.8 % (at 0.1 mg/L) and 94.2 % (at 1 mg/L).

Furthermore a FELS test with *O. latipes* and testing duration of 42 days showed no significant histopathological effects on the liver at concentrations of up to 40 mg/L (Shin et al., 2022), (see section 7.8.1.1.2). There was an increasing trend in atrophy and abnormalities of liver plasma with increasing exposure concentrations. However, this trend was not statistically significant from the untreated control. Effects on the kidney were significantly increased at 40 mg/L. The LOEC in this study was 40 mg/L (NOEC 4 mg/L) based on significantly failed or reduced inflation of sac-fry swim-bladder (13 dpf) and significantly increased death, abnormal appearance and significantly decreased weight and length (30 dph).

A study by Liang et al. 2017 showed histopathological effects on the liver (hypertrophy of the hepatocytes, nuclei pyknosis, and increases in cellular vacuolization) at the concentrations 50, 500 and 5000 µg/L after 42 days exposure on male adults of rare minnow. Information on mortality or growth in this study were not given. After 28 days only at 5000 µg/L the above-described changes were seen (changes specified in Liang et al. 2014).

The FSDT study authors (Connect Chemicals GmbH, 2021b) suggest that BTA delays the development of fish. The eMSCA considers this as very unlikely, as the number of juveniles (less developed than stage 0) in the treatments is in the same range as in the control or slightly lower. A delay of development is also unlikely, as the growth of exposed fish (including undifferentiated fish) is not significantly decreased in comparison to control, (cf. Table 17: Table 17).

***In vivo* studies with invertebrates**

A study with the marine mollusk scallop (*Chlamys nobilis)* is available (He et al., 2019). *Chlamys nobilis* is a gonochoristic species for which is known to possess VTG gene and can produce the protein VTG (Shi et al., 2018), (Sigang, 2021).

He et al. (2019) exposed *Chlamys nobilis* to nominal 10, 100 and 1000 µg/L BTA for 60 days in seawater. Concentrations were not analytically verified. Three replicates per treatment were used with 12 males and 12 females in each tank. The testing water was renewed daily to 66.7% and the dosing solutions were renewed daily. Effects of the Substance on the VTG content in the gonads and haemolymph of males and females were determined. In addition, the gonadosomal index [GSI (%) = gonad weights (g) / body weights (g) x 100], Ethoxyresorufin-O-deethylase (EROD) activity (to measure metabolic capacity) and catalase (to examine oxidative stress) in digestive glands and gills, acetylcholinesterase activity in the digestive glands and gills were measured. The study was assessed with Klimisch 2.

Results:

VTG content was significantly increased at 10 µg/L BTA in male gonads after 35 days exposure (LOEC (35 d) = 10 µg/L). However, after 60 days exposure at 10 µg/L, the VTG content in male gonads was lower than after 10 and 35 days. At 100 and 1000 µg/L there was a duration-dependent increase in VTG concentration, with significant effects after 60 days exposure at 100 and 1000 µg/L (LOEC (60d) = 100 µg/L BTA in male gonads). In male haemolymph, the VTG concentration was significantly increased at 1000 µg/L after 35 and 60 days of exposure.

In female gonads, no significant effects on VTG level were seen, whereas in female hemolymph the VTG concentration was significantly increased at 1000 µg/L BTA after 60 days exposure. For further information see Table 21.

The authors of the study concluded that BTA may exert oestrogenic effects on marine filter-feeding scallops.

Table 21

***In vivo* data on fish and invertebrates, relevant for ED assessment**						
Life stage/ duration	**Conc. / test condition / solvent**	**Vitellogenin**	**Histology / Sex ratio**	**Others**	**Refer ence**	**Reli- ability (Klimisc h) by eMSCA**
			Fish			
Zebrafish *Danio rerio* Eleuthero embryos (exposure from 2-4 hpf for 6 days)	10, 100, 1000 µg/L (n), 7.9, 97.3; 1197.3 µg/L (m) Semi-static, renewal of water every 24 h, 5 replicates consisting of 80 eggs, 27 °C	VTG mRNA slightly up-regulated, but not significantly		No effect on mortality, hatching time or hatching rate. Transcriptional effects: No significant effects on on endocrine sensitive/mediated parameters (vtg1 up-regulated, but not significantly). ARNT2 up-regulated (metabolism) gstp1 and ugt1a down-regulated (Phase II enzymes, metabolism)	(Fent et al., 2014)	2
Rare minnow *(Gobiocypris rarus)* Sexually mature fish (age approx. 10 months) Exposure: 28 d	50, 500, 5000 µg/L (n), 20 fish per vessel, 2 replicates	VTG mRNA significantly up-regulated in the liver from 50µg/L to 5000µg/L), in males the VTG up-regulation was considerably more pronounced than in females	Females (at 5000 µg/L): degenerated ovaries at 5000 µg/L, cortical alveolus stage and perinucleolar oocytes were predominant pointing to undeveloped gonad, Control females: completely mature gonads (oocytes generally developed into vitellogenic oocytes); Males (at 5000 µg/L): disorganisation of the lobular structure, an abnormal predominance of spermatozoa at 5000 µg/L, scarce intermediate germ cell stages, indication for stimulated spermatogenesis Control males: normal cytoarchitecture of the seminiferous tubules, different germ cell cysts during the spermatogenic process	Hormone content: Females: E2 sign. decreased at 5000 µg/L (but no dose-response) Males: E2 sign. increased at 5000µg/L No effect on 11-ketotestosterone level in males and females Gene expression: Females (at all conc.): ERα up-regulated in liver; CYP19A and AR upregulated in gonads; LHß upregulated in brain; Males: LHß highly upregulated in brain (at all conc.); No effect on body weight and length, GSI and HSI (only increased GSI in males at 0.5 but not 5 mg/L), No mortality Histopathology liver at 5000 µg/L: hypertrophy of hepatocytes and nuclei pyknosis; increase in cellular vacuolization in females and males, no effects at 50 and	(Liang et al., 2014)	2

