National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Environmental risk limits for benzotriazoles

Proposal for quality standards for surface water

RIVM letter report 2025-0051
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Colophon

DOI 10.21945/RIVM-2025-0051

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This study was commissioned by the Dutch ministry of Infrastructure and Water Management in the context of the project 'Chemische waterkwaliteit, normering en richtlijn prioritaire stoffen'

Published by:
National Institute for Public Health and the Environment, RIVM
PO Box 1 | 3720 BA Bilthoven
The Netherlands
www.rivm.nl/en

Synopsis

Environmental risk limits for benzotriazoles

Proposal for quality standards for surface water

Benzotriazoles are chemical substances that are widely used in industrial cooling water systems to prevent corrosion. They are also present in antifreeze and dishwasher tablets. These substances are emitted to surface waters through industrial and household discharges.

The national task force to address emerging substances (Dutch: werkgroep Aanpak Opkomende Stoffen) would like more information on the environmental impact of these discharges.
In response, the Ministry of Infrastructure and Water Management (I&W) commissioned RIVM to derive environmental risk limits for this group of substances.

Benzotriazoles include hundreds of different compounds. RIVM selected eight of them for which research has demonstrated widespread use. Some of these eight substances are comparable in terms of chemical structure, environmental behaviour and effects. In this case, the derived risk limits apply to the sum of the respective benzotriazoles. This report therefore includes risk limits for four benzotriazoles (or groups of benzotriazoles): 1H-benzotriazole and sodium 1H-benzotriazolide, methylbenzotriazoles, hydroxybenzotriazole and sodium 5-N-butyltriazole.

The risk limits in this report are scientific advisory values. Based on the present research, the Ministry of I&W may decide to set quality standards for benzotriazoles in surface water. An indicative water quality standard for 1H-benzotriazole is already in force. RIVM has collected more information that enables derivation of a more reliable value, which may replace the current indicative standard.

Keywords: environmental quality standards, surface water, benzotriazoles

Publiekssamenvatting

Milieurisicogrenzen voor benzotriazolen

Voorstel voor waterkwaliteitsnormen

Benzotriazolen zijn chemische stoffen. Ze worden veel gebruikt in industriële koelwatersystemen om aantasting van metaal tegen te gaan. Ze zitten ook in antivries en in vaatwastabletten. Via lozingen uit de industrie en afvalwater uit huishoudens komen deze stoffen in het oppervlaktewater terecht.

De nationale werkgroep Aanpak Opkomende Stoffen wil weten of deze lozingen schadelijk zijn voor het milieu. Het ministerie van Infrastructuur en Waterstaat (IenW) heeft het RIVM daarom gevraagd om risicogrenzen te bepalen voor deze groep stoffen.

Er bestaan honderden verschillende benzotriazolen. Het RIVM heeft er acht geselecteerd waarvan uit onderzoek blijkt dat ze veel worden gebruikt. Bij een aantal van deze acht stoffen lijken de chemische structuur, hoe ze zich in het milieu 'gedragen' en de effecten erg op elkaar. In deze gevallen geldt de risicogrens voor de opgetelde hoeveelheden van de desbetreffende benzotriazolen. Dit rapport bevat daarom risicogrenzen voor vier (groepen) benzotriazolen: 1H-benzotriazool en natrium 1H-benzotriazolide, methylbenzotriazolen, hydroxybenzotriazool en natrium 5-N-butyltriazool.

De risicogrenzen in dit rapport zijn wetenschappelijke advieswaarden. Het ministerie van Infrastructuur en Waterstaat kan op basis van dit onderzoek besluiten om waterkwaliteitsnormen voor benzotriazolen vast te stellen. Voor 1H-benzotriazool bestaat al een indicatieve norm. Het RIVM heeft nu meer informatie gevonden waarmee een betrouwbaarder waarde kon worden bepaald. Deze kan de huidige indicatieve norm vervangen.

Kernwoorden: milieukwaliteitsnormen, oppervlaktewater, benzotriazolen

Contents

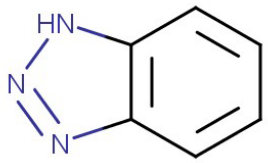
1 Introduction

1.1 Benzotriazole and its derivatives

Benzotriazoles are compounds which are mainly used as corrosion inhibitors. They are applied in e.g., cooling system liquids (Berbee & Rutten, 2022), dishwasher detergents (Vetter & Lorenz, 2013), and de-icers for aircraft and airports (Olds et al., 2022). Niche uses include the stabilisation of copper and copper alloy for archaeological artifacts (Golfomitsou & Merkel, 2004).

Benzotriazoles are compounds that are characterised by a benzene ring (C_6H_6) fused to a triazole ring featuring two carbon atoms and three nitrogen atoms ($C_2H_3N_3$). The structural formula of benzotriazole, also known as 1H-benzotriazole (CAS no. 95-14-7) is given in Figure 1.

Figure 1 Structural formula of benzotriazole (ECHA, 2023).



Benzotriazole is undergoing an endocrine disrupting (ED) and persistent, mobile and toxic (PMT) assessment. Since February 21st, 2023 benzotriazole is listed on the PBT[1] assessment list of the European Chemicals Agency (ECHA), while the listing for ED assessment goes back to September 13rd, 2016. Currently, there is no harmonised classification for benzotriazole listed in Annex VI of the CLP regulation yet; a proposal for classification as 'Toxic to aquatic life with long lasting effects' (Aquatic Chronic 2, H411) was submitted by Germany in 2021 (BAuA, 2021) and adopted by RAC in 2022 (ECHA, 2022a), but the process is not finalised yet. Notified classifications of various suppliers also include Aquatic Chronic 2. These properties indicate a potential hazard to the aquatic environment.

The use of azoles is associated with resistant fungal species in humans and the environment (Assress et al., 2021). Some benzotriazole derivatives have antifungal, antimicrobial and antiviral activity (Briguglio et al., 2015), but it is not clear whether environmental emissions of benzotriazoles also induce azole resistance. This is not further investigated in this study.

1.2 Study incentive

Questions have been raised by the Dutch national working group on Emerging Substances ('werkgroep Aanpak Opkomende Stoffen'[2])

[1] Persistent, Bioaccumulative and Toxic. Besides substances evaluated for PBT the list also includes substances evaluated for other properties, such as PMT.

[2] The working group investigates the harmfulness of unknown substances. The working group uses data on occurrence, use and toxicity to find groups of substances that can pose a threat to water quality. For these

whether the use of benzotriazoles leads to risks for the aquatic environment. Rijkswaterstaat (the Dutch executive organization of the Ministry of Infrastructure and Water Management) carried out an inventory on additives used in industrial cooling systems over 2014-2017, which showed the widespread use of benzotriazoles (Berbee & Rutten, 2022). The blowdown of these systems is discharged into national waters, and potentially also into regional waters. From the inventory it appeared that large quantities of nitrogen-containing corrosion inhibitors are used in cooling systems, with an estimated maximum yearly discharge of 130 tonnes year^{-1} into Dutch national surface waters (Dutch: Rijkswateren). Recent evaluation of monitoring data showed nationwide presence of benzotriazoles in surface water, including drinking water intake points (Harmsma, 2022; Osté et al., 2022). In the context of the policy on emerging substances, the consultancy agent Arcadis was commissioned to make an inventory of the state of knowledge of the characteristics and use of the various benzotriazoles, emission sources and management options (Harmsma, 2023). In parallel, RIVM performed present study on (eco)toxicological risk limits of benzotriazoles in surface water.

For benzotriazole (CAS no. 95-14-7) indicative Environmental Quality Standards (i-EQS) for freshwater and saltwater were derived in the Netherlands in 2014.[3] For other benzotriazoles no EQS are available. In order to assess the potential impact of benzotriazoles on surface water quality, the Dutch ministry of Infrastructure and Water Management (I&W) commissioned RIVM to propose (updated) risk limits for benzotriazole and its derivatives in surface water.

1.3 Scope of the report

In this report it is investigated whether (indicative) EQS can be proposed for benzotriazole and its derivatives. At present, ECHA lists a total of 313 compounds with a benzotriazole structure[4]. To limit the scope, this report focuses on a small group of benzotriazoles which are considered chemically most similar to benzotriazole and which are potentially relevant for Dutch surface waters. The current i-EQS for benzotriazole is also re-assessed incorporating the most recent knowledge on its ecotoxicity. The selection of compounds for which EQS are proposed is further explained in Chapter 2.

For the ease of reading, we use the terms 'EQS' and 'i-EQS' throughout this report. It should be noted, however, that the values derived in this report are advisory values proposed by RIVM. They have no formal status until approved by the ministry of I&W.

1.4 Methodology for EQS-derivation

The Dutch EQS methodology makes a distinction between indicative and formal EQS ('gedegen' in Dutch). Indicative EQS are derived using data from predefined databases, such as the US EPA Ecotox Knowledgebase

substances, the working group collects information about harmfulness, information about sources and the group advises on a possible approach to tackle the potential threats.

[3] https://rvs.rivm.nl/sites/default/files/2018-05/Benzotriazole.pdf.

[4] Based on a search query with 'benzotriazole', d.d. 05-06-2023.

and ECHA's dissemination of REACH registration data. Data are generally used without consulting underlying literature, although limited evaluation may take place in case of e.g., conflicting results. The formal EQS derivation follows the European technical guidance for derivation of environmental quality standards under the Water Framework Directive (WFD) including collection and evaluation of all available literature (EC, 2018).

For both benzotriazole and a group of four benzotriazoles containing a methyl-group (further referred to as methylbenzotriazoles), it is known that other countries derived EQS according to the WFD-methodology. From a scientific point of view it is considered appropriate to take account of official WFD-EQS derived by a recognised international institute, and use these derivations as a basis for the current assessment. Therefore, the data and outcomes of the available EQS derivations were used as a starting point to propose updated EQS in this report. To that end, the methodologies used in the underlying study reports were assessed for consistency with both the current WFD methodology and the RIVM methodology for indicative EQS[5].

For the other benzotriazole derivatives selected (see Chapter 2), the RIVM methodology to derive indicative EQS was followed. Ecotoxicity data were collected from the available REACH-registration dossiers and from the US EPA ECOTOX Knowledgebase (ECHA, 2023b,c,d,e,f,g,h; US EPA, 2023). All data were collected between July and August 2023, and in April 2024 the databases were accessed once more to check for new data.

We noted that in existing EQS derivations for benzotriazole and methylbenzotriazoles data gaps were identified for fish toxicity, resulting in the use of higher assessment factors. Therefore, a limited, non-systematic literature search was performed in Google Scholar to find data on fish that would potentially allow for lowering the assessment factor[6]. The authors are aware that not all available, potentially relevant literature may have been found in this literature search. However, based on the collated data it is possible to determine whether or not fish toxicity would be critical for EQS derivation (i.e., lower effect concentrations than the lowest values already available). For benzotriazole and methylbenzotriazoles it is also discussed if the EQS, as proposed by RIVM in this report, may be considered as 'formal' EQS, or as indicative EQS (i-EQS). Further details can be found in the respective chapters.

1.5 Reliability assessment ecotoxicity data

For benzotriazole and methylbenzotriazoles, all retrieved fish studies were assessed for reliability with the CRED (Criteria for reporting and evaluating ecotoxicity data) evaluation method (Moermond et al., 2016) in mind, to determine whether abovementioned adjustment of the assessment factor was appropriate. For other taxa, which were already represented in the dataset, new acute and chronic data were only

[5] https://rvs.rivm.nl/onderwerpen/normen/milieu/handleiding-normafleiding.

[6] The search was performed August 2023 using the term 'benzotriazole'.

assessed if effect concentrations were lower than the previous reported ones.

The CRED method helps to evaluate aquatic ecotoxicity data in a reproducible, transparent, and consistent way. A reliability score is assigned to each ecotoxicity study (or alternatively each endpoint in case of multiple endpoints and differing scores). The potential outcomes are R1 (reliable without restrictions), R2 (reliable with restrictions), R3 (not reliable) and R4 (not assignable). According to the methodology of WFD only reliable (and relevant) results should be considered in setting EQS (thus studies with a score R1/R2). However, ecotoxicity studies found in scientific literature or via databases (for example REACH) often lack sufficient details to thoroughly assess the reliability, resulting in reliability scores R4. This could lead to exclusion of many, potentially reliable, studies.

From earlier EQS-derivations it is known that often the same details are missing in different studies. Some of these details are not substance-specific (for example whether control validity was met in the experiment, or whether reported test concentrations are nominal/measured concentrations), while some information impact reliability on a substance-specific basis (for example water renewal, which is more important for a readily biodegradable compound than a stable compound). Especially for the details such as reporting nominal/measured concentrations it can be arguable whether the lack of such information should lead to assigning a reliability score R2 or R4. Therefore, the reliability score was assigned based on expert judgement. When necessary, authors were contacted for additional information to support reliability scoring. For each (i-)EQS-derivation an appendix is available with reported endpoints, and brief explanations on assigned reliability scores are given for relevant studies.

1.6 Reading guide

In Chapter 2 the selection of benzotriazole derivatives is presented for which (i-)EQS are derived. In Chapters 3 to 6 (i-)EQS are proposed for the selected benzotriazole derivatives, with the corresponding appendices (Appendix 3 to 6) for complete overviews of the ecotoxicity data and assessments of reliability. In Chapter 7 the conclusions are reported, including an overview of all proposed (i-)EQS.

2 Selection of benzotriazole derivatives

2.1 Approach

As many benzotriazole derivates exist, it was decided to prioritise individual compounds and select those that might be expected to have a similar mode of action, thereby contributing to mixture toxicity. Mixture toxicity is relevant because compounds with similar modes of action may meet their individual EQS while posing a cumulative risk when present in combination.