***In vivo* data on fish and invertebrates, relevant for ED assessment**						
Life stage/ duration	**Conc. / test condition / solvent**	**Vitellogenin**	**Histology / Sex ratio**	**Others**	**Reference**	**Reli-ability (Klimisch) by eMSCA**
				500 µg/L		
Fathead minow *Pimephales promelas,* Sexual mature fish, exposure: 14 d	10, 100, 1000 µg/L, mean measured values between 80 and 86% of nominal; Flow-through, Males: exposure to BTA + E2 (100ng/L); Solvent for E2: DMF (67µL/L) Females: only exposed to BTA 8 fish per treatment	Plasma VTG: Males (exposed to BTA + E2): no decrease of VTG-level, VTG in the same range like the E2-treatment (no inhibition) Females (exposed to BTA): no decrease or increase of VTG		Positive Control: E2 (100 ng/L nominal)	(Harris et al., 2007)	2
Marine medaka *Oryzias melastigma* Male and female Age: 3 months Sampling at days 4 and 35	10, 100, 1000 µg/L (n), Without solvent Salt water Daily reneval of water	VTG gene expression, sampling of liver, gills, intestine and gonads Males: VTG mRNA upregulated at 10 µg/L in liver (after 4 and 35 d), at 10 µg/L in gills and intestine after 35 d). Females: VTG mRNA upregulated at 10 µg/L (liver and intestine after 4 and 35 d)			(Tangtian et al., 2012)	3 without replicates and unknown number of fish
Zebrafish, Danio rerio, OECD TG 234 (FSDT) Duration: 63 d Determination	0.1, 0.32, 1, 3.2, 10 mg/L (nom); 0.1, 0.33, 1.07, 3.34, 11.0 mg/L (measured)	No effect on VTG in females, males and undiff. fish, but high standard deviation	Sex ratio: - Males (including transition phase): At 0.1 mg/L 6.7 % (sign. decreased), - Undifferentiated fish (consists of juveniles and protogynic gonads): At 0.1 and 1 mg/L sign. increased,	No effect on hatching Post-hatch survival (35 dpf): NOEC 1.0 mg/L, LOEC 3.2 mg/L Length (35 dpf) sign. decreased at 0.1 and 11 mg/L, differences less than 5 %, not	(Connect Chemicals GmbH, 2021b)	1

***In vivo* data on fish and invertebrates, relevant for ED assessment**						
Life stage/ duration	**Conc. / test condition / solvent**	**Vitellogenin**	**Histology / Sex ratio**	**Others**	**Reference**	**Reli-ability (Klimisch) by eMSCA**
of metabolites 4-hydroxy-1H-benzotriazole and 5-hydroxy-1H-benzotriazole			Sex ratio based on detailed histological analysis: - Males in transition phase: At 0.1 to 10 mg/L sign. decreased No males in transition phase were at 0.1 mg/L	considered relevant At 63 dpf: unexpected mortality 3 days before test termination in control and at 0.1 mg/L; no statistical determination of survival (Survival rate in control: 78.3 %; survival rate in treatments between 75.8 % (at 0.1 mg/L) and 94.2% (1 mg/L)) Length and weight increased in males, females and undiff. fish (no statistical determination as not considered adverse by the study authors) Histopathology of liver, severity of lesions: Hydropic degeneration sign. increased at 3.2 and 10 mg/L; bile duct hyperplasia sign. increased at 0.1 mg/L; no effects on single cell necrosis, hyalinised hepatocytes, vacuolation, infiltration, karyomegaly, multinucleated cells; Prevalence (% of fish) of lesions: single cell necrosis sign. increased at 0.1, 3.2 and 10 mg/L		
Invertebrates						
Chlamys nobilis Duration: 60 d 12 males and females in each vessel, 3 replicates per treatment,	0.01, 0.1, 1 mg/L	VTG content significantly increased - at 10 µg/L in male gonads after 35 d, after 60 d slightly lower value at 10 µg/L - at 100 and 1000 µg/L in male gonads after 35 and 60 d (LOEC 100 µg/L), no effect in female		GSI: in males LOEC 0.1 mg/L, in females LOEC 1 mg/L BTA. Catalase: LOEC in male digestive glands and gills: 1 mg/L; LOEC in female digestive glands and gills: 1 and 0.1 mg/L, respectively. Ethoxyresorufin-O-deethylase (EROD) activity: in digestive glands LOEC in males 0.01 mg/L, in females 0.1 mg/L, no effect in gills.	(He et al., 2019)	2

***In vivo* data on fish and invertebrates, relevant for ED assessment**						
Life stage/ duration	**Conc. / test condition / solvent**	**Vitellogenin**	**Histology / Sex ratio**	**Others**	**Reference**	**Reli-ability (Klimisch) by eMSCA**
Semi-static		gonads; - at 1000 µg/L in male haemolymph after 35 d and female haemolymph after 60 d - generally the VTG content did increase with longer exposure time				

7.10.1.3. *Supportive evidence from in vivo studies with mammals*

The mammalian data presented in this section are solely included to support the assessment of environmental ED properties as the concern for ED (human health) or further human health concerns were not in the scope of the substance evaluation.

Carcinogenicity study (US NCI, 1978)

For BTA, a two-year carcinogenesis study comparable to OECD TG 451 was performed in rats (Fischer 344) and mice (B6C3F1). The test compound was administered via diet in time-weighted average doses of 0, 6700, and 12100 ppm for rats (corresponding to approximately 0, 335, and 605 mg/kg bw/d) or 0, 11700, and 23500 ppm for mice (corresponding to 0, 1755, and 3525 mg/kg bw/d). All surviving animals were terminated at weeks 104 - 106.

Body weights of dosed rats and mice were lower than the corresponding controls throughout most of the study. Mortality was not affected by treatment.

In rats, non-neoplastic lesions were observed in both sexes at the low and high doses in the liver (increased incidence in basophilic/eosinophilic cytological changes, and clear cell changes), and kidney (nephrosis in both sexes and epithelial hyperplasia in females). In the endocrine organs, potential findings include increased incidences of focal pituitary hyperplasia in males (1/45 (2.2%), 2/40 (5.0%), 5/45 (11.1%) at 0, 6700, and 12,100 ppm, respectively), and C-cell hyperplasia in males (0/43 (0%), 3/40 (7.5%), and 2/44 (4.6%) at 0, 6700, and 12,100 ppm, respectively). In reproductive/endocrine organs, increased incidences of (acute focal) prostate inflammation in males (acute: 0/45 (0%), 3/43 (7.0%), and 9/45 (20.0%); acute focal: 0/45 (0%), 21/43 (48.8%), and 12/45 (26.7%) at 0, 6700, and 12100 ppm, respectively), and uterus/endometrium inflammation (acute + not-otherwise-specified: 1/48 (2.1%), 11/45 (24.4%), and 12/50 (24.0%) at 0, 6700 and 12100 ppm, respectively), cystic endometrial hyperplasia (0/48 (0%), 4/45 (8.9%), and 4/50 (8.0%) at 0, 6700 and 12100 ppm, respectively), and hydrometra (0/48 (0%), 4/45 (8.9%), and 4/50 (8.0%) at 0, 6700, and 12100 ppm, respectively) in female rats occurred at the low and high dose. Statistical analysis of incidences of non-neoplastic lesions was not performed by the study authors.

In mice, possible treatment-related non-neoplastic lesions were observed in the lung (haemorrhage in high-dose males; focal inflammation in low- and high-dose females), mesenteric lymph nodes (haemorrhage in the low- and high-dose males and females), and bone marrow (myelofibrosis in the low and high-dose females). In reproductive organs, findings in mice were confined to hydrometra in low and high-dose females (0/44 (0%), 12/46 (26.1%), and 9/46 (19.6%) at 0, 11700, and 23500 ppm, respectively).

In rats, a significant increase of neoplastic nodules in males occurred in liver (0/48 (0%), 0/46 (0%), and 5/45 (11.1%) at 0, 6700 and 12100 ppm, respectively; within historical control incidences). Brain tumours occurred in low-dose males (0/46 (0%), 3/44 (6.8%), and 0/46 (0%) at 0, 6700 and 12100 ppm, respectively) and in one high-dose female (0/50 (0%), 0/47 (0%), and 1/50 (2.0%) at 0, 6700 and 12100 ppm, respectively). Because the historical control data of this lab show a very low incidence of brain tumours, this finding is considered by the authors suggestive of, but not of sufficient evidence for carcinogenicity. In female rats, the incidence of endometrial polyps was significantly higher at the low-dose and non-significantly increased at the high-dose (2/48 (4.2%), 10/45 (22.2%), and 8/50 (16.0%) at 0, 6700, and 12100 ppm, respectively). In addition, non-significant increases in C-cell adenoma at the low-dose (0/43 (0%), 4/43 (9.3%), and 0/50 (0%) at 0, 6700 and 12100 ppm, respectively) and C-cell carcinoma at the low- and high-dose (0/43 (0%), 1/43 (2.3%), and 3/50 (6.0%)) were observed in female rats. In mice, the incidence of alveolar/bronchiolar carcinomas was significantly higher in low-dose females (0/49 (0%), 9/49 (8.2%), 3/49 (6.2%) at 0, 11700 and 23500 ppm, respectively). The data show neoplastic findings in two species in both sexes in different organs. However, it is questionable, whether the neoplastic changes in the liver and uterus of rats and in the lungs of mice are substance-related. A

substance-related origin of C-cell adenomas is similarly uncertain. In the case of the occurring brain tumours, due to the extraordinarily low spontaneous incidence in historical control data, it cannot be excluded that this is a substance-related effect, despite the low incidence and the lack of dose-dependence.

With regard to ED, some observations (neoplastic and non-neoplastic) were reported in sexual organs which are in general sensitive towards the oestrogen/androgen/ steroidogenesis (EAS)-modality. However, incidences were either low or no dose-response was observed, and a relationship with treatment is questionable. Therefore, this carcinogenesis study does not provide evidence for EAS-sensitive/mediated adversity. Regarding non-EAS-mediated/sensitive adversity, the increase in the incidence of C-cell adenomas in female rats was statistically not significant and not dose-dependent, and the slight increase in C-cell hypertrophy in males did not show dose-dependency either. Thus, a clear substance-related effect cannot be concluded from this study alone. However, increased incidences of C-cell hyperplasia were observed in an OECD TG 414 study (Biological Testing Laboratory, 2020) in maternal rats (see below).

OECD TG 421 (vivo Science GmbH, 2013)

A reproductive/developmental toxicity screening study (oral gavage) according to OECD TG 421 with rats (Wistar) tested BTA in doses of 0, 12.5, 50, and 200 mg/kg bw/d.

Mild discomfort in the mid- and high-dose animals *("wiping of nose and mouth through the cage bedding, salivation after application, bleeding of mucous membranes at nose and mouth, respirators sounds"*) was reported. Otherwise, there was no treatment-related systemic toxicity observed. Regarding reproductive/endocrine-sensitive findings, the only potential treatment-related effect was a slightly higher rate of stillborn pups in the high-dose group (0.00%, 0.77%, 0.00%, and 3.01% at 0, 12.5, 50, and 200 mg/kg bw/d, respectively; not significant). Since this study was performed before the update of OECD TG 421, anogenital distance (AGD), nipple retention, and thyroid hormone or thyroid-stimulating hormone levels, or thyroid histology were not analysed.

OECD TG 414 (Biological Testing Laboratory, 2020)

A prenatal developmental toxicity study (oral gavage) according to OECD TG 414 with rats (Sprague-Dawley) tested BTA in doses of 0, 36, 120, and 330 mg/kg bw/d.

Behavioural changes in the high-dose dams occurred within 1.5 – 3 h after dosing (*"hypokinesia, staggering gait or flatness as a more severe behaviour disorder, excessive vocalisation, chromodacryorrhea, ptyalism, nasal discharge").* Maternal body weight effects were mild and confined to the high-dose group (–5 % lower body weight compared to control on gestation day 20). However, corrected maternal body weight was similar to controls in all dose-groups. C-cell hyperplasia (minimal severity) was observed in the mid- and high-dose dams and is considered a test item-related finding (0/24, 0/21, 3/24, and 4/23 at 0, 36, 120, and 330 mg/kg bw/d, respectively).

Toxicologically relevant developmental effects were observed in the mid- and high-dose groups including significantly increased post-implantation losses (2.8, 2.1, 7.4, and 6.3% at 0, 36, 120, and 330 mg/kg bw/d, respectively), and a trend for lower litter size (13.0, 13.2, 12.6, and 12.2 at 0, 36, 120, and 330 mg/kg bw/d, respectively). Foetal weight was significantly lower in the high-dose (-1.9, +2.8, and -11.5% vs control at 36, 120, and 330 mg/kg bw/d, respectively), and normalised AGD, also known as anogenital index (AGI), was increased in high-dose female foetuses (+5.7 % vs. controls). Furthermore, foetal as well as litter incidences of a variety of skeletal and soft tissue variations, and malformation were increased in the mid- and high-dose foetuses, though some of the findings showed no dose-response. Given the findings observed in this study, a

harmonised classification of BTA for the endpoint developmental toxicity is considered adequate by the eMSCA.