One of the options for grouping of chemicals is the similar property principle, according to which structural similar chemicals are likely to have similar effects. To that end, a group of benzotriazoles was analysed on structural similarity using a predefined methodology (Extended Fingerprint with Jaccard-Tanimoto coefficient). Similarity scores reflect the extend of similarity; a similarity score of 0 indicates no similarity and a similarity score of 1 indicates that two chemicals are identical. While for specific groups of chemicals similarity threshold scores have been derived to consider chemicals structurally similar (Wassenaar et al., 2022), currently no uniform threshold score is available to compare benzotriazoles. Derivation of a threshold score was beyond the scope of the EQS-derivation, nevertheless the methodology can be used to identify potentially similar benzotriazoles. Here we used an indicative threshold value of half the maximum score (≥0.50) to identify benzotriazoles 'similar' to benzotriazole (CAS no. 95-14-7).

2.2 Overview selected benzotriazoles

To collect an initial list of benzotriazoles to assess, the search function of the ECHA website was used (ECHA, 2023h). Only registered substances were selected for the assessment since these represent benzotriazoles with higher production or import volume[7]. As the SMILES codes are needed as input for the similarity model, all compounds for which a SMILES code could be retrieved were assessed.

From the total ECHA of 313 compounds with a benzotriazole structure listed in ECHA's database, only 39 (12.4%) have a registration under REACH (see Appendix 1). For 26 compounds the SMILES could be retrieved, allowing to calculate similarity scores with the method described in section 2.1. Compounds which could not be assessed where mainly reaction products and mixtures of benzotriazole derivatives. In Appendix 2 all similarity scores are given. In total eight compounds, including benzotriazole, were selected based on the similarity score of ≥0.50 (see Table 2.1).

[7] If substances are not registered within REACH, this means that compounds are manufactured or imported in the European Economic Area (EEA) in less than 1 tonne per year per registrant.

Table 2.1 Benzotriazoles with a similarity score of ≥0.50 and for which (indicative) EQS are derived.

No	Compound	CAS no.
1	Benzotriazole (=1H-Benzotriazole)	95-14-7
2	Sodium 1H-benzotriazolide (=1H-Benzotriazole, sodium salt)	15217-42-2
3	Methyl-1H-benzotriazole (=Tolyltriazole)	29385-43-1
4	4-Methyl-1H-benzotriazole	29878-31-7
5	5-Methyl-1H-benzotriazole (=6-Methylbenzotriazole)	136-85-6
6	Sodium 4(or 5)-methyl-1H-benzotriazolide (=Sodium tolyltriazolide)	64665-57-2
7	1-Hydroxybenzotriazole	2592-95-2
8	Sodium 5-N-butylbenzotriazole[a]	118685-34-0

a: it should be noted that the chemical name, as used in the REACH registration dossier, incorrectly indicates that the butyl-group is linked to the nitrogen atom of the triazole. The butyl-group is linked to the benzene ring (5-Butyl-1H-benzotriazole sodium salt). Throughout this report we use the registered name.

The parallel study of Arcadis focused on many of the same benzotriazole derivatives as listed above (no. 1, 2, 3, 4, 6 and 7) (Harmsma, 2023). In addition, Arcadis listed two other compounds (5,6-Dimethyl-1H-benzotriazole; CAS no. 4184-79-6 and 2-Methylbenzotriazole; CAS no. 16584-00-2) and a mixture of benzotriazoles (Chloroalkylbenzotriazoles[8]; CAS no. 202420-04-0). These benzotriazoles are not further discussed in this report since they are not registered under REACH (ECHA, 2023h).

It is noted that 4-methyl-1H-benzotriazole (CAS no. 29878-31-7) does not have an individual REACH registration either. The compound is included because the registered substance methyl-1H-benzotriazole (tolyltriazole; CAS no. 29385-43-1) is a mixture of 4-methyl-1H-benzotriazole and 5-methyl-1H-benzotriazole (CAS no. 136-85-6).

Note that in the literature and databases, different terms are used to indicate the same benzotriazole compound or benzotriazole mixture. This may cause confusion regarding which compound is discussed. Therefore, to avoid unclarities, we added CAS numbers where needed.

2.3 Chemical identity and classification and substance properties

Table 2.2 shows the relevant chemical identifiers of the selected benzotriazoles, Table 2.3 shows the relevant physico-chemical properties. The authors are not aware that the selected benzotriazoles exhibit a specific mode of action in terms of ecotoxicity, however as mentioned before some benzotriazoles are known to act as antifungal, antimicrobial and/or antiviral agent.

[8] 1H-Benzotriazole, C-chloro-C-methyl-, sodium salt.

Table 2.2 Chemical identity, classification and regulatory status of the selected benzotriazoles for which EQS are derived in the present study. All information was retrieved from the REACH registration dossiers (ECHA, 2023b,c,d,e,f,g,h).

	benzotriazole		methyl				hydroxy	other
Property	Benzotriazole	Sodium 1H-benzo-triazolide	Methyl-1H-benzotriazole	4-Methyl-1H-benzotriazole	5-Methyl-1H-benzotriazole =6-Methyl-benzotriazole	Sodium 4(or 5)-methyl-1H-benzo-triazolide	1-Hydroxy-benzotriazole	Sodium 5-N-butylbenzo-triazole
CAS number	95-14-7	15217-42-2	29385-43-1	29878-31-7	136-85-6	64665-57-2	2592-95-2	118685-34-0
EC number	202-394-1	239-296-6	249-596-6	249-921-1	205-265-8	265-004-9	219-989-7	404-450-2
Molecular formula	$C_6H_5N_3$	$C_6H_4N_3Na$	$C_7H_7N_3$	$C_7H_7N_3$	$C_7H_7N_3$	$C_7H_6N_3Na$	$C_6H_5N_3O$	$C_{10}H_{12}N_3Na$
SMILES	C1=CC2=NNN=C2C=C1	C1=CC=C2C(=C1)[N-]N=N2.[Na+]		CC1=CC=CC2=NNN=C12	CC1=CC2=NNN=C2C=C1	CC1=CC2[N-]N=NC2=C=C1.[Na+]	C1=CC=C2C(=C1)N=NN2O	CCCCC1=CC2=C(C=C1)[N-]N=N2.[Na+]
Type of constituent	Mono	Mono	Multi[c]	Mono	Mono	Multi	Mono	Mono
Molecular weight	119.13	141.11	133.15	133.15	133.15	155.14	135.15	197.22
Notified CLP[a]			Repro 2 H361d			Repro 2 H361d		
REACH assessment	PMT[b], ED[b]		PMT[b]					
Tonnage band	>1000-<10000	>100-<1000	>1000-<10000	[d]	>1-<10	>1000-<10000	>10-<100	>10

a: only those classifications that are relevant for EQS derivation, see the REACH registration dossiers for all classifications.
b: PMT – Persistent, Mobile and Toxic. ED – Endocrine Disrupting.
c: the REACH registration for methyl-1H-benzotriazole with CAS no. 29385-43-1 refers to a mixture of 4- and 5-methylbenzotriazole.
d: not individually registered but part of methyl-1H-benzotriazole with CAS no. 29385-43-1.

Table 2.3 Physico-chemical properties of the selected benzotriazoles for which EQS are derived in the present study. All data were retrieved from the REACH Registration dossiers (ECHA, 2023b,c,d,e,f,g,h), except for the molecular weights and BCF values which were retrieved by using EPI Suite (US EPA, 2000-2012).

		benzotriazole		methylbenzotriazole				hydroxy	other
Property	**Source/ Remark**	Benzotriazole	Sodium 1H-benzo-triazolide	Methyl-1H-benzo-triazole[a]	4-Methyl-1H-benzotriazole	5-Methyl-1H-benzotriazole =6-Methyl-benzotriazole	Sodium 4(or 5)-methyl-1H-benzo-triazolide	1-Hydroxy-benzotriazole	Sodium 5-N-butylbenzo-triazole
Physical state (20 °C; 1013 hPa)	ECHA	solid	solid	solid	No REACH registration dossier available	solid	solid	solid	solid
Melting point [°C]	ECHA	100	245	76-87		79.1	230	148-154	170
Boiling point [°C]	ECHA	204	decomp.	decomp.		301-364	decomp.	>300	decomp.
Solubility [$g.L^{-1}$]	ECHA	19.8	>500	4.0		6	625-664	4.2	>500
Vapour pressure [Pa]	ECHA	10	0.001	14		0.001	0.001	0.009	0.016
Dissociation constant pK_a	ChemAxon acidic	8.32	8.32	8.79		8.85	8.85	6.89[b]	8.81
	ChemAxon basic	0.58	0.58	1.05		0.78	0.78	-0.47	0.67
Octanol/water partition coefficient log K_{ow}	ECHA	1.34	0.48	1.08		-	1.09	0.15	0.65
	EpiSuite (exp.)	1.44	1.44	n.a.		n.a.	n.a.	0.69	n.a.
	EpiSuite (QSAR)	1.17	1.17	1.71		1.71	1.71	0.11	3.19
	ChemAxon neutral	1.3	1.3	1.81		1.81	1.81	0.63	3.15[c]
	logD at pH7	1.29	1.29	1.81		1.81	1.81	0.27	2.76[c]
Bioconcentration factor [$L.kg^{-1}$]	EpiSuite	4.14	4.14	6.28		6.28	6.28	3.1	58.9
Biomagnification factor		1	1	1		1	1	1	1
Ready biodegradable	ECHA	NO	NO	NO		YES[d]	NO	NO	NO
Half-life hydrolysis [d]	ECHA	stable	stable	stable		stable	stable	stable	stable
Half-life water [d]	ECHA	831 (12 °C)	n.a.	n.a.		n.a.	1000 (10 °C)	n.a.	n.a.
Adsorption coefficient log K_{oc}	ECHA	1.89	0.77	2.04		n.a.	2.04	0.55	n.a.
	Epsuite (exp)	1.69	1.69	n.a.		n.a.	n.a.	n.a.	n.a.
	Epsuite (QSAR)	1.80	1.80	1.94		1.94	1.94	1.55	2.76

n.a. = not available
a: methyl-1H-benzotriazole is a mixture of 4-methyl-1H-benzotriazole and 5-methyl-1H-benzotriazole.
b: largely ionised at pH 7.
c: because of the butyl group, the neutral log K_{ow} is much higher than for the other benzotriazoles; the small proportion of ionisation at pH 7 has a larger effect than for example 4- or 5-methyl-1H-benzotriazole.
d: the biodegradability potential is not as expected based on the biodegradability of the other benzotriazoles selected in the present study. EPI Suite estimates that the compound is not ready biodegradable.

2.4 Grouping of benzotriazoles for EQS derivation

2.4.1 Benzotriazole and sodium 1H-benzotriazolide

Sodium 1H-benzotriazolide (CAS no. 15217-42-2) is the sodium salt of benzotriazole, with the sodium group being the only difference between the compounds. When released into the aquatic environment, sodium 1H-benzotriazolide dissociates into sodium and benzotriazole. Benzotriazole itself is also a dissociating compound, but with a pK_a of 8.37 it will primarily be present in the neutral form at environmentally relevant pH values.[9] In the aquatic environment organisms will be exposed to the same (neutral) compound, and thus comparable ecotoxicological effects are expected. Therefore, both compounds are grouped for EQS derivation.

2.4.2 Methylbenzotriazoles

The registered substance methyl-1H-benzotriazole (tolyltriazole; CAS no. 29385-43-1) is a mixture of 4-methyl-1H-benzotriazole (CAS no. 29878-31-7) and 5-methyl-1H-benzotriazole (CAS no. 136-85-6), which makes it reasonable to consider these compounds together for EQS derivation. Similar to what is described above for benzotriazole and sodium 1H-benzotriazolide, dissolving sodium 4(or 5)-methyl-1H-benzotriazolide (sodium tolyltriazolide; CAS no. 64665-57-2) into water will lead to one and the same compound to which aquatic organisms are exposed. Therefore, these four methylated compounds are taken together for EQS derivation, in line with the Swiss EQS derivation for methylbenzotriazole (see section 4.1).

2.4.3 1-Hydroxybenzotriazole and Sodium 5-N-butylbenzotriazole

1-Hydroxybenzotriazole (CAS no. 2592-95-2) and sodium 5-N-butylbenzotriazole (CAS no. 118685-34-0) are distinct compounds for which it is not expected beforehand that their behaviour and ecotoxicity in surface water is comparable to any of the benzotriazoles investigated in this study. Therefore, these compounds are initially considered separately in this report.

[9] It is acknowledged that dissociation impacts the ecotoxicity of a compound, however as most selected benzotriazoles have pK_a >8 the impact of dissociation has not been taken into account when assessing the reliability of individual studies.

3 Benzotriazole and sodium 1H-benzotriazolide

3.1 Overview existing EQS

EQS for surface water for benzotriazole (CAS no. 95-14-7) have been derived by RIVM[10] in 2014 and the Swiss Centre for Applied Ecotoxicology (Oekotoxzentrum, 2015). The background of these EQS is briefly summarised below.

3.1.1 Indicative EQS RIVM

RIVM has derived indicative EQS for benzotriazole for freshwater (fw) and saltwater (sw) in 2014. Separate i-EQS values were derived to cover long-term (i-AA-EQS; 'i-JG-MKN' in Dutch) and short-term (i-MAC-EQS; 'i-MAC-MKN' in Dutch) effects of benzotriazole. The EQS values were derived in line with the at-the-time existing draft version of the former guidance (De Poorter et al., 2015). This means that limited sources were explored for ecotoxicity data, and that endpoints were not assessed for reliability. Data were retrieved by using the OECD toolbox (OECD, 2013) and by collecting data from the REACH registration dossier (ECHA, 2023a)[11]. Food chain effects were not considered in the absence of relevant toxicological classifications.

i-MAC-EQS

The i-MAC-EQS_{fw} was derived by applying an AF on the lowest acute effect concentration. Data were available for the three standard trophic levels, with L(E)C_{50} values of 180 mg L^{-1} for the fish *Danio rerio*, 15.8 mg L^{-1} for the crustacean *Daphnia galeata*[12] and 75 mg L^{-1} for the algae *Raphidocelis subcapitata*[13], all taken from the REACH registration dossier for benzotriazole. The effect concentrations for fish and algae originate from industry studies, the effect concentration for *D. galatea* originates from an open literature study by Seeland et al. (2012) which is used as key-study by the registrant. An AF of 100 was applied to the lowest 48-h EC_{50} of 15.8 mg L^{-1} resulting in an i-MAC-EQS_{fw} of 158 µg L^{-1} (rounded to 160 µg L^{-1}).