With regard to ED, the only treatment-related changes in parameters mediated by or sensitive to the EAS-modality were increased post-implantation losses and increased AGI in female offspring. According to Schwartz et al. (2019), an increased AGI in females may be considered a masculinisation effect, e.g. resulting from the presence of excess androgen levels or ectopic activation of androgen receptors. There is no evidence for such a mode-of-action, since *in vitro*, BTA displayed no androgenic but anti-androgenic (Fent et al., 2014) and anti-oestrogenic activity (Harris et al., 2007). Whereas a decrease of male AGI is a well-established marker for reduced foetal androgen signalling, associated with genital malformations and reproductive disorders later in life, the toxicological relevance (in males as well as females) of AGI-increases is unclear. Moreover, the effect size is considered small, and other specific adverse effects were not observed in any study which could be clearly related to the EAS-modality. Although the increased post-implantation losses are considered adverse and EAS-sensitive, it is unclear whether there is any mechanistic relationship with the *in vitro* findings or the increased AGI in female foetuses.

Besides the EAS-modality, the increased incidence of C-cell hyperplasia in the OECD TG 414 study together with the occurrence of C-cell neoplasia in the carcinogenesis study indicate non-EAS mediated endocrine activity related to a disturbance of calcitonin/calcium homeostasis. Considering the increased incidence of skeletal findings in the OECD TG 414 study, it seems possible that a substance-induced change in calcium homeostasis in dams might be associated with the altered skeletal development of offspring. However, since no further mechanistic data are available with regard to this concern (i.e. calcitonin and calcium levels, specific bone investigations), a relationship between C-cell hyperplasia and the skeletal findings remains speculative.

Conclusion of *in vivo* studies with mammals

The adverse findings in the OECD TG 414 (Biological Testing Laboratory, 2020) are considered by the eMSCA to warrant classification for reproductive toxicity.

With regard to ED, treatment-related changes in EAS-sensitive/mediated parameters were observed in the OECD TG 414 study, including increased post-implantation losses and increased AGI in female offspring. On the other hand, the findings in reproductive/endocrine organs in the carcinogenesis study are of questionable relationship with treatment. No clear MoA can be formulated with regard to the EAS-modality. Additionally, the increased incidence of C-cell hyperplasia in the OECD TG 414 study together with the occurrence of C-cell neoplasia in the carcinogenesis study indicate non-EAS mediated endocrine activity related to a disturbance of calcitonin/calcium homeostasis. Due to the lack of further mechanistic data, a relationship between C-cell hyperplasia and the skeletal findings in offspring remains speculative.

In conclusion, BTA induces effects on some endocrine sensitive organs/parameters but the evidence for a plausible relationship between endocrine activity and the observed adverse effects is insufficient to conclude on the ED properties of BTA in mammals. With regard to the environment, the findings in the mammalian studies show some effects on EAS-sensitive/mediated parameters but provide only limited supporting mechanistic evidence for the ED-properties of BTA seen in fish studies. Although the increased post-implantation losses in the OECD TG 414-study are potentially population-relevant, this finding alone is too unspecific and cannot be plausibly linked to an endocrine mode of action. Therefore, the mammalian data per se provide no evidence for endocrine disruptive population-relevant effects.

7.10.1.4. *Discussion of in vitro and in vivo studies*

***In vitro* studies:**

The *in vitro* studies (Harris et al., 2007), (Fent et al., 2014) and (Seeland et al., 2012) did not show receptor mediated oestrogenic activity in YES assays. No androgenic activity was seen by (Fent et al., 2014).

In the anti-oestrogen screen (YES) (Harris et al. 2007), BTA may have anti-oestrogenic activity, however, no definitive conclusion can be drawn due to the lack of statistical evaluation and information on cytotoxicity.

In the anti-androgen screen (YAS) by Fent et al. (2014) antiandrogenic activity in the YAS assay probably existed. Personal communication showed that cytotoxicity appeared only at the highest concentration tested. However, there was no statistical evaluation and information on the cytotoxicity assay used. Therefore the result is not definitely conclusive.

The YES assay performed by Seeland et al., 2012 showed significant cytotoxicity of BTA to the yeast cells used at 50 µg/L and higher.

Therefore, from *in vitro* studies, no conclusive results on the anti-oestrogenic or anti-androgenic activity of BTA can be drawn.

***In vivo* studies in fish and invertebrates:**

- Histopathology: The FSDT (Connect Chemicals GmbH, 2021b) showed that BTA impaired the development of males at an early stage of gonadal development, as at 0.1 mg/L there were no males in transition phase (hence the ratio of males at 0.1 mg/L was significantly decreased) and significantly less males in transition phase were in the treatments 0.1 to 10 mg/L by statistical calculation using Cochran-Armitage and at 0.1 to 3.2 mg/L by calculation using CPFISH. Further there were seen significantly more undifferentiated fish (protogynic gonads and juveniles) at 0.1 and 1 mg/L and also at the other concentrations (but not significantly increased). These observations above can be explained by an impact of BTA on the steroidogenesis leading to an oestrogenic mode of action of the Substance acting via HPG-axis.

- Plasma VTG: Plasma VTG levels in the FSDT were not significantly changed, however the standard deviation is very high in this test (VTG measurement via ELISA kit). In a study with fathead minnow (exposure 14 d) and testing male mature fish in an anti-oestrogen test no effect on plasma VTG was seen, and no effect in female fish (Harris et al., 2007). It might be possible that in this study no effect on plasma VTG was seen, as BTA interacts with the hormonal circuit at an earlier point, direct on the E2 level. Moreover, the testing duration of 14 days was rather short. And females are generally in a vitellogenin test less sensitive than males, since they have typically a higher VTG value than males. Therefore, the results cannot be considered as reliable and conclusive endpoints.

- VTG gene expression: The upregulation of VTG on the gene level observed by (Liang et al., 2014) in rare minnows and (Tangtian et al., 2012) in marine medaka (however reliability 3) may be a response to an oestrogenic MoA or a response in the hormonal control circuit. There were no effects on VTG mRNA in zebrafish eleuthero embryos seen (Fent et al., 2014) after exposure to the Substance for 6 days, aside from a not significant elevation of VTG mRNA.