For saltwater, the i-MAC-EQS_{sw} was derived by applying an AF of 10 to the i-MAC-EQS_{fw}, resulting in a value of 15.8 µg L^{-1} (rounded to 16 µg L^{-1}).

i-AA-EQS

For long-term effects, data were available for two standard trophic levels, namely algae and crustaceans, and a macrophyte. The lowest chronic effect concentration was a 21-d EC_{10} of 0.97 mg L^{-1} for *D. galeata*, while the lowest effects concentrations for algae and macrophytes were E_rC_{10}-values of 1.18 mg L^{-1} for *Desmodesmus subspicatus* and 3.94 mg L^{-1} for *Lemna minor*. These effect

[10] https://rvs.rivm.nl/sites/default/files/2018-05/Benzotriazole.pdf.

[11] The same registration dossier has been accessed, however in 2014.

[12] The RIVM report with background information (https://rvs.rivm.nl/sites/default/files/2018-05/Benzotriazole.pdf) specifies that the effects were found for *Daphnia magna*. However, based on the information in the REACH registration dossier, the correct species is *Daphnia galeata.*

[13] Former name is *Pseudokirchneriella subcapitata*.

concentrations were taken from the REACH registration dossier and all originate from the aforementioned study by Seeland et al. (2012). Since the acutely most sensitive species was also tested chronically, an assessment factor (AF) of 50 was applied to the most sensitive endpoint to derive an i-AA-EQS$_{fw}$ of 19 µg L^{-1}.

The i-AA-EQS$_{sw}$ was derived by applying an AF of 10 to the i-AA-EQS$_{fw}$, resulting in a value of 1.9 µg L^{-1}.

3.1.2 *EQS Swiss Centre for Applied Ecotoxicology*

The Swiss Centre for Applied Ecotoxicology (Oekotoxzentrum) derived AA-EQS and MAC-EQS for freshwater (Oekotoxzentrum, 2015) in line with the then applicable technical guidance for deriving environmental quality standards under the Water Framework Directive (WFD) (EC, 2011). This implies that ecotoxicity data are gathered from a broad number of sources, including scientific literature, and that all endpoints are assessed for reliability. Solely reliable data were used to derive EQS; non-reliable data were only used as supporting information to decide on the AF. The evaluation by Swiss Centre for Applied Ecotoxicology did not consider EQS for saltwater, secondary poisoning was not deemed relevant in view of the log K_{ow}.

MAC-EQS$_{fw}$

Reliable data were available for algae and crustaceans, with EC$_{50}$-values of 189 mg L^{-1} for *D. subspicatus* from Baumann et al. (2013) and 15.8 mg L^{-1} for *D. galeata* from Seeland et al. (2012). Additional data were available for bacteria and protozoa. For fish various data were found, however none of the relevant results were assessed as reliable (R1) or reliable with restrictions (R2). The reliability of the LC$_{50}$ of 180 mg L^{-1} for *D. rerio* used in the RIVM-evaluation was considered unassignable (R4), as was the E$_r$C$_{50}$ of 75 mg L^{-1} for *R. subcapitata*, as information in the REACH dossier was deemed insufficient. While the guidance indicates that no MAC-EQS$_{fw}$ can be derived if the acute base set is incomplete, the authors derived a MAC-EQS anyway considering that the compilation of data suggested that fish are not the most sensitive taxon. Therefore, an AF of 100[14] was applied to the most sensitive endpoint of 15.8 mg L^{-1} for *D. galeata*, which resulted in an MAC-EQS$_{fw}$ of 158 µg L^{-1}.

AA-EQS$_{fw}$

Reliable data were available for algae and crustaceans. For algae, a chronic NOEC of 4.76 mg L^{-1} for *D. subspicatus* was presented. This is the geometric mean of two NOECs of 1.2 and 18.9 mg L^{-1} from the above mentioned studies by Baumann et al. (2013) and Seeland et al. (2012). The lowest effect concentration for crustaceans was the previously mentioned EC$_{10}$ of 0.97 mg L^{-1} for reproduction of *D. galeata* that was also used by RIVM. Since chronic data for fish were not available, the corresponding AF of 50 was applied to the most sensitive endpoint to derive an AA-EQS$_{fw}$. Based on the 21-d EC$_{10}$ of 0.97 mg L^{-1} for *D. galeata*, this resulted in an AA-EQS$_{fw}$ of 19 µg L^{-1}.

[14] Note that the current EQS-guidance allows for an AF of 10 when at least one short-term L(E)C$_{50}$ is available for each of three trophic levels of the base set (fish, crustaceans and algae), and potentially sensitive species are included in the dataset.

3.1.3 *Comparison of existing EQS*

While the EQS derived by RIVM and Swiss Centre for Applied Ecotoxicology have a different status (indicative and formal) and were derived according to different guidance documents, similar AA- and MAC-EQS_{fw} were derived for benzotriazole by RIVM and Swiss Centre for Applied Ecotoxicology. In both cases *D. galeata* was considered the most sensitive species, with the same endpoints being used to derive the EQS (see Table 3.1). Food chain effects via human fish consumption and secondary poisoning of birds and mammals were considered irrelevant.

Table 3.1 Data used by RIVM and by Swiss Centre for Applied Ecotoxicology (Oekotoxzentrum) to derive EQS, including the obtained (i-)EQS.

Institute	**EQS type**	**Lowest effect concentration [mg L^{-1}]**	**Assessment factor**	**Derived EQS [µg L^{-1}]**
Oekotoxzentrum	MAC-EQS_{fw}	EC_{50} 15.8	100	160/158
RIVM	i-MAC-EQS_{sw}		1000	16
Oekotoxzentrum	AA-EQS_{fw}	EC_{10} 0.97	50	19
RIVM	i-AA-EQS_{sw}		500	1.9

a: Note that the RIVM report mistakenly indicates that *Daphnia magna is the most sensitive species,* while the original data source indicates that *Daphnia galeata was used as test species*.

3.2 Collection of existing and new ecotoxicity data

Since a formal EQS from a recognised international institute is available for benzotriazole, we used the data from the Swiss report as a starting point for the new EQS proposal. An additional data search was performed to retrieve relevant data published over the last years[15], especially focussing on filling the data-gap for fish (see section 3.1). In addition, data were collected for sodium 1H-benzotriazolide.

In the US EPA Ecotoxicology Knowledgebase (ECOTOX) no (new) data were available (US EPA, 2023). In the REACH registration dossiers (ECHA, 2023a,d) and in the RAC-opinion proposing a harmonised classification and labelling of benzotriazole as Aquatic Chronic 2, H411 (ECHA, 2022a) additional studies with algae and fish were found. In scientific literature both acute and chronic data were found. A complete overview of all data relevant for EQS derivation is given in Appendix 3, and the potentially critical acute and chronic data are discussed in more detail. A summary of the effect concentrations relevant for EQS derivation is presented in the next section.

3.3 Summary of critical ecotoxicity data

Table 3.2 and 3.3 summarise the relevant acute and chronic ecotoxicity data for benzotriazole, showing the lowest effect concentration per taxonomic group based on the dataset from the Swiss EQS report and additional literature. The REACH registration dossiers for benzotriazole and sodium 1H-benzotriazolide refer to the existing acute EC_{50} and chronic EC_{10} for *Daphnia galatea* of 15.8 and 0.97 mg L^{-1} as key values for derivation of the PNEC for intermittent release and chronic exposure, respectively. A lower reliable acute E_rC_{50} of 8.3 mg L^{-1} for growth rate of

[15] The latest data search of Swiss Centre for Applied Ecotoxicology was performed on 04-12-2015.

the algal species *Chlorella sorokiniana* was retrieved from the open literature.

One acute LC_{50} for the fish species *Danio rerio* was considered reliable with restrictions, which means that the acute base set is complete. Also the chronic base set is complete; reliable chronic NOEC for *D. rerio* and *Oryzias latipes* were found in the open literature. The data do not indicate that fish are more sensitive than algae and crustacea.

3.4 Proposal for updated environmental quality standards

3.4.1 Treatment of freshwater and marine data

According to the EQS-guidance, datasets for freshwater and marine species are pooled for organic compounds unless there is evidence for differences in sensitivity between both groups and/or environmental behaviour of the compound differs between both environments. It is not expected that the environmental behaviour of benzotriazole is influenced by differences in salinity. Although not discussed in detail, the consulted literature includes some data for marine species, among which *Vibrio fischeri* (bacteria), *Americamysis bahia* (crustacea) and *Menidia beryllina* (fish). However, these species are not considered to be specific marine species, because their taxonomic position, life form and/or feeding strategy are similar to their freshwater counterparts, in accordance with the WFD Guidance (EC, 2018). Therefore, data for all species are pooled in one single dataset.

3.4.2 Derivation of the MAC-EQS

The MAC-$QS_{fw,eco}$ is determined based on the lowest acute $L(E)C_{50}$ effect concentration available and an assessment factor. The lowest acute effect concentration is an 72-h E_rC_{50} of 8.3 mg L^{-1} for *C. sorokiniana*.

In line with EC (2018) and RIVM (2024a) an AF of 100 is selected by default, which can be lowered to 10 when the specific mode of action of the substance is known and the most sensitive taxonomic group is represented in the dataset, or when for non-specifically acting substances (for example, acting by narcosis only) the interspecies variation in toxicity is low (standard deviation of the log_{10} transformed $L(E)C_{50}$ values is <0.5). The present study focuses on a pragmatic use of existing and additional data in order to select the critical values for EQS derivation and did not aim to collect a complete overview of all available data. This makes it difficult to draw definitive conclusions on the variation between species. However, taking the reliable data together, the level of variation meets the criterion of the EQS-guidance for a reduction of the assessment factor, and adding the data which could not be assessed for reliability (assigned R4) does not change the outcome. In addition, for benzotriazoles no specific mode of action in terms of ecotoxicity is known. Therefore, it is considered appropriate to use an AF of 10.

With the EC_{50} of 8.3 mg L^{-1} and an AF of 10, the proposed MAC-$QS_{fw,\ eco}$ is **0.83 mg L^{-1}** (**830 µg L^{-1}**).

An additional AF of 10 is applied to calculate an MAC-$QS_{sw,\ eco}$ of **0.083 mg L^{-1}** (**83 µg L^{-1}**).

Table 3.2 Overview of lowest reliable acute effect concentrations for benzotriazole for each taxonomic group.

Taxonomic group	**Species**	**Endpoint**	**Parameter**	**Value [mg L^{-1}]**	**Source**
Bacteria	*Vibrio fischeri*[a]	Luminescence	5-min EC_{50}	41.1	Oekotoxzentrum (2015)
Protozoa	*Tetrahymena pyriformis*	Growth	60-h EC_{50}	259	Oekotoxzentrum (2015)
Algae	*Chlorella sorokiniana*	Growth rate	72-h E_rC_{50}	8.3	Gatidou et al. (2019)
Crustacea	*Daphnia galeata*	Immobility	48-h EC_{50}	15.8	Oekotoxzentrum (2015) RIVM (2014)[b]
Pisces	*Danio rerio*	Mortality	96-h LC_{50}	180	ECHA (2022a) / ECHA (2023d)

a: marine species
b: https://rvs.rivm.nl/sites/default/files/2018-05/Benzotriazole.pdf

Table 3.3 Overview of lowest reliable chronic effect concentrations for benzotriazole for each taxonomic group.

Taxonomic group	**Species**	**Endpoint**	**Parameter**	**Value [mg L^{-1}]**	**Source**
Algae	*Raphidocelis subcapitata*	Growth rate	72-h E_rC_{10}	10.5	ECHA (2022a) ECHA (2023a) Oekotoxzentrum (2015)
Crustacea	*Daphnia galeata*	Reproduction	21-d EC_{10}	0.97	Oekotoxzentrum (2015) RIVM (2014)[a]
Fish	*Danio rerio*	Post-hatch survival	35-dpf NOEC	1.07	ECHA (2022a) ECHA (2023a)
Plants	*Lemna minor*	Growth	7-d E_rC_{10}	7.4	Newly derived, based on Seeland et al. (2012)

a: https://rvs.rivm.nl/sites/default/files/2018-05/Benzotriazole.pdf

3.4.3 Derivation of the AA-EQS

For derivation of the AA-EQS, three routes have to be considered: direct ecotoxicity, secondary poisoning and human fish consumption. Inclusion of the latter two depends on whether bioaccumulation is expected and/or there is a relevant CLH classification for human-toxicological effects. The log K_{ow} of benzotriazole is around 1.3-1.4 and lower than the trigger value for deriving the $QS_{water, sec\ pois}$ (log K_{ow} >3). Benzotriazole is assessed under REACH for PMT and ED properties[16]. This implies that benzotriazole may have hazardous properties which may adversely affect humans. Therefore, protecting humans from consuming contaminated seafood products is also relevant and a $QS_{water, hh\ food}$ is derived in section 3.4.3.2.

3.4.3.1 Direct ecotoxicity

The $QS_{fw, eco}$ and $QS_{sw, eco}$ are determined based on the lowest chronic effect concentration available and an assessment factor. The lowest chronic effect concentration is the 21-d EC_{10} value of 0.97 mg L^{-1} for *D. galeata*, also used in the EQS-derivations by RIVM in 2014 and the Swiss in 2015. However, with the addition of the fish studies, reliable chronic data are available for all three standard trophic levels. In line with EC (2018) and RIVM (2024a) an AF of 10 may be applied when data are available for these trophic levels and the potentially most sensitive taxonomic group is included in the chronic dataset.