- Gene expression of other genes related to HPG axis: In a study with sexually mature rare minnows up-regulation of CYP19A and CYP11A gene expressions at the concentrations 50 to 5000 µg/L in the gonads of females was seen (Liang et al. 2014). CYP19A is responsive to oestrogenic substances and can lead to enhanced production of

E2. Also CYP11A can lead to enhanced production of E2. There were yet other genes significantly upregulated in females as ERα, ERβ2, FSHR (follicle stimulating hormone receptor), LHR (luteinising hormone receptor) and 17β-hydroxysteroid dehydrogenase (17βHSD) which is involved in steroidogenesis and LHß was significantly upregulated in males (Liang 2014).

- Hormone content: The E2 level was affected in rare minnows (Liang et al., 2014): E2 was significantly decreased at 5000 µg/L in females (but no dose-response, as there was a not significant increase at 500 µg/L); and in males E2 was sign. increased at 5000 µg/L. The increase in males was possibly caused by an oestrogenic MoA.

In the invertebrate marine scallop *Chlamys nobilis* (He et al., 2019) a significant increase of VTG in male gonads and hemolymph and female hemolymph was seen, suggesting an oestrogenic MoA in these invertebrates.

7.10.2. Conclusions of the endocrine disrupting properties for the environment and related classification and labelling

From the available *in vitro* studies, no conclusive results regarding anti-oestrogenic or anti-androgenic modes of action of BTA can be drawn. The available *in vitro* tests suggest that the assumed oestrogenic MoA is not receptor mediated, which fits to the observed effects on aromatase gene induction in the *in vivo* fish studies.

In summary, *in vivo* studies in fish show that there are indications for oestrogenic activity of BTA. There was seen an up-regulation of CYP19A and CYP11A gene expressions at the concentrations 50 to 5000 µg/L in the gonads of females (Liang et al. 2014). For CYP19A it is known that it is responsive to oestrogenic substances. Both gene products can lead directly (Cyp19A) or indirectly (Cyp11A) to enhanced production of E2. VTG and ERα gene expressions were up-regulated in the liver also at 50 to 5000 µg/L in females. In males the upregulation of VTG gene expression in the liver was very pronounced at these concentrations.

The available FSDT study requested during substance evaluation of BTA showed that there were significantly less males compared to the control at 0.1 mg/L. The decrease of males is a strong indication for the above proposed oestrogenic MoA. Furthermore, there was a significant increase in undifferentiated fish at 0.1 and 1 mg/L as well as a significant decrease of males in transition phase at 0.1 to 10 mg/L. These effects on sexual development can also be explained via interference with steroidogenesis leading to an oestrogenic mode of action of the Substance.

Regarding the plasma VTG values in the FSDT: the standard deviation was very high in females and males and thus cannot be considered as reliable and conclusive endpoint in this study.

Moreover, it is possible that there might be varying mechanisms due to specific conditions, like internal body burden of the Substance or different E2 levels in males and females.

An oestrogenic MoA of BTA is further supported by results obtained in the invertebrate marine scallop *Chlamys nobilis* (He et al., 2019), where an increase of VTG content in male gonads and haemolymph of males and females were seen.

The available mammalian data provide limited supporting evidence (increased post-implantation losses and increased AGI in female offspring) for the ED properties of BTA seen in fish studies related to the EAS modality. Potentially population relevant findings in the mammalian studies (i.e. increased post-implantation losses) cannot be plausibly linked to endocrine activity.

The skewed sex ratio with less males and more undifferentiated fish observed in the available OECD TG 234 study is an adverse and population relevant effect and indicative

for an underlying oestrogenic MoA via the HPG axis in fish (according to OECD TG 234 *"the sex is defined as female, male, intersex (both oocytes and spermatogenetic cells in one gonad) or undifferentiated, determined in individual fish via histological examination of the gonads"*). An indication for endocrine disruption of BTA is also that no males in transition phase were observed at 0.1 mg/L and significantly less males in transition phase were seen at 0.1 up to 10 mg/L, pointing to impairment of development of males at an early stage of gonadal development and also acting via an oestrogenic MoA.

Taking into account the adverse effect observed on sex ratio in the available long-term fish study (OECD TG 234) and further *in vivo* fish and invertebrate data pointing to an endocrine activity, the eMSCA concludes that BTA fulfils the WHO/IPCS definition for an endocrine disruptor in the environment. Furthermore, the eMSCA concludes that BTA can be classified as ED ENV Cat. I according to the criteria for ED laid out in CLP.

7.11. PBT and vPvB assessment

Persistence:

The degradation of BTA has been tested within different test systems and media, which all indicated an incomplete degradation. Thus, the eMSCA concludes that BTA is very persistent in the environment according to Regulation 1907/2006 (REACH), as well as the revised version of Regulation 1272/2008 (CLP) (EC, 2022).

Bioaccumulation:

Based on a measured log K_{ow} of 1.34 (at 22.7 °C, pH 5.7), a bioaccumulation testing in aquatic species was waived. However, it was concluded by the eMSCA that the bioaccumulation potential of BTA is low.

Ecotoxicity:

Toxicity data with fish, Daphnia and algae do not fulfil the T-criterion of 0.01 mg/L of Annex XIII of the EU-REACH Regulation. The most sensitive organism is the invertebrate *D. galeata* with an EC_{10} of 0.97 mg/L (see section 7.8.1.2.2). BTA has a harmonised classification as Aquatic chronic 2.

The intended classification of BTA due to the endocrine disruptive properties category 1 for the environment is also leading to fulfilment of T criteria under the new CLP regulation.

Overall Conclusion of the PBT/ vPvB Assessment

Based on available data, BTA is concluded to be very persistent (P and vP), not bioaccumulative (not B or vB) and toxic (T).

Therefore, BTA cannot be considered a PBT or vPvB substance (not PBT/ vPvB).

Mobility:

In accordance to the data presented in section 7.7.3, the eMSCA concludes that BTA is very mobile (vM) according to the new hazard criteria introduced into Regulation 1272/2008 (CLP) (EC, 2022) and can be expected to contaminate the aqueous environment.

Overall Conclusion of the PMT/ vPvM Assessment

Under consideration of the information for persistency and mobility, in the perspective of the eMSCA there is sufficient evidence that BTA fulfils the CLP criteria for very persistent and very mobile (vPvM) and persistent, mobile and toxic (PMT).