While the lowest chronic effect concentration was found for the crustacean *D. galeata*, the lowest acute effect concentration was found for the alga *C. sorokiniana* from a newly retrieved study (Gatidou et al., 2019). The E_rC_{50} of 8.3 mg L^{-1} is almost 10 times lower than the lowest E_rC_{50} from the CLH report for benzotriazole (75 mg L^{-1} for *R. subcapitata*), suggesting that *C. sorokiniana* is more sensitive than the standard algae species.
For *C. sorokiniana* no valid chronic endpoint is provided in the study report. In the supporting information of the study it can be seen that <5% inhibition occurred at 0.2 mg L^{-1}, and ~12% inhibition at 2 mg L^{-1}, which may indicate that the EC_{10} for *D. galeata* of 0.97 mg L^{-1} is protective for effects on algal growth rate. An attempt to contact the authors for additional data failed, therefore it cannot be concluded with certainty that algae are less sensitive than invertebrates.

However, since the acute data indicate that variation in sensitivity between species is generally low, it is considered appropriate to use an AF of 10 to derive a $QS_{fw, eco}$.

With the EC_{10} of 0.97 mg L^{-1} and an AF of 10, the proposed $QS_{fw, eco}$ is **0.097 mg L^{-1}** (**97 µg L^{-1}**).

The QS for saltwater is derived with an additional AF of 10 resulting in a $QS_{sw, eco}$ of **0.0097 mg L^{-1}** (**9.7 µg L^{-1}**).

3.4.3.2 Indirect exposure of humans

To determine whether an EQS based on food consumption of fish and shellfish would be lower than an EQS based on protecting freshwater

[16] PACT - Public Activities Coordination Tool - ECHA.

organisms ($QS_{fw, eco}$, see section above), a tentative calculation is made based on the indicative health-based limit value of 0.1 mg kg^{-1} body weight day^{-1} used by RIVM for derivation of a drinking water limit (van Leerdam et al., 2018). Using an allocation factor of 0.2, a fish and shellfish consumption of 115 gram day^{-1} and a standard body weight of 70 kg (RIVM, 2024a), the $QS_{hh\ food}$ is calculated as 12.2 mg kg^{-1} food. Using the estimated BCF value of 4.14 L kg^{-1} (see Table 2.3), the corresponding concentration in surface water would be **2.94 mg L^{-1}**, which is higher than the proposed QS_{eco}. As a high assessment factor was applied to derive the indicative health-based limit value, it is not expected that generated additional human health data would lead to lower $QS_{hh,food}$, and therefore it is concluded that the EQS based on direct ecotoxicity is protective for human fish consumption.

3.5 Conclusion benzotriazole/sodium 1H-benzotriazolide

Below an overview of the proposed EQS for benzotriazole and sodium 1H-benzotriazolide is given. When both compounds are emitted, the EQS applies to the sum, expressed as benzotriazole. The EQS values refer to dissolved concentrations of benzotriazole, but are also valid for the total concentration in non-filtered samples. Direct ecotoxicity is the critical route for derivation of the AA-EQS.

Because an additional data search was performed, and critical studies have been evaluated for reliability, it is proposed to consider these values as 'formal'.

Table 3.4 Proposed EQS for the sum of benzotriazole and sodium 1H-benzotriazolide. All values in µg L^{-1} expressed as benzotriazole, for dissolved and total concentrations.

Freshwater [µg L^{-1}]		**Saltwater [µg L^{-1}]**	
AA-EQS_{fw}	**MAC-EQS_{fw}**	**AA-EQS_{sw}**	**MAC-EQS_{sw}**
97	830	9.7	83

4 Methylbenzotriazoles

4.1 Overview available EQS

Water quality standards for the group of methylated benzotriazoles have not been set in the Netherlands. The Swiss Centre for Applied Ecotoxicology (Oekotoxzentrum, 2016) derived EQS for surface water for a group of methylbenzotriazoles in line with the then applicable WFD-guidance (EC, 2011). This concerns the same methylbenzotriazoles that were selected for EQS derivation in the present study (see section 2.2), namely:

- Methyl-1H-benzotriazole (CAS no. 29385-43-1)
- 4-Methyl-1H-benzotriazole (CAS no. 29878-31-7)
- 5-Methyl-1H benzotriazole (=6-Methylbenzotriazole; CAS no. 136-85-6)
- Sodium 4(or 5)-methyl-1H-benzotriazolide (sodiumtolyltriazole; CAS no. 64665-57-2)

The Swiss derivation is briefly summarised below. Note that the evaluation by Swiss Centre for Applied Ecotoxicology did not consider EQS values for saltwater, secondary poisoning was not considered relevant in view of the log K_{ow}.

MAC-EQS$_{fw}$
Reliable data were available for the basic three trophic levels, namely algae, crustaceans and fish. Additional data were found for bacteria and flatworms. The lowest effect concentration was found for the marine bacterial species *Vibrio fischeri* exposed to 5-methyl-1H-benzotriazole. The 15-min EC_{50} of 4.25 mg L^{-1} was around two-fold lower than the second lowest EC_{50} of 8.58 mg L^{-1} for crustacean *D. galeata* (also exposed to 5-methyl-1H-benzotriazole). Lowest L(E)C_{50} for algae, fish and flatworms were 57.5, 53, and 74.0 mg L^{-1}, respectively. The sensitivity of freshwater bacterial species to methylbenzotriazoles could not be assessed due to a lack of reliable data. Therefore, the EC_{50} for *V. fischeri* was used to derive the MAC-EQS$_{fw}$, even though the marine species was not considered relevant to determine freshwater ecotoxicity. It should be noted, however, that for organic chemicals, the EQS-guidance prescribes that freshwater and marine species are to be combined into one dataset. Using the data for *V. fischeri* to derive the MAC-EQS$_{fw}$ is therefore in line with current procedures (EC, 2018).

As data were available for three basic trophic levels and the standard deviation of the log_{10} transformed (reliable) L(E)C_{50} values was <0.5, an AF of 10 was applied to the EC_{50} of 4.25 mg L^{-1}, resulting in a MAC-EQS$_{fw}$ of 0.425 mg L^{-1} (425 µg L^{-1}).

AA-EQS$_{fw}$
Reliable chronic data were available for two basic trophic levels, namely algae and crustaceans. For algae, a NOEC of 4.2 mg L^{-1} for *D. subspicatus* was used, which is the geometric mean of two NOECs of 2.5 and 7.5 mg L^{-1} from Baumann et al. (2013) and Seeland et al. (2012). For the crustacean *D. galeata* an 21-d NOEC of 1 mg L^{-1} from

Seeland et al. (2012) was used. It was noted that the corresponding EC_{10} value of 0.4 mg L^{-1} for *D. galeata* is lower, but this value was considered less reliable by the Swiss, because effects were only seen in the highest concentration tested. As reliable data for fish were missing, the AA-EQS_{fw} was derived by applying an AF of 50 to the lowest chronic value. Based on the 21-d EC_{10} of 1 mg L^{-1} for *D. galeata*, an AA-EQS_{fw} of 0.02 mg L^{-1} (20 µg L^{-1}) was derived.

4.2 Collection of existing and new ecotoxicity data

Since a formal EQS from a recognised international institute is available for the (same) group of methylbenzotriazoles, we used the data from the Swiss report as a starting point for the new EQS derivation. An additional data search was performed to retrieve relevant data published over the last years, especially focussing on filling the data-gap for chronic toxicity to fish.

From the US EPA Ecotox Knowledgebase (US EPA, 2021) no new data (data generated during or after 2016[17]) were retrieved on any of the four methylbenzotriazoles. In the REACH registration dossiers (ECHA, 2023b,c,g) and in the RAC-opinion proposing a harmonised classification and labelling of methyl-1H-benzotriazole (ECHA, 2022b), new studies with algae, crustaceans and fish were found, also studies from before 2016. Data from the registration dossiers were not taken into account in the EQS derivation by Oekotoxzentrum (2016), and therefore added in the present study. Recent ecotoxicity data were also found in the open literature. In Appendix 4, a complete overview of data relevant for EQS derivation is given, and the potentially critical acute and chronic data are discussed in more detail. A summary of the effect concentrations relevant for EQS derivation is presented in the next section.

4.3 Summary of critical ecotoxicity data

Table 4.1 and 4.2 summarise the relevant acute and chronic ecotoxicity data for methylbenzotriazoles, showing the lowest value per taxonomic group based on the dataset from the Swiss EQS report and additional literature. The REACH registration dossier for methyl-1H-benzotriazole considers the EC_{50} of 8.58 mg L^{-1} for *D. galeata* from Seeland et al., (2012) as acute key value, and the EC_{10} of 0.4 mg L^{-1} from the same study considered as key value for chronic toxicity.

In Table 4.1 a geometric mean acute effect concentration for *V. fischeri* is presented. In the Swiss derivation the lowest EC_{50} for *V. fischeri* was used (Oekotoxzentrum, 2016). The values for the other taxa are similar.

[17] The latest data search of Swiss Centre for Applied Ecotoxicology was performed on 17-08-2016.

Table 4.1 Overview of lowest acute effect concentrations for the group of methylbenzotriazoles for each taxonomic group.

Taxonomic group	**Species**	**Endpoint**	**Parameter**	**Value [mg L^{-1}]**	**Source**
Bacteria	*Vibrio fischeri*[a]	Luminescence	15-min EC_{50}	7.67[b]	-
Algae	*Desmodesmus subspicatus*	Growth rate	72-h E_rC_{50}	58	Oekotoxzentrum (2016)
Crustacea	*Daphnia galeata*	Immobility	48-h EC_{50}	8.58	Oekotoxzentrum (2016)
Platyhelminthes	*Dugesia japonica*	Mortality	96-h EC_{50}	74	Oekotoxzentrum (2016)

a: marine species
b: geometric mean value

Table 4.2 Overview of lowest chronic effect concentrations for the group of methylbenzotriazoles for each taxonomic group.

Taxonomic group	**Species**	**Endpoint**	**Parameter**	**Value [mg L^{-1}]**	**Source**
Algae	*Desmodesmus subspicatus*	Growth rate	72-h NOEC	7.0	Baumann et al. (2013) ECHA (2022b) Oekotoxzentrum (2016)
Crustacea	*Daphnia galeata*	Reproduction	21-d EC_{10}	0.40	Oekotoxzentrum (2016) / Seeland et al. (2012)
Macrophyta	*Lemna minor*	Growth rate	7-d E_rC_{10}	11.0	Newly derived, based on Seeland et al. (2012)

For chronic toxicity the lowest NOEC for algae from Baumann et al. (2013) is presented, instead of the calculated geometric mean value by the Swiss. In addition, for crustacea we use the lowest EC_{10} from the same study as the NOEC used by the Swiss, as we consider the endpoint more relevant, and we add a reliable endpoint for macrophyta. Similar to the Swiss derivation, no reliable chronic endpoint could be obtained for fish.

4.4 Proposal for environmental quality standards

4.4.1 Treatment of freshwater and marine data

As explained previously for benzotriazole (see section 3.4.1), data for organic chemicals are pooled unless there is evidence for differences in sensitivity between freshwater and marine species or differences in environmental fate. The available data do not indicate that this is the case, and datasets are pooled.

4.4.2 Derivation of the MAC-EQS

Reliable data are available for bacteria, algae, crustaceans, and for a flatworm. In the absence of reliable acute data for fish, derivation of the MAC-$QS_{fw,\ eco}$ is in principle not possible. The WFD-guidance gives the option to use non-testing methods[18], provided that these are not critical. Based on the information for other benzotriazoles and taking into account the results of the R4 fish studies for methylbenzotriazoles (11 studies, with LC_{50} ranging 14.6-128 mg L^{-1}), there is some confidence that an EQS based on data for algae and crustacea will be protective for fish as well. Therefore, we propose to derive the MAC-$QS_{fw,\ eco}$ with an AF of 100 on the lowest acute effect concentration, which is the geometric mean 15-min EC_{50} of 7.67 mg L^{-1} for *Vibrio fischeri*. The resulting MAC-$QS_{fw,\ eco}$ of **76.7 µg L^{-1}** (rounded to **77 µg L^{-1}**) is about a factor of 10 higher than the corresponding AA-$QS_{fw,eco}$ (8 µg L^{-1}, see below); This difference is also consistent with benzotriazole. An additional AF of 10 is applied to calculate an MAC-$QS_{sw,\ eco}$ of **7.7 µg L^{-1}**.

4.4.3 Derivation of the AA-EQS

The log K_{ow} of the methylbenzotriazoles do not raise concerns about bioaccumulation, and derivation of an $QS_{water,\ sec\ pois}$ is not triggered. Food chain effects are not considered in the absence of relevant toxicological classifications. Therefore, only direct ecotoxicity is assessed.

4.4.3.1 Direct ecotoxicity

The QS_{eco} is determined based on the lowest chronic effect concentrtion and an assessment factor. The lowest chronic effect concentrations is the 21-d EC_{10} of 0.40 mg L^{-1} for *Daphnia galeata*. In the absence of reliable data for fish, an assessment factor of 50 is applied.

With the EC_{10} of 0.40 mg L^{-1} and an AF of 50, the proposed $QS_{fw,eco}$ is **0.008 mg L^{-1}** (**8 µg L^{-1}**).

An additional AF of 10 is applied to calculate an $QS_{sw,eco}$ of **0.0008 mg L^{-1}** (**0.8 µg L^{-1}**).

[18] e.g. read-across or quantitative structure–activity relationships (QSARs).

4.5 Conclusion methylbenzotriazoles

Below an overview of the proposed EQS for a group of methylbenzotriazoles are given. The EQS values refer to the sum of different methylated benzotriazoles. They are presented in µg L^{-1} as dissolved concentrations, but are also valid for total concentration in non-filtered samples. It is proposed to consider the values as 'formal' EQS.

It is noted that the EQS is influenced by the absence of data for fish, which leads to a higher assessment factor. In this way the EQS may reflect data gaps rather than 'true' toxicity. The issue of data gaps in relation to assessment factors and mixture assessment is further discussed in Chapter 7.

Table 4.3 Proposed EQS for the sum of methylbenzotriazoles[a]. All values in µg L^{-1}, for dissolved and total concentrations.

Freshwater [µg L^{-1}]		**Saltwater [µg L^{-1}]**	
AA-EQS$_{fw}$	**MAC-EQS$_{fw}$**	**AA-EQS$_{sw}$**	**MAC-EQS$_{sw}$**
8	77	0.8	7.7

a: 4-methyl-1H-benzotriazole (tolyltriazole) (CAS no. 29878-31-7);
5-methylbenzotriazole (6-methylbenzotriazole) (CAS no. 136-85-6);
Methyl-1H-benzotriazole (CAS no. 29385-43-1);
Sodium 4(or 5)-methyl-1H-benzotriazolide (sodiumtolyltriazole) (CAS no. 64665-57-2)

5 1-hydroxybenzotriazole

5.1 Introduction

The authors are not aware of any existing EQS for 1-hydroxy-benzotriazole (CAS no. 2592-95-2).

The chemical structure of 1-hydroxybenzotriazole is almost similar to benzotriazole, the difference is that 1-hydroxybenzotriazole has an alcohol (OH-group) bound to the triazole group. The physico-chemical and biological properties of both compounds are also quite similar (see section 2.3), however it is noted that 1-hydroxybenzotriazole biodegrades quite well in a screening test, while benzotriazole hardly biodegrades. In practice this could imply that similar emissions would result into reduced exposure to 1-hydroxybenzotriazole as compared to benzotriazole. It is also noted that the pK_a of 1-hydroxybenzotriazole is around 6.89 and that the compound is largely ionised at environmentally relevant pH. Moreover, due to limitations in the ecotoxicity dataset (see section 5.2) it is not possible to judge whether the data and derived EQS for benzotriazole are representative for 1-hydroxytriazole. Therefore data for 1-hydroxybenzotriazole are presented below, and these data are used to derive EQS. In the concluding section the EQS for 1-hydroxybenzotriazole and benzotriazole are compared.

5.2 Summary of critical ecotoxicity data

Acute data for algae, crustacea and fish were retrieved (see Table 5.1 and Appendix 5). For the alga *Desmodesmus subspicatus* a 72-h E_rC_{50} of 87.3 mg L^{-1} from an OECD TG 201 study is available. For the crusteacean *Daphnia magna,* a 48-h EC_{50} of 75.5 mg L^{-1} was obtained in an OECD TG 202 study. Lastly in an OECD TG 203 study, a 96-h LC_{50} of >100 mg L^{-1} for *Danio rerio* was reported. No chronic toxicity data were found for 1-hydroxybenzotriazole.

5.3 Proposal for environmental quality standards

In view of the limited data and the absence of chronic effect concentrations, derivation of 'formal' EQS was not deemed possible and indicative values have been derived according to current procedures (i-MAC-QS and i-AA-EQS)[19].

5.3.1 Derivation of the i-MAC-EQS

The i-MAC-$QS_{fw, eco}$ is derived from the lowest acute effect concentration and an assessment factor. The lowest acute effect concentration is a 48-h EC_{50} of 75.5 mg L^{-1} for *Daphnia magna*. Data are also available for algae and fish. In line with the national guidance on indicative EQS derivation (RIVM (2024a) an AF of 100 can be applied, resulting in an i-MAC-$QS_{fw, eco}$ of **0.755 mg L^{-1}** (**755 µg L^{-1}**). An additional AF of 10 is applied to derive the i-MAC-$QS_{sw, eco}$ of **76 µg L^{-1}**.

[19] Handleiding normafleiding | Risico's van stoffen (rivm.nl)

Table 5.1 Overview of the lowest acute effect concentrations for 1-hydroxy-benzotriazole for each taxonomic group.

Taxonomic group	**Species**	**Endpoint**	**Parameter**	**Value [mg L^{-1}]**	**Source**
Algae	*Desmodesmus subspicatus*	Growth rate	72-h E_rC_{50}	87.3	ECHA (2023e)
Crustacea	*Daphnia magna*	Immobility	48-h EC_{50}	75.5	ECHA (2023e)
Fish	*Danio rerio*	Mortality	96-h LC_{50}	>100	ECHA (2023e)

5.3.2 Derivation of the i-AA-EQS

For derivation of the i-AA-EQS, three routes have to be considered: direct ecotoxicity, secondary poisoning and human fish consumption. Inclusion of the latter two depends on whether bioaccumulation is expected and/or there is a classification for human-toxicological effects.

The log K_{ow} of 1-hydroxybenzotriazole is 0.15, which is lower than the trigger value for deriving the $QS_{water, sec\ pois}$ (log K_{ow} >3). There are no specific relevant triggers of concern for the compound (see Table 2.2). Therefore, food chain effects need not be considered, only direct ecotoxicity.

The i-$QS_{fw, eco}$ and i-$QS_{sw, eco}$ are determined based on the lowest chronic or acute effect concentrations available and an assessment factor. As only acute data are available, the same acute value as for the i-MAC-EQS is used (75.5 mg L^{-1}). For derivation of the i-$QS_{fw, eco}$, an assessment factor of 1000 is used, resulting in an i-$QS_{fw, eco}$ of **0.0755 mg L^{-1}** (**76 µg L^{-1}**). The i-QS_{sw} is derived by applying an extra AF of 10 and is **7.6 µg L^{-1}**.

5.4 Conclusion 1-hydroxybenzotriazole

Below, an overview of the proposed indicative EQS values for 1-hydroxybenzotriazole are given. The EQS values refer to dissolved concentrations, but are also valid for total concentration in non-filtered samples. It is proposed to consider the values as indicative EQS.

Table 5.2 Proposed i-EQS for 1-hydroxybenzotriazole in this study. All values in µg L^{-1}, for dissolved and total concentrations.

Freshwater [µg L^{-1}]		**Saltwater [µg L^{-1}]**	
i-AA-EQS_{fw}	**i-MAC-EQS_{fw}**	**i-AA-EQS_{sw}**	**i-MAC-EQS_{sw}**
76	755	7.6	76

It should be noted that the data for benzotriazole (and methylbenzotriazole) show that *D. magna* is less sensitive than *D. galatea*, and *D. rerio* is less sensitive than other fish species. This means that the acute data for 1-hydroxybenzotriazole are likely not representative for the effects of this substance on aquatic organisms. It is not possible to assess whether this is sufficiently compensated for by the AFs used for i-EQS derivation. On the other hand, the i-EQS values for 1-hydroxybenzotriazole are almost similar to the EQS for benzotriazole ((i-)MAC-QS_{fw}: 755 vs 830 µg L^{-1}; (i-)QS_{fw}: 76 vs 97 µg L^{-1}), which may be an indication that the derived i-EQS are sufficiently protective.

6 Sodium 5-N-butylbenzotriazole

6.1 Introduction

For sodium 5-N-butylbenzotriazole (CAS no. 118685-34-0) the authors are not aware of any EQS available.

The chemical structure of sodium 5-N-butylbenzotriazole is related to sodium 1H-benzotriazolide, but the presence of a butylgroup attached to the benzene ring is expected to have a larger influence on the toxicity than the hydroxyl group of 1-hydroxybenzotriazole in the previous chapter. According to EPI Suite estimates, the uptake in organisms is considerably higher than that of benzotriazole (BCF 58.9 vs 4.14 L kg^{-1}). Based on the chemical structure and physico-chemical properties, it may be assumed that uptake of sodium 5-N-butylbenzotriazole from water at environmentally relevant pH is higher than for the other benzotriazoles, which might also lead to higher toxicity. Therefore, data for sodium 5-N-butylbenzotriazole are presented below, and these data are used to derive EQS.

6.2 Overview of ecotoxicity data

Acute toxicity data are available for bacteria, algae, crustacea and fish (See Table 6.1 and Appendix 6). For *Vibrio* fischeri a 15-min EC_{50} of 0.88 mg L^{-1} was reported in a Microtox assay with 5-butylbenzotriazole (5-butyl-1H-benzotriazole). For the crustacean *Ceriodaphnia dubia,* a 48-h LC_{50} of 1.1 mg L^{-1} is available. For the alga *Raphidocelis subcapitata,* a 72-h E_rC_{50} of 5.7 mg L^{-1} was reported in an OECD TG 201 study with sodium 5-N-butylbenzotriazole, and lastly, in an OECD 203 study, a 96-h LC_{50} of 2.8 mg L^{-1} for fish species *Oncorhynchus mykiss* was derived for the salt. Corresponding effect concentrations for the base form 5-butyl-1H-benzotriazole are 5.04 and 2.47 mg L^{-1} for the alga and fish species, respectively.

One chronic effect concentration was found for sodium 5-N-butylbenzotriazole (see Table 6.2). In an OECD 201 study with *Desmodesmus subspicatus* a 72-h NOEC of 0.27 mg L^{-1} was found, which equals to 0.24 mg L^{-1} for the non-salt form 5-butyl-1H-benzotriazole.

Table 6.1 Overview of the lowest acute effect concentrations for sodium 5-N-butylbenzotriazole for each taxonomic group.

Taxonomic group	**Species**	**Endpoint**	**Parameter**	**Value [mg L^{-1}] [a]**	**Source**
Bacteria	*Vibrio fischeri*	Luminescence	15min EC_{50}	0.88	Pillard et al. (2001)
Algae	*Raphidocelis subcapitata*	Growth rate	72h E_rC_{50}	5.04	ECHA (2023f)
Crustacea	*Ceriodaphnia dubia*	Mortality	48h LC_{50}	1.1	Pillard et al. (2001)
Fish	*Oncorhynchus mykiss*	Mortality	96-h LC_{50}	2.47	ECHA (2023f)

a: given as concentration of the base form 5-butyl-1H-benzotriazole.

Table 6.2 Overview of the lowest chronic effect concentrations for sodium 5-N-butylbenzotriazole for each taxonomic group.

Taxonomic group	**Species**	**Endpoint**	**Parameter**	**Value [mg L^{-1}] [a]**	**Source**
Algae	*Desmodesmus subspicatus*	Growth rate	72-h NOE_rC	0.24	ECHA (2023f)

a: given as concentration of the base form 5-butyl-1H-benzotriazole.

6.3 Proposal for indicative environmental quality standards

6.3.1 Derivation of the i-MAC-EQS

The i-MAC-$QS_{fw,eco}$ is determined based on the lowest acute effect concentration available and a safety factor. The lowest acute effect concentration is the 15-min EC_{50} of 0.88 mg L^{-1} for *Vibrio fischeri*. In line with RIVM (2024a) an AF of 100 should be selected to derive the i-MAC-$QS_{fw, eco}$, or alternatively an AF of 10 when there is also data for the most sensitive taxonomic group. The available data for the bacteria, alga, crustaceans and fish demonstrate a low variety in toxicity, however the number of available studies is limited. Based on the limited data it is not clear whether the most sensitive taxonomic group is tested. Therefore, the AF of 100 is considered most appropriate.

With the EC_{50} of 0.88 mg L^{-1} and an AF of 100, the proposed i-MAC-$QS_{fw,eco}$ is **0.0088 mg L^{-1}** (**8.8 µg L^{-1}**).

An additional AF of 10 is applied to calculate an i-MAC-$QS_{sw,eco}$ of **0.00088 mg L^{-1}** (**0.88 µg L^{-1}**).

6.3.2 Derivation of the i-AA-EQS

For derivation of the i-AA-EQS, three routes have to be considered: direct ecotoxicity, secondary poisoning and human fish consumption. Inclusion of the latter two depends on whether bioaccumulation is expected and/or there is a classification for human-toxicological effects.

There are different log K_{ow} estimates available, ranging from 2.76-3.19. As some estimates are higher than 3, derivation of both i-$QS_{water, sec pois}$ and i-$QS_{water, hh food}$ is triggered. In line with RIVM (2024a) only the latter is derived, because it assumed that this EQS is also protective for birds and mammals. Therefore, direct ecotoxicity and indirect exposure of humans are considered for EQS derivation.

6.3.2.1 Direct ecotoxicity

The i-$QS_{fw, eco}$ is determined based on the lowest chronic NOEC/EC_{10} value and a safety factor. The lowest and only chronic value is a 72-h NOE_rC value of 0.24 mg L^{-1} for *R. subcapitata*. In line with RIVM (2024a) an AF of 1000 is selected to calculate an i-$QS_{fw,eco}$.

With the NOE_rC of 0.24 mg L^{-1} and an AF of 1000, the proposed i-$QS_{fw,eco}$ is **0.00024 mg L^{-1}** (**0.24 µg L^{-1}**).

An additional AF of 10 is applied to calculate an i-$QS_{sw,eco}$ of **0.00024 mg L^{-1}** (**0.024 µg L^{-1}**).

6.3.2.2 Indirect exposure of humans

To determine whether EQS based on food consumption of fish and shellfish would be more stringent than EQS based on direct ecotoxicity, an indicative health-based limit value is needed. For (sodium) 5-butyl-1H-benzotriazole such value is currently not available.

A pragmatic approach was taken by collecting surrogate values and selecting the lowest value available. An option is to use the indicative health-based limit value of 0.1 mg kg^{-1} body weight day^{-1} for

benzotriazole as reported in section 3.4.3.2. From the same source (van Leerdam et al., 2018) also a value of 0.05 of mg kg^{-1} body weight day^{-1} is available for the sum of 4-methyl-1H-benzotriazole and 5-methyl-1H-benzotriazole. Another approach is to use the Threshold of Toxicological Concern (TTC). Based on predictions from a QSAR toolbox[20] and ToxTree[21], sodium 5-butyl-1H-benzotriazole is classified as a class III chemical, which equals a threshold value of 1.5 µg kg^{-1} body weight day^{-1}. The REACH registration dossier and the open literature were also screened by the authors. One relevant 28-day oral repeated dose toxicity study with mice was found in the REACH registration dossier, with a NOAEL of 55 mg kg^{-1} body weight day^{-1}. By applying the highest possible assessment factor of 6000[22] an indicative health-based limit value of 9.2 µg kg^{-1} body weight day^{-1} would be derived in line with RIVM (2024b).