7.12. Exposure assessment

7.12.1. Human health

Not evaluated.

7.12.2. Environment

Information on uses and Summary of Monitoring data

BTA is registered under REACH and is manufactured in and/or imported to the European Economic Area in an aggregated tonnage of 1 000 to 10 000 tpa. It is used by consumers, by professional workers (widespread uses) and in formulation or re-packing and at industrial sites.

The Substance and its derivatives mainly are used as anticorrosive and anti-scaling agent especially for non-ferrous metals and silver. The anticorrosive properties are needed e.g. in coolants, antifreezers, de-icing agents and in decalcification products. BTA is used in many dishwasher detergents for silver protection (Römpp, 2014). During industrial metal processing the Substance is used in cooling lubricants. Furthermore, the Substance is used in solar panels as UV protection, used in medical applications or in photographic developers (Bajaj and Sakhuja, 2016).

Environmental Occurrence - Monitoring Studies

BTA is frequently detected in the aquatic environment. According to Harris et al. (2007), it enters groundwater and surface waters due to its use as anticorrosive, deicing and antifreeze agent for airplanes via runoff from airports. Concentrations up to 126 µg/L were measured in runoff waters from airports (Cancilla et al., 1998). In addition, BTA is used in dishwashing detergents for silver protection leading to high concentrations in rivers (e.g. (Voutsa et al., 2006)). The elimination in water treatment plants is poor (< 30 %, (Voutsa et al., 2006)), and BTA was therefore found in sewage effluents up to 100 mg/L (Voutsa et al., 2006). The hydroxy-metabolites of BTA were not in the focus of monitoring studies. BTA is also frequently found in marine waters up to 135 ng/L (e.g. (Nodler et al., 2014), (Xie et al., 2020)).

An overview on the available monitoring studies for the aquatic compartment including surface waters, marine waters and groundwater, waste treatment plants and sediments is presented below. No data were found for marine sediments, the atmospheric and the terrestrial environment.

Surface waters

The most comprehensive study was performed by JRC (Loos et al., 2009) providing data from 100 rivers in 27 EU countries. Therein, the maximum concentration was 7.997 µg/L and the mean concentration amounted to 0.43 µg/L. Concentrations measured in other studies are all in the low µg/L range (see Table 22). Concentrations in runoff from airport may be extremely high with up to 126 µg/L (Cancilla et al., 1998). Also, concentrations measured in rivers downstream or near wastewater treatment plants (WWTPs) are higher than upstream (Janna et al., 2011).

Table 22

Overview results from monitoring studies for BTA: surface waters		
Details	**Concentrations**	**Reference**
100 rivers in 27 EU countries	LOD 1 ng/L, **Freq. 94%** **max. 7.997 µg/L** **mean: 0.493 µg/L** **median 0.226 µg/L** Per90 1.225 µg/L	(Loos et al., 2009)
Danube River (longest river in the EU)	LOD 1 ng/L, **freq 100%,** **max. 0.38 µg/L,** **mean 0.213 µg/L** **median 0.185** µg/L Per90 0.324 µg/L	(Loos et al., 2010b)
German river surface water: Rhine, Elbe, Havel	Rhine: 0.13 to 0.39 µg/L Elbe <0.05 to 0.68 µg/L Havel upstream of Berlin: < 0.05 µg/L Havel downstream of Berlin: 1.57 µg/L	(Reemtsma et al., 2010)
Glatt River Switzerland	0.36 – 3.69 µg/L	(Voutsa et al., 2006)
Glatt River Switzerland	max. 5.0 µg/L	(Schaffner, 2004)
Glatt River Switzerland	max. 6.3 µg/L median: 1.0 µg/L	(Giger et al., 2006)
Rivers, Berlin, Germany	0.9 µg/L Lake Tegel 3.4 µg/L Landwehr canal 0.2 µg/L bank filtrate used for drinking water	(Weiss and Reemtsma, 2005)
Surface waters Berlin, Germany 2010-2015	11 sites 391 samples, **max. 12.0 µg/L** **mean 1.77 µg/L** **median 1.22 µg/L** LOD 0.02 - 0.05 µg/L	(Abgeordnetenhaus Berlin, 2015)
Rhine, Germany	0.05 - 0.5 µg/L	(Schaffner, 2004)
Rhine, Germany	0.13 - 3.5 µg/L	(Reemtsma et al., 2010)
Main, Hengstbach, Germany	0.072-0.472 µg/L 0.383-1.47 µg/L	(Kiss and Fries, 2009)
Rivers, UK (5 samples)	0.013, 0.021, 0.023, 0.118, 1.96 µg/L	(Janna et al., 2011)
Yangtze River Delta, China	min. 0.074 µg/L max. 18.303 µg/L mean 2.475 µg/L	(Peng et al., 2018)
Pearl River, South China	0.112–1.279 µg/L (dry sezon) 0.023–0.482 µg/L (wet sezon)	(Han et al., 2020)
Modeled concentrat. surface waters	0.4 - 1 µg/L overall 0.001 – 1 µg/L below STP inputs	(Janna et al., 2011)
Airport Run off to Surface water	Up to 126 µg/L	(Cancilla et al., 1998)

Table 23

Overview results from monitoring studies for BTA: marine waters		
Details	**Concentrations**	**Reference**
San Francisco Bay, USA	**freq. 90 %** **max. 135 ng/L** **median 29 ng/L**	(Nodler et al., 2014)
Aegean Sea & Dardanelles, Greece and Turkey	freq. 30 %, max. 53 ng/L, median 11 ng/L	(Nodler et al., 2014)
Northern Adriatic Sea, Italy	freq. 100 %, max. 113 ng/L, median 104 ng/L	(Nodler et al., 2014)
Around Liaodong Peninsula, China	freq. 100 %, min. 7,9 ng/L, max. 30 ng/L,	(Xie et al., 2020)

Groundwater

For groundwater only one comprehensive study was done by JRC (Loos et al., 2010a), providing data from 164 samples in 23 EU countries. Here, BTA was detected in 53% samples with a maximum concentration of 1.032 µg/L. Measured concentrations in tap water are in the ng/L range (Janna et al., 2011).