The TTC of 1.5 µg kg^{-1} body weight day^{-1} is used as lowest and therefore most stringent value for 5-butyl-1H-benzotriazole. Using an allocation factor of 0.2, a fish and shellfish consumption of 115 g day^{-1} and a standard body weight of 70 kg (RIVM, 2024a), the $QS_{hh\ food}$ is calculated as 183 µg kg^{-1} food. Using the highest estimated BCF value of 58.9 L kg^{-1} (see Table 2.3), the corresponding concentration in surface water would be **3.1 µg L^{-1}**, which is lower than the proposed i-MAC-$QS_{fw,eco}$, but higher than the i-MAC-$QS_{sw,eco}$ and i-QS_{eco}. It is concluded that the EQS based on direct ecotoxicity are protective for human fish consumption.

6.4 Conclusion sodium 5-N-butylbenzotriazole.

Below an overview of the proposed EQS values for sodium 5-butyl-1H-benzotriazole are given. The EQS are expressed as 5-butyl-1H-benzotriazole given the fate of the compound in water. The EQS values refer to dissolved concentrations, but are also valid for total concentration in non-filtered samples. It is proposed to consider the values as indicative EQS.

It is noted that the i-MAC-EQS_{fw} is more than a factor of 35 higher than the proposed i-AA-EQS_{fw}. When performing a compliance check, a single value close to the i-MAC-EQS_{fw} would automatically lead to exceedance of the i-AA-EQS_{fw}. This is due to the high AF needed for derivation of the latter, and indicates that the i-AA-EQS reflect uncertainty due to data gaps rather than by 'true' toxicity (see further Chapter 7).

Table 6.3 Proposed i-EQS for sodium 5-butyl-1H-benzotriazole in this study. All values in µg L^{-1}, for dissolved and total concentrations 5-butyl-1H-benzotriazole.

Freshwater [µg L^{-1}]		**Saltwater [µg L^{-1}]**	
i-AA-EQS_{fw}	**i-MAC-EQS_{fw}**	**i-AA-EQS_{sw}**	**i-MAC-EQS_{sw}**
0.24	8.8	0.024	0.88

[20] https://toolbox.oasis-lmc.org/WebClient/.

[21] https://toxtree.sourceforge.net/.

[22] 6 (exposure duration) x 10 (interspecies variation) x 10 (intraspecies variation) x 10 (missing data on reproductive toxicity).

7 Discussion and conclusions

In this report EQS for freshwater and saltwater have been derived for several benzotrazioles. In Table 7.1 an overview of all EQS values is given. All values presented are for dissolved and total concentrations.

For benzotriazole and sodium 1H-benzotriazolide, and for the group methylbenzotriazoles, formal EQS are proposed. For these substances EQS_{fw} were already available from an evaluation by Switzerland. In this report the data and outcomes from the Swiss derivations were used as a basis, and additional literature and data were collected.
The newly proposed EQS values differ from the Swiss derivations. For benzotriazole, a lower reliable acute effect concentration and lower assessment factor (AF) were used, leading to a higher MAC-EQS_{fw}, while the AA-EQS_{fw} derived in the present study is higher as a lower AF was applied. For the group of methylbenzotriazoles a lower MAC-EQS_{fw} was derived due to the use of a higher AF and higher effect concentration, and the proposed AA-EQS_{fw} is lower because a lower reliable effect concentration was used in the derivation.

For 1-hydroxybenzotriazole and sodium 5-N-butylbenzotriazole indicative EQS are proposed. The acute toxicity of sodium 5-N-butylbenzotriazole clearly differs from the other compounds, while chronic toxicity data is scarce, leading to the application of a high AF. The structural difference between 1-hydroxybenzotriazole and benzotriazole is limited to the presence of an OH-group, and similar toxicity might be assumed. However, too few ecotoxicity data are available for 1-hydroxybenzotriazole to make a sound comparison and it was decided not to pool both datasets. In the end, the derived i-EQS for 1-hydroxytriazole are comparable to those of benzotriazole, but due to the different AFs it is difficult to judge whether the ecotoxicity of both compounds is indeed comparable. However, it cannot be excluded that different benzotriazoles exhibit a similar ecotoxicological action, thereby contributing to a mixture effect when present simultaneously.

From a scientific point of view it would be interesting to further investigate whether or not patterns of benzotriazoles' toxicity could be explained by physico-chemical properties of the individual compounds. If reliable Quantitative Structure Activity Relationships (QSARs) could be established to fill data gaps, this would potentially allow for derivation of one combined EQS for benzotriazoles. The clear advantage of that approach is that the combined action of benzotriazoles is taken into account irrespective of differences in assessment factors. The US EPA ECOSAR program[23] includes Structure Activity Relationships (SARs) for benzotriazoles, establishing relationships between log K_{ow} and toxicity for fish, daphnids and algae. However, underlying datasets are relatively small and date back to decades ago.

[23] Ecological Structure Activity Relationships (ECOSAR) Predictive Model | US EPA.

Table 7.1 Overview of derived environmental quality standards in this report. For each benzotriazole or group of benzotriazoles the proposed EQS status is given. All values in µg L^{-1}, for dissolved and total concentrations.

Substance	**CAS number**	**Status proposed EQS**	**Freshwater [µg L^{-1}]**		**Saltwater [µg L^{-1}]**	
			AA-EQS$_{fw}$	**MAC-EQS$_{fw}$**	**AA-EQS$_{sw}$**	**MAC-EQS$_{sw}$**
Benzotriazole / Sodium 1H-benzotriazolide	95-14-7 15217-42-2	Formal	97	830	9.7	83
Methylbenzotriazoles	29385-43-1 64665-57-2 136-85-6 29878-31-7	Formal	8	77	0.8	7.7
1-hydroxybenzotriazole	2592-95-2	Indicative	76	755	7.6	76
Sodium 5-N-butylbenzotriazole	118685-34-0	Indicative	0.24	8.8	0.024	0.88

Moreover, identity of the underlying compounds and ecotoxicity data included in the SARs is mostly unknown since the data is based on confidential business information, limiting the possibility to evaluate the SARs. In order to establish QSARs, a database should be set-up including reliable ecotoxicity and physico-chemical data for as many benzotriazoles as possible. This is a time-consuming and costly activity.

From a pragmatic point of view, it may therefore be considered for the time being to sum-up the risk ratios of individual benzotriazoles according to the hazard index method (Bodar et al., 2024). If there is an indication of a mixture risk, it may be considered to further explore the options for derivation of a group-EQS. Investment of resources for this activity would be justified if there are clear indications that different benzotriazoles are present in surface waters which significantly contribute to the toxicity of the mixture. It should be noted, however, that existing monitoring data mainly concern benzotriazole. While some data are present for methylbenzotriazoles as well (Harmsma, 2023), relevance of other benzotriazole compounds will likely not become clear.

Currently only reliable and relevant studies may be used for EQS-derivation according to the EQS-methodology of the Water Framework Directive. During the reliability assessment of the consulted studies a contradiction was observed. Studies that provide extensive details tend to receive a lower reliability score than those with more limited details. This can be attributed to a more critical evaluation of details or data which may not be apparent in other studies. While transparent and elaborate reporting is encouraged for scientifical understanding, it may impact the perceived reliability of a study. A more balanced approach is therefore needed to avoid unequal reliability assessments and to keep promoting transparency in ecotoxicity testing and reporting.

References

Assress HA, Selvarajan R, Nyoni H, Mamba BB, Msagati TA. 2021. Antifungal azoles and azole resistance in the environment: current status and future perspectives—a review. Reviews in Environmental Science and Bio/Technology: 1-31.

BAuA. 2021. Federal Institute for Occupational Safety and Health.

Baumann M, Weiß K, Schüssler W, Kopf W. 2013. Ecotoxicological evaluation of benzotriazole, 4-methyl-l H-benzotriazole and 5-methyl-l H-benzotriazole in surface waters. Vom Wasser 111 (1): 1-30.

Berbee RPM, Rutten B. 2022. Hulpstoffen in open circulatie koelsystemen lozend op Rijkswater. Rijkswaterstaart.

Briguglio I, Piras S, Corona P, Gavini E, Nieddu M, Boatto G, Carta A. 2015. Benzotriazole: An overview on its versatile biological behavior. European Journal of Medicinal Chemistry 97: 612-648.

Bodar CWM, ter Burg W, Faber M, van Herwijnen R, Hof M, van Leeuwen L, Naus M, Pronk M. 2024. Cumulatie ZZS en vergunningverlening (vervolgonderzoek 2023). RIVM rapport 2023-0411.

Cancilla DA, Baird JC, Geis SW, Corsi SR. 2003. Studies of the environmental fate and effect of aircraft deicing fluids: Detection of 5 - methyl - 1H - benzotriazole in the fathead minnow (*Pimephales promelas*). Environmental Toxicology and Chemistry: An International Journal 22 (1): 134-140.

Cancilla DA, Holtkamp A, Matassa L, Fang X. 1997. Isolation and characterization of Microtox® - active components from aircraft de - icing/anti - icing fluids. Environmental Toxicology and Chemistry: An International Journal 16 (3): 430-434.

Corsi SR, Geis SW, Loyo-Rosales JE, Rice CP. 2006. Aquatic toxicity of nine aircraft deicer and anti-icer formulations and relative toxicity of additive package ingredients alkylphenol ethoxylates and 4, 5-methyl-1H-benzotriazoles. Environmental science & technology 40 (23): 7409-7415.

Damalas DE, Bletsou AA, Agalou A, Beis D, Thomaidis NS. 2018. Assessment of the acute toxicity, uptake and biotransformation potential of benzotriazoles in zebrafish (*Danio rerio*) larvae combining HILIC-with RPLC-HRMS for high-throughput identification. Environmental science & technology 52 (10): 6023-6031.

De Poorter L, van Herwijnen R, Janssen P, Smit CE. 2015. Handleiding voor de afleiding van indicatieve milieurisicogrenzen. RIVM Rapport 2015-0057.

Durjava MK, Kolar B, Arnus L, Papa E, Kovarich S, Sahlin U, Peijnenburg W. 2013. Experimental assessment of the environmental fate and effects of triazoles and benzotriazole. Alternatives to Laboratory Animals 41 (1): 65-75.

EC. 2011. Technical Guidance For Deriving Environmental Quality Standards. Common Implementation Strategy for the Water Framework Directive (2000/60/EC), Guidance Document No. 27. European Commission (EC).

EC. 2018. Technical guidance for deriving environmental qualilty standards. Guidance Document No. 27. Updated version 2018. Document endorsed by EU Water Directors at their meeting in Sofia on 11-12 June 2018. Brussel: Europese Commissie.
ECHA. 2022a. European Chemicals Agency.
ECHA. 2022b. European Chemicals Agency.
ECHA. 2023a. Registration dossier Benzotriazole. Publication date: 25-06-2013. Last update: 20-10-2022. https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14234. Accessed: 10 August, 2023.
ECHA. 2023b. Registration dossier Methyl-1H-benzotriazole. Publication date: 25-06-2013. Last update: 20-10-2022. https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14272. Accessed: 10 August, 2023.
ECHA. 2023c. Registration dossier Sodium 4(or 5)-methyl-1H-benzotriazolide. Publication date: 15-07-2013. Last update: 13-07-2022. https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/10426. Accessed: 10 August, 2023.
ECHA. 2023d. Registration dossier Sodium 1H-benzotriazolide. Publication date: 25-06-2013. Last update: 07-07-2022. https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/10204. Accessed: 10 August, 2023.
ECHA. 2023e. Registration dossier 1-hydroxybenzotriazole. Publication date: 14-05-2017. Last update: 30-11-2020. https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/19265. Accessed: 10 August, 2023.
ECHA. 2023f. Registration dossier Sodium 5-N-butylbenzotriazole. Publication date: 06-07-2012. Last update: 13-10-2016. https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/15644. Accessed: 10 August, 2023.
ECHA. 2023g. Registration dossier 6-methylbenzotriazole. Publication date: 17-04-2018. Last update: 10-01-2020. https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/23746. Accessed: 10 August, 2023.
ECHA. 2023h. Database. https://echa.europa.eu/nl/home. Accessed: 10 August, 2023.
Gatidou G, Anastopoulou P, Aloupi M, Stasinakis AS. 2019. Growth inhibition and fate of benzotriazoles in *Chlorella sorokiniana* cultures. Science of the total environment 663: 580-586.
Giraudo M, Douville M, Cottin G, Houde M. 2017. Transcriptomic, cellular and life-history responses of *Daphnia magna* chronically exposed to benzotriazoles: Endocrine-disrupting potential and molting effects. PloS one 12 (2): e0171763.
Golfomitsou S, Merkel J. 2004. Synergistic effects of corrosion inhibitors for copper and copper alloy archaeological artefacts. Proceedings of Metal: 344-368.
Harmsma S. 2022. Brononderzoek drinkwaterrelevante stoffen. Tien stoffen in het Rijnstroomgebied. Rijkswaterstaat, WVL. Arcadis.
Harmsma S. 2023. Benzotriazolen - typen, toepassingen, zuivering, metingen en emissiebronnen. Rijkswaterstaat, WVL. Arcadis.
Im H, Kang J, Jacob MF, Bae H, Oh J-E. 2023. Transgenerational effects of benzotriazole on the gene expression, growth, and reproduction of *Daphnia magna*. Environmental Pollution 323: 121211.

Li M-H. 2013. Acute toxicity of industrial endocrine-disrupting chemicals, natural and synthetic sex hormones to the freshwater planarian, *Dugesia japonica*. Toxicological & Environmental Chemistry 95 (6): 984-991.
Moermond CT, Kase R, Korkaric M, Ågerstrand M. 2016. CRED: Criteria for reporting and evaluating ecotoxicity data. Environmental Toxicology and Chemistry 35 (5): 1297-1309.
OECD. 2011. Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test.
OECD. 2013. QSAR Toolbox (computer program). Version 3.1. Paris, OECD (computer program). Version
Oekotoxzentrum. 2015. EQS - Vorschlag des Oekotoxzentrums für: Benzotriazol.
Oekotoxzentrum. 2016. EQS - Vorschlag des Oekotoxzentrums für: Methylbenzotriazole.
Olds HT, Corsi SR, Rutter TD. 2022. Benzotriazole concentrations in airport runoff are reduced following changes in airport deicer formulations. Integrated Environmental Assessment and Management 18 (1): 245-257.
Osté L, Ouwerkerk K, Derksen A. 2022. NORMAN prioritering Nederlandse waterkwaliteitsdata. Versie 2.2. Deltares en AD eco advies. Available via https://publications.deltares.nl/11206216_010_0001.pdf.
Pillard DA, Cornell JS, DuFresne DL, Hernandez MT. 2001. Toxicity of benzotriazole and benzotriazole derivatives to three aquatic species. Water research 35 (2): 557-560.
RIVM. 2024a. Handleiding voor de afleiding van indicatieve milieurisicogrenzen. Deel 5: Afleiding van risicogrenzen per compartiment. Versie 1.1.
RIVM. 2024b. Handleiding voor de afleiding van indicatieve milieurisicogrenzen. Deel 3. MRT Humaan. Humane toxiciteit - Toelichting. Versie 1.0.
Seeland A, Oetken M, Kiss A, Fries E, Oehlmann J. 2012. Acute and chronic toxicity of benzotriazoles to aquatic organisms. Environmental Science and Pollution Research 19: 1781-1790.
Shin Y-J, Kim B, Kim H, Kim K, Park K, Kim J, Kim H-j, Kim P. 2022. 1, 2, 3-benzotriazole adversely affects early-life stage of *Oryzias latipes*. Science of The Total Environment 815: 152846.
US EPA. 2000-2012. Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11. United States Environmental Protection Agency, Washington, DC, USA. (computer program). Version 4.11.
US EPA. 2023. ECOTOX Knowledgebase. https://cfpub.epa.gov/ecotox/search.cfm. Accessed: 6 July, 2023.
van Leerdam R, Janssen P, van der Aa N, Versteegh J. 2018. Risicobeoordeling 42 opkomende stoffen in oppervlaktewaterbronnen voor drinkwaterbereiding: Probleemstoffen op basis van Protocol monitoring en toetsing drinkwaterbronnen KRW.
Vetter W, Lorenz J. 2013. Determination of benzotriazoles in dishwasher tabs from Germany and estimation of the discharge into German waters. Environmental Science and Pollution Research 20: 4435-4440.

Wassenaar PN, Rorije E, Vijver MG, Peijnenburg WJ. 2022. ZZS similarity tool: The online tool for similarity screening to identify chemicals of potential concern. Journal of Computational Chemistry 43 (15): 1042-1052.

Appendix 1 Registered Benzotriazoles

Table A1.1 Different structural analogues of benzotriazole, sorted by the amount manufactured/imported per year in the EEA. Only registered benzotriazoles (analogues with a tonnage) in REACH are shown in the table.

Name	**CAS no.**	**EC no.**	**Molecular formula**	**Manufactured/ imported in EEA per year (tonnes)**
Benzotriazole / 1H-benzotriazole	95-14-7	202-394-1	$C_6H_5N_3$	≥ 1 000 to < 10 000
Methyl-1H-benzotriazole	29385-43-1	249-596-6	$C_7H_7N_3$	≥ 1 000 to < 10 000
Bumetrizole	3896-11-5	223-445-4	$C_{17}H_{18}ClN_3O$	≥ 1 000 to < 10 000
2-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol	3147-75-9	221-573-5	$C_{20}H_{25}N_3O$	≥ 1 000 to < 10 000
2-(2H-Benzotriazol-2-yl)-6-(1-methyl-1-phenylethyl)-4-(1,1,3,3-tetramethylbutyl)phenol	73936-91-1	422-600-5	$C_{29}H_{35}N_3O$	≥ 1 000 to < 10 000
Sodium 4(or 5)-methyl-1H-benzotriazolide	64665-57-2	265-004-9	-	≥ 1 000 to < 10 000
2-(2H-benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl)phenol	70321-86-7	274-570-6	$C_{30}H_{29}N_3O$	≥ 1 000 to < 10 000
2-(2H-benzotriazol-2-yl)-p-cresol	2440-22-4	219-470-5	$C_{13}H_{11}N_3O$	≥ 1 000 to < 10 000
Sodium 1H-benzotriazolide	15217-42-2	239-269-6	$C_6H_4N_3Na$	≥ 100 to < 1 000
2-(2H-benzotriazol-2-yl)-4,6-ditertpentylphenol	25973-55-1	247-384-8	-	≥ 100 to < 1 000
Benzotriazol, ar-methyl-, reaction product with formaldehyde and Diethanolamine	1474044-75-1	939-703-0	-	≥ 100 to < 1 000
Reaction mass of 1H-Benzotriazole-1-methanamine, N,N-bis(2-ethylhexyl)-6-methyl- and 2H-Benzotriazole-2-methanamine, N,N-bis(2-ethylhexyl)-5-methyl- and N,N-bis(2-ethylhexyl)-4-methyl-1H-benzotriazole-1-methylamine and 2H-	-	939-700-4	-	≥ 100 to < 1 000

Name	CAS no.	EC no.	Molecular formula	Manufactured/ imported in EEA per year (tonnes)
Benzotriazole-2-methanamine, N,N-bis(2-ethylhexyl)-4-methyl- and N,N-bis(2-ethylhexyl)-5-methyl-1H-benzotriazole-1-methylamine				
2,2'-methylenebis(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol)	103597-45-1	403-800-1	$C_{41}H_{50}N_6O_2$	≥ 100 tonnes
A mixture of: α-3-(3-(2H-benzotriazol-2-yl)-5-tert-butyl-4-hydroxyphenyl)propionyl-ω-hydroxypoly(oxyethylene); α-3-(3-(2H-benzotriazol-2-yl)-5-tert-butyl-4-hydroxyphenyl)propionyl-ω-3-(3-(2H-benzotriazol-2-yl)-5-tert-butyl-4-hydroxyphenyl)propionyloxypoly(oxyethylene)	-	400-830-7	-	≥ 100 tonnes
A mixture of branched and linear C7-C9 alkyl 3-[3-(2H-benzotriazol-2-yl)-5-(1,1-dimethylethyl)-4-hydroxyphenyl]propionates	127519-17-9	407-000-3	-	≥ 100 tonnes
A mixture of: isomers of 2-(2H-benzotriazol-2-yl)-4-methyl-(n)-dodecylphenol; isomers of 2-(2H-benzotriazol-2-yl)-4-methyl-(n)-tetracosylphenol; isomers of 2-(2H-benzotriazol-2-yl)-4-methyl-5,6-didodecyl-phenol. n=5 or 6	125304-04-3	401-680-5	-	≥ 100 tonnes
1-hydroxybenzotriazole	2592-95-2	219-989-7	$C_6H_5N_3O$	≥ 10 to < 100
Reaction mass of sodium 4-chloro-5-alkylbenzotriazolide and sodium 5-chloro-4-alkylbenzotriazolide and sodium 4-chloro-7-alkylbenzotriazolide and sodium 5-chloro-6-alkylbenzotriazolide	-	-	$C_7H_5ClN_3Na$	≥ 10 to < 100
Sodium 3-(2H-benzotriazol-2-yl)-5-sec-butyl-4-hydroxybenzenesulfonate	92484-48-5	403-080-9	$C_{16}H_{16}N_3NaO_4S$	≥ 10 tonnes

Name	CAS no.	EC no.	Molecular formula	Manufactured/ imported in EEA per year (tonnes)
Sodium 5-N-butylbenzotriazole	118685-34-0	404-450-2	$C_{10}H_{12}N_3Na$	≥ 10 tonnes
6-methylbenzotriazole	136-85-6	205-265-8	$C_7H_7N_3$	≥ 1 to < 10
Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate	128625-52-5	603-290-2	-	≥ 1 to < 10
Reaction mass of Octyl-3-[3-tert-butyl-4-hydroxy-5-(5-chloro-2H-benzotriazole-2-yl)phenyl]propionate and 2-Ethylhexyl-3-[3-tert-butyl-4-hydroxy-5-(5-chloro-2H-benzotriazole-2-yl)phenyl]propionate	-	916-914-6	-	≥ 1 to < 10
2-METHYL-2-PROPENOIC ACID, 2-[3-(2H-BENZOTRIAZOL-2-YL)-4HYDROXYPHENYL]ETHYL ESTER 2-[3-(2H-Benzotriazol-2-yl)-4-hydroxyphenyl]ethyl methacrylate	96478-09-0	424-240-4	$C_{18}H_{17}N_3O_3$	≥ 1
O-2-(1-H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate	94790-37-1	423-020-5	$C_{11}H_{16}F_6N_5OP$	Intermediate use only
2-benzotriazol-2-yl-4-methyl-6-(2-methylallyl)phenol	98809-58-6	419-750-9	$C_{17}H_{17}N_3O$	Intermediate use only
Methyl 6-methoxy-1H-benzotriazole-5-carboxylate	59338-86-2	261-705-9	$C_9H_9N_3O_3$	Intermediate use only
O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate	125700-67-6	603-087-9	$C_{11}H_{16}BF_4N_5O$	Intermediate use only
2-(2H-benzotriazol-2-yl)-4-tert-butylphenol	3147-76-0	221-574-0	$C_{16}H_{17}N_3O$	Is not currently being manufactured in and / or imported to the European Economic Area
O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate	148893-10-1	604-662-7	-	Is not currently being manufactured in and / or imported to the

Name	CAS no.	EC no.	Molecular formula	Manufactured/ imported in EEA per year (tonnes)
				European Economic Area
4,5,6,7-tetrahydro-1H-benzotriazole	6789-99-7	229-858-6	$C_6H_9N_3$	Is not currently being manufactured in and / or imported to the European Economic Area
1-tetradecanoylbenzotriazole	-	437-790-5	$C_{20}H_{31}N_3O$	Confidential
4-chloro-5-methylbenzotriazole sodium salt;5-chloro-4-methylbenzotriazole sodium salt;reaction mass of: 4-chloro-7-methylbenzotriazole sodium salt	202420-04-0	427-730-6	$C_{21}H_{15}Cl_3N_9Na_3$	Confidential
2-(2-hydroxy-4-octyloxyphenyl)-2H-benzotriazole	3147-77-1	448-630-9	$C_{20}H_{25}N_3O_2$	Confidential
COPOLYMER OF 3-(2H-1,2,3-BENZOTRIAZOLE-2-YL)-4-	-	443-470-6	-	Confidential
N-[(benzotriazole-1-yl)methyl)]-4-carboxybenzenesulfonamide	170292-97-4	416-470-9	$C_{14}H_{12}N_4O_4S$	Confidential
1-[di(4-octylphenyl)aminomethyl]-4-methyl-1H-benzotriazole;N-[(4-methyl-1H-benzotriazol-1-yl)methyl]-4-octyl-N-(4-octylphenyl)aniline;reaction mass of: 1-[di(4-octylphenyl)aminomethyl]-5-methyl-1H-benzotriazole;reaction mass of: N-[(5-methyl-1H-benzotriazol-1-yl)methyl]-4-octyl-N-(4-octylphenyl)aniline	-	420-720-2	-	Confidential
A mixture of: phenyl 1-(1-[2-chloro-5-(hexadecyloxycarbonyl)phenylcarbamoyl]-3,3-dimethyl-2-oxobutyl)-1H-2,3,3a,7a-tetrahydrobenzotriazole-5-carboxylate;	-	421-480-1		Confidential

Name	CAS no.	EC no.	Molecular formula	Manufactured/ imported in EEA per year (tonnes)
phenyl 2-(1-(2-chloro-5-(hexadecyloxycarbonyl)phenylcarbamoyl)-3,3-dimethyl-2-oxobutyl)-1H-2,3,3a,7a-tetrahydrobenzotriazole-5-carboxylate; phenyl 3-(1-(2-chloro-5-(hexadecyloxycarbonyl)phenylcarbamoyl)-3,3-dimethyl-2-oxobutyl)-1H-2,3,3a,7a-tetrahydrobenzotriazole-5-carboxylate				
A mixture (50:50) of: 2-[2-acetylamino-4-[N,N-bis[2-ethoxycarbonyloxy)ethyl]amino]phenylazo]-5,6-dichloro-1,3-benzothiazole; 2-[2-acetylamino-4-[N,N-bis[2-ethoxycarbonyloxy)ethyl]amino]phenylazo]-6,7-dichloro-1,3-benzotriazole	143145-93-1	411-600-0		Confidential

Appendix 2 ZZS-Similarity Tool scores Benzotriazoles

Table A2.1 Similarity scores for the different benzotriazole compounds.[a] [b]