Table 24

Overview results from monitoring studies for BTA: ground and tap water		
Details	**Concentrations**	**Reference**
Groundwater 23 EU-member states, 164 samples	LOD 1 ng/L, **freq. 53 %,** **max. 1.032 µg/L,** **mean 0.024 µg/L,** **median 1 ng/L** P90 0.04 µg/L	(Loos et al., 2010a)
Tap water, UK	0.0006 -0.0794 µg/L (0.031 µg/L mean)	(Janna et al., 2011)

Waste Water Treatment effluents

For WWTPs, JRC analysed samples from 90 European sites in 2010 in a comprehensive study (Loos et al., 2013). BTA was detected in 97% of the samples with a maximum concentration of 221 µg/L and a mean of 6.3 µg/L. In other studies, similar concentrations were detected with a rather broad range (cf. Table 25).

Table 25

Overview results from monitoring studies for BTA: waste water treatment effluents		
Details	**Concentrations**	**Reference**
90 European WWTPs (in 2010)	LOQ 40 ng/L, **freq. 97%,** **max. 221 µg/L** **mean 6.3 µg/L** **median 2.7 µg/L**	(Loos et al., 2013)

	Per90 10.9 µg/L	
Municipal sewage effluents (33 primary, 61 secondary)	Prim. effluent: mean 18 µg/L (22 - 75 µg/L) Sec. effluent: mean 10 µg/L (11 - 100 µg/L)	(Voutsa et al., 2006)
STP effluents in Europe	1 - 10 mg/L	(Reemtsma et al., 2006)
Municipal STP effluents Germany, 7 samples	4.1 – 8.8 µg/L	(Coors, 2016)
1 WTP sewage effluent, Berlin, Germany	11.9 µg/L influent 9.6 µg/L effluent	(Weiss and Reemtsma, 2005)
8 sewage effluents UK	0.84 – 3.65 µg/L	(Janna et al., 2011)
Sewage sludge from WTP China, 5 sites	Detected in all samples 17.2 – 198 µg/kg mean 142 µg/kg	(Zhang et al., 2011)

Sediment

For freshwater sediments, only 2 studies were found from China and the USA (Table 26).

Table 26

Overview results from monitoring studies for BTA: freshwater sediments		
Details	**Concentrations**	**Reference**
Songhua river China, 6 sites	Frequency: 1/6 samples 0.385 µg/kg dw	(Zhang et al., 2011)
Sagnew and Detroit River USA	Frequency: 6/6 sites 0.424 - 33.4 µg/kg dw mean 9.43 µg/kg dw	(Zhang et al., 2011)

7.13. References

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7.14. Abbreviations

4/5-OH-BTA	4-hydroxy-benzotriazole or 5-hydroxy-benzotriazole
11-KT	11-ketotestosterone
AGD	Anogenital distance
AGI	Anogenital index
AR	Androgen receptor
BTA	1H-Benzotriazole
bw	body weight
dpf	days post fertilisation
dph	days post hatch
E2	Estradiol
EAS	Oestrogen/androgen/steroidogenesis (modality)
ELISA	Enzyme-linked immunosorbent assay
ER	Oestrogen receptor
FSH	Follicle-stimulating hormone
eMSCA	evaluating Member State competent authority
GnRH	Gonadotropin-releasing hormone
GSI	gonadosomatic index
EC_{50}	Half maximal effective concentration
LC_{50}	Half maximal lethal concentration
LHβ	Luteinising-hormone β
LHR	Luteinising-hormone receptor
LOEC	Lowest observed effect concentration
LOQ	Limit of quantification
NOEC	No observed effect concentration
PCR	polymerase chain reaction
QSAR	Quantitative Structure-Activity Relationship
RMOA	Regulatory Management Option Analysis
STP	Sewage treatment plant
VTG	Vitellogenin
WWTP	Waste water treatment plant

Annex I – statistical calculation (FSDT)

Analysis transgenity patterns in Zebrafish for Benzotriazole

Hypotheses Tests for quantal data sets

Cochran-Armitage test is a one-sided trend test based on Pearsons X^2-test and designed for binomial proportions. It assumes that the subjects are independent within and among the treatment groups and the treatment proportions are monotonic to the dose score. Because Cochran- Armitage ignores replicates it can only be applied to data set without extra binomial- variance. In that case Rao-Scott test could be applied. Several author (Green et al. 2018; Hirji and Tang, 1998) have analyzed the statistical power of the two tests and found adequately powered in many applications.

In a step-down procedure sequentially, different hypotheses tests were conducted to the data set, which will be reduced in each step by one treatment starting with the highest and ending with the lowest concentration. Abort criterium is that the test is not significant otherwise the step-down procedure will be continued.

In case of unclear dose response, the two-sided CPFISH test (Lehmann et al. 2017) could be applied. The test combines the closure principle (CP) to consider the α- inflation and the Fisher- Freeman- Halton test (FISH). Lehmann et al. discusses the statistical power and highly recommend the use of the test for quantal data sets with or without trend.

Data set

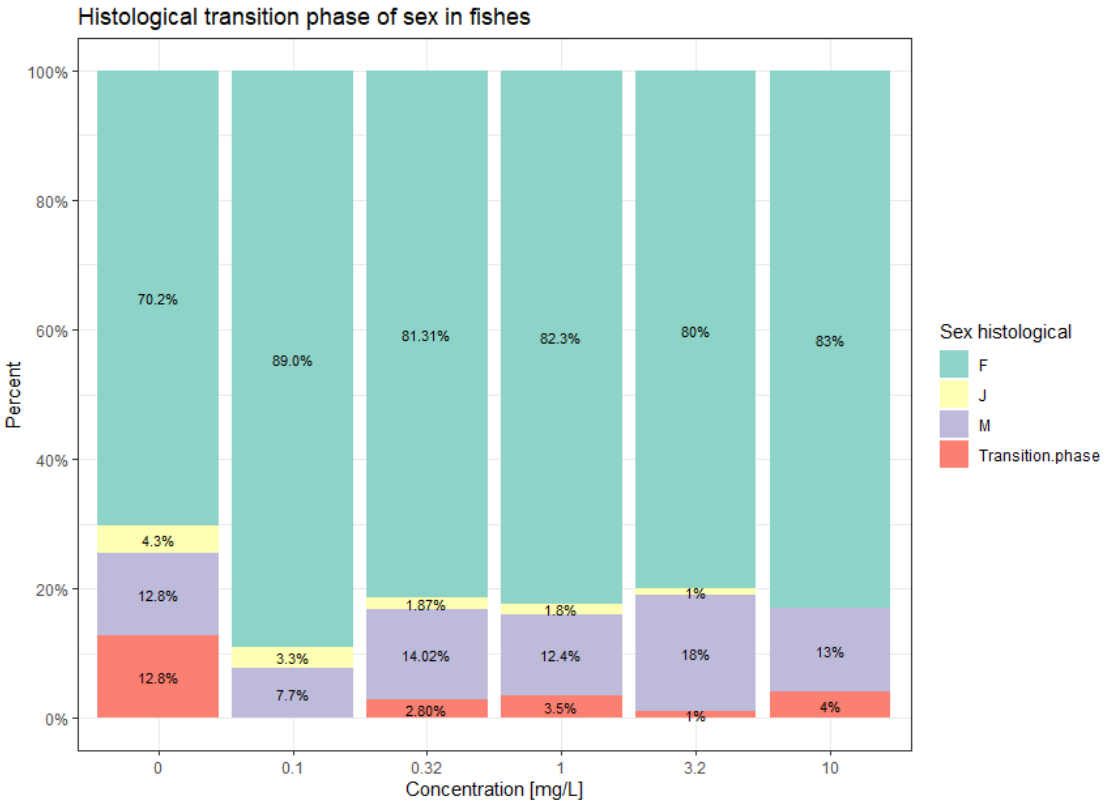

Figure A6: Distribution of histological sex in Danio reiro for the control and 5 treatment levels

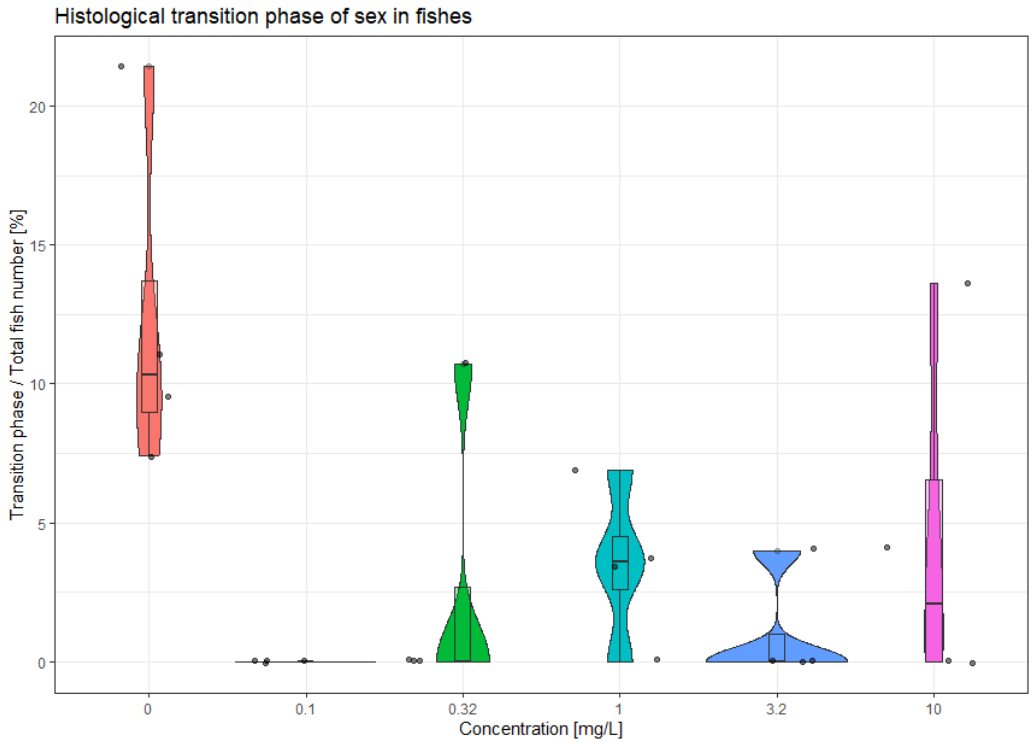

Figure A7: Violina-boxplots of the proportion of males in transition phase in the *Danio rerio* test for the different treatments and replicates.

Pre-Condition for Cochran- Armitage test

The test has the pre-conditions of a trend and variance homogeneity. The selected test to prove variance homogeneity depends on the distribution of the underlying residuals. In case of normal distribution, the Rao Scott test could be applied which has a high statistical power. In case of non-normal distribution, the Brown-Forsythe-Levene' s test could be applied.

Test for monotony:

Test	t-value	Pr(>|t|)
Linear	-1.72	0.103
Quadratic	1.99	0.062

The H_0 is in that case that there is no trend in the data set. The H_0 hypotheses cannot be rejected. There is no trend detectable. In case of the normal procedure of the Cochran-Armitage test the pre-condition for the test were not fulfilled. In the specific case Cochran Armitage test can be conducted because each single treatment is compared with the control. The p- values were Bonferroni correction to avoid Alpha inflation.

Test for normality and variance homogeneity:

shapiro.test(stdres(lm(Transition.Phase/Starting ~ Treatment)))

Shapiro-Wilk normality test

W = 0.85393, p-value = 0.002588

The H_0 of normality can be refused. The p- value is significant. Therefore, a Brown-Forsythe Levene' s test a robust alternative to Bartlett's test that is less sensitive to deviations from normality can be used to analyse the variance distribution.

Levene's Test for Homogeneity of Variance (center = median)

	Df	F value	Pr(>F)
Group	5	0.9244	0.4879
	18		

The H_0 hypotheses of homogeneity could not be rejected. As Cochran-Armitage test does not take into account the variance structure it implies variance homogeneity, which is fulfilled by the data. The Rao- Scott correction has not to be conducted, because of lower power compared to Cochran- Armitage.

Results

Cochran Armitage-test:

Table A27: Test statistics of Cochran Armitage test of each treatment compared to the control with Bonferroni corrected p values

Treatment [mg/L]	Z	p- value uncorrected	p- value Bonferroni
10	2.218	0.013	0.066
3.2	3.276	0.001	0.003
1	2.475	0.007	0.033
0.32	2.682	0.004	0.018
0.1	3.525	0.000	0.001

Treatment 3.2 mg/L, 1 mg/L, 0.32 mg/L and 0.1 mg/L are significant different from the control.

Table A2: CPFISH test

Treatment [mg/L]	p-Value
10	0.036
3.2	0.004
1	0.022
0.32	0.017
0.1	0.001

CPFISH shows the significant p-values for all treatments.

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