Compound no.	1	2	3	4	5	6	7	8	9	10	11	12	13
1	1.000	0.694[a]	0.333	0.362	0.315	0.756[a]	0.343	0.436	1.000[a]	0.347	0.708[a]	0.272	0.576[a]
2	0.694[a]	1.000	0.373	0.402	0.365	0.918[b]	0.396	0.477	0.694[b]	0.387	0.540[b]	0.308	0.688[b]
3	0.333	0.373	1.000	0.766	0.750	0.374	0.811	0.765	0.333	0.802	0.316	0.599	0.388
4	0.362	0.402	0.766	1.000	0.870	0.404	0.804	0.830	0.362	0.901	0.327	0.685	0.515
5	0.315	0.365	0.750	0.870	1.000	0.354	0.917	0.722	0.315	0.890	0.289	0.629	0.452
6	0.756[a]	0.918[b]	0.374	0.404	0.354	1.000	0.385	0.482	0.756[b]	0.388	0.576[b]	0.308	0.677[b]
7	0.343	0.396	0.811	0.804	0.917	0.385	1.000	0.788	0.343	0.858	0.313	0.612	0.398
8	0.436	0.477	0.765	0.830	0.722	0.482	0.788	1.000	0.436	0.796	0.385	0.611	0.412
9	1.000[a]	0.694[b]	0.333	0.362	0.315	0.756[b]	0.343	0.436	1.000	0.347	0.708[b]	0.272	0.576[b]
10	0.347	0.387	0.802	0.901	0.890	0.388	0.858	0.796	0.347	1.000	0.315	0.689	0.454
11	0.708[a]	0.540[b]	0.316	0.327	0.289	0.576[b]	0.313	0.385	0.708[b]	0.315	1.000	0.254	0.466[b]
12	0.272	0.308	0.599	0.685	0.629	0.308	0.612	0.611	0.272	0.689	0.254	1.000	0.363
13	0.576[a]	0.688[b]	0.388	0.515	0.452	0.677[b]	0.398	0.412	0.576[b]	0.454	0.466[b]	0.363	1.000
14	0.773[a]	0.898[b]	0.377	0.408	0.357	0.894[b]	0.388	0.488	0.773[b]	0.392	0.586[b]	0.310	0.746[b]
15	0.321	0.325	0.268	0.290	0.274	0.313	0.273	0.296	0.321	0.283	0.453	0.255	0.299
16	0.301	0.339	0.654	0.711	0.637	0.339	0.683	0.690	0.301	0.688	0.278	0.566	0.354
17	0.113	0.168	0.264	0.269	0.254	0.164	0.261	0.273	0.113	0.263	0.109	0.251	0.173
18	0.374	0.458	0.331	0.341	0.318	0.447	0.338	0.385	0.374	0.340	0.337	0.286	0.415
19	0.395	0.436	0.825	0.915	0.796	0.440	0.869	0.907	0.395	0.878	0.354	0.661	0.450
20	0.257	0.272	0.173	0.233	0.209	0.286	0.177	0.189	0.257	0.206	0.229	0.178	0.314
21	0.347	0.324	0.527	0.587	0.526	0.324	0.539	0.630	0.347	0.556	0.327	0.477	0.319
22	0.264	0.319	0.269	0.304	0.281	0.318	0.274	0.302	0.264	0.282	0.255	0.330	0.315
23	0.756[a]	0.918[b]	0.374	0.404	0.354	1.000[b]	0.385	0.482	0.756[b]	0.388	0.576[b]	0.308	0.677[b]
24	0.374	0.414	0.856	0.869	0.843	0.417	0.919	0.857	0.374	0.929	0.337	0.649	0.429
25	0.327	0.366	0.981	0.768	0.767	0.367	0.829	0.750	0.327	0.820	0.310	0.590	0.381
26	0.347	0.387	0.802	0.901	0.890	0.388	0.858	0.796	0.347	1.000	0.315	0.689	0.454

[a] All values ≥0.50 compared to benzotriazole (compound no. 1) are given in green
[b] The similarity scores of the compounds having a score ≥0.50 to each other are given in orange

Continuation Table A.2.1 [a] [b]

Compound no.	14	15	16	17	18	19	20	21	22	23	24	25	26
1	0.773[a]	0.321	0.301	0.113	0.374	0.395	0.257	0.347	0.264	0.756[a]	0.374	0.327	0.347
2	0.898[b]	0.325	0.339	0.168	0.458	0.436	0.272	0.324	0.319	0.918[b]	0.414	0.366	0.387
3	0.377	0.268	0.654	0.264	0.331	0.825	0.173	0.527	0.269	0.374	0.856	0.981	0.802
4	0.408	0.290	0.711	0.269	0.341	0.915	0.233	0.587	0.304	0.404	0.869	0.768	0.901
5	0.357	0.274	0.637	0.254	0.318	0.796	0.209	0.526	0.281	0.354	0.843	0.767	0.890
6	0.894[b]	0.313	0.339	0.164	0.447	0.440	0.286	0.324	0.318	1.000[b]	0.417	0.367	0.388
7	0.388	0.273	0.683	0.261	0.338	0.869	0.177	0.539	0.274	0.385	0.919	0.829	0.858
8	0.488	0.296	0.690	0.273	0.385	0.907	0.189	0.630	0.302	0.482	0.857	0.750	0.796
9	0.773[b]	0.321	0.301	0.113	0.374	0.395	0.257	0.347	0.264	0.756[b]	0.374	0.327	0.347
10	0.392	0.283	0.688	0.263	0.340	0.878	0.206	0.556	0.282	0.388	0.929	0.820	1.000
11	0.586[b]	0.453	0.278	0.109	0.337	0.354	0.229	0.327	0.255	0.576[b]	0.337	0.310	0.315
12	0.310	0.255	0.566	0.251	0.286	0.661	0.178	0.477	0.330	0.308	0.649	0.590	0.689
13	0.746	0.299	0.354	0.173	0.415	0.450	0.314	0.319	0.315	0.677[b]	0.429	0.381	0.454
14	1.000	0.316	0.342	0.156	0.484	0.444	0.256	0.327	0.311	0.894[b]	0.421	0.370	0.392
15	0.316	1.000	0.266	0.160	0.263	0.289	0.168	0.283	0.237	0.313	0.279	0.265	0.283
16	0.342	0.266	1.000	0.262	0.369	0.761	0.160	0.540	0.301	0.339	0.729	0.656	0.688
17	0.156	0.160	0.262	1.000	0.189	0.275	0.129	0.224	0.177	0.164	0.275	0.261	0.263
18	0.484	0.263	0.369	0.189	1.000	0.362	0.179	0.340	0.333	0.447	0.358	0.327	0.340
19	0.444	0.289	0.761	0.275	0.362	1.000	0.197	0.600	0.295	0.440	0.945	0.827	0.878
20	0.256	0.168	0.160	0.129	0.179	0.197	1.000	0.188	0.144	0.286	0.189	0.170	0.206
21	0.327	0.283	0.540	0.224	0.340	0.600	0.188	1.000	0.268	0.324	0.575	0.519	0.556
22	0.311	0.237	0.301	0.177	0.333	0.295	0.144	0.268	1.000	0.318	0.287	0.266	0.282
23	0.894[b]	0.313	0.339	0.164	0.447	0.440	0.286	0.324	0.318	1.000	0.417	0.367	0.388
24	0.421	0.279	0.729	0.275	0.358	0.945	0.189	0.575	0.287	0.417	1.000	0.875	0.929
25	0.370	0.265	0.656	0.261	0.327	0.827	0.170	0.519	0.266	0.367	0.875	1.000	0.820
26	0.392	0.283	0.688	0.263	0.340	0.878	0.206	0.556	0.282	0.388	0.929	0.820	1.000

[a] All values ≥0.50 compared to benzotriazole (compound no. 1) are given in green
[b] The similarity scores of the compounds having a score ≥0.50 to each other are given in orange

Supporting table for Table A2.1

No.	Compound	CAS. no.
1	Benzotriazole / 1H-benzotriazole	95-14-7
2	Methyl-1H-benzotriazole	29385-43-1
3	Bumetrizole	5-11-3896
4	2-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol	3147-75-9
5	2-(2H-Benzotriazol-2-yl)-6-(1-methyl-1-phenylethyl)-4-(1,1,3,3-tetramethylbutyl)phenol	73936-91-1
6	Sodium 4(or 5)-methyl-1H-benzotriazolide	64665-57-2
7	2-(2H-benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl)phenol	70321-86-7
8	2-(2H-benzotriazol-2-yl)-p-cresol	2440-22-4
9	Sodium 1H-benzotriazolide	15217-42-2
10	2-(2H-benzotriazol-2-yl)-4,6-ditertpentylphenol	25973-55-1
11	1-hydroxybenzotriazole	2592-95-2
12	Sodium 3-(2H-benzotriazol-2-yl)-5-sec-butyl-4-hydroxybenzenesulfonate	92484-48-5
13	Sodium 5-N-butylbenzotriazole	118685-34-0
14	6-methylbenzotriazole	136-85-6
15	Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate	128625-52-5
16	2-METHYL-2-PROPENOIC ACID, 2-[3-(2H-BENZOTRIAZOL-2-YL)-4HYDROXYPHENYL]ETHYL ESTER 2-[3-(2H-Benzotriazol-2-yl)-4-hydroxyphenyl]ethyl methacrylate	96478-09-0
17	2-benzotriazol-2-yl-4-methyl-6-(2-methylallyl)phenol	98809-58-6
18	Methyl 6-methoxy-1H-benzotriazole-5-carboxylate	59338-86-2
19	2-(2H-benzotriazol-2-yl)-4-tert-butylphenol	3147-76-0
20	4,5,6,7-tetrahydro-1H-benzotriazole	6789-99-7
21	2-(2-hydroxy-4-octyloxyphenyl)-2H-benzotriazole	3147-77-1
22	N-[(benzotriazole-1-yl)methyl)]-4-carboxybenzenesulfonamide	170292-97-4
23	4-methyl-1H-benzotriazole	29878-31-7
24	2-benzotriazol-2-yl-4,6-di-tert-butylphenol	3846-71-7
25	2,4-di-tert-butyl-6-(5-chlorobenzotriazol-2-yl)phenol	3864-99-1
26	2-(2H-benzotriazol-2-yl)-4-(tert-butyl)-6-(sec-butyl)phenol	36437-37-3

Appendix 3 Toxicity data benzotriazole and sodium 1H-benzotriazolide

Table A3.1 Overview of ecotoxicity data for benzotriazole (BT; CAS no. 95-14-7) and sodium 1H-benzotriazolide (NaBT; CAS no. 15217-42-2). Presented are the relevant reliable studies and studies of which we regard the reliability differently from the Swiss EQS derivation, supplemented with new and relevant data from the REACH registration dossiers and open literature. Results are expressed in mg L^{-1} benzotriazole. Lowest reliable and relevant effect concentration per taxon are indicated on a grey background. The results are ordered by taxon and alphabetically by species.

ACUTE									
Substance	**Taxon**	**Species**	**Duration**	**Criterion**	**Value [mg L^{-1}]**	**Remarks**	**R**	**New**	**Source**
BT	Bacteria	*Vibrio fischeri*	5min	EC_{50}	41.13		2		Cancilla et al. (1997) / Oekotoxzentrum (2015)
BT	Protozoa	*Tetrahymena pyriformis*	60 h	EC_{50}	258.5		2		Oekotoxzentrum (2015)
BT	Algae	*Chlorella sorokiniana*	72 h	EC_{50}	8.3	see text	2	Y	Gatidou et al. (2019)
BT	Algae	*Desmodesmus subspicatus*	72 h	E_rC_{50}	189	see text	2		Baumann et al. (2013) / Oekotoxzentrum (2015)
BT	Algae	*Desmodesmus subspicatus*	72 h	E_rC_{50}	231	see text	4		ECHA (2023a) / Oekotoxzentrum (2015)
BT	Algae	*Raphidocelis subcapitata*	72 h	E_rC_{50}	75	see text	2		ECHA (2022a) / ECHA (2023a) / Oekotoxzentrum (2015)
NaBT	Algae	*Raphidocelis subcapitata*	72 h	E_rC_{50}	126.6	see text	2	Y	ECHA (2023d)
NaBT	Crustacea	*Americamysis bahia* (saltwater)	96 h	LC_{50}	95.9	see text	4	Y	ECHA (2023d)

ACUTE									
Substance	**Taxon**	**Species**	**Duration**	**Criterion**	**Value [mg L^{-1}]**	**Remarks**	**R**	**New**	**Source**
NaBT	Crustacea	*Americamysis bahia* (saltwater)	96 h	LC_{50}	119.1	see text	4	Y	ECHA (2023d)
BT	Crustacea	*Daphnia galeata*	48 h	EC_{50}	15.8		2		ECHA (2022a) / ECHA (2023a) / Oekotoxzentrum (2015) / Seeland et al. (2012)
BT	Crustacea	*Daphnia magna*	48 h	EC_{50}	50		2		Baumann et al. (2013) / Oekotoxzentrum (2015)
BT	Crustacea	*Daphnia magna*	48 h	EC_{50}	91	see text	2		ECHA (2022a) / ECHA (2023a) / Oekotoxzentrum (2015)
BT	Crustacea	*Daphnia magna*	48 h	EC_{50}	93.3	see text	4	Y	Giraudo et al. (2017)
BT	Crustacea	*Daphnia magna*	48 h	EC_{50}	107	see text	2		ECHA (2022a) / ECHA (2023a) / Oekotoxzentrum (2015) / Seeland et al. (2012)
BT	Crustacea	*Daphnia magna*	48 h	EC_{50}	137	see text	2		ECHA (2022a) / ECHA (2023a) / Oekotoxzentrum (2015)
BT	Crustacea	*Daphnia magna*	48 h	EC_{50}	155.4	see text	4	Y	Durjava et al. (2013)
NaBT	Crustacea	*Daphnia magna*	48 h	LC_{50}	165.1	see text	4	Y	ECHA (2023d)
BT	Pisces	*Danio rerio* (embryo)	72 h	LC_{50}	0.67-16 (mean = 6.43)	see text	3	Y	Durjava et al. (2013) / ECHA (2023a)

ACUTE									
Substance	**Taxon**	**Species**	**Duration**	**Criterion**	**Value [mg L^{-1}]**	**Remarks**	**R**	**New**	**Source**
BT	Pisces	*Danio rerio*	96 h	LC_{50}	170	see text	4	Y	Damalas et al. (2018) / ECHA (2023a)
BT	Pisces	*Danio rerio*	96 h	LC_{50}	180	see text	2		ECHA (2022a) / ECHA (2023a) / Oekotoxzentrum (2015)
NaBT	Pisces	*Lepomis macrochirus*	96 h	LC_{50}	64	see text	4	Y	ECHA (2023d)
NaBT	Pisces	*Menidia beryllina* (saltwater)	96 h	LC_{50}	26	see text	4	Y	ECHA (2023d)
NaBT	Pisces	*Menidia beryllina* (saltwater)	96 h	LC_{50}	33.2	see text	4	Y	ECHA (2023d)
BT	Pisces	*Oncorhynchus mykiss*	96 h	LC_{50}	21.6	see text	4		Oekotoxzentrum (2015)
NaBT	Pisces	*Oncorhynchus mykiss*	96 h	LC_{50}	12.5	see text	4	Y	ECHA (2023d)
BT	Pisces	*Oryzias latipes*	96 h	LC_{50}	143.47	see text	4	Y	Shin et al. (2022)
BT	Pisces	*Pimephales promelas*	96 h	LC_{50}	65	see text	4		Oekotoxzentrum (2015) / Pillard et al. (2001)
NaBT	Pisces	*Pimephales promelas*	96 h	LC_{50}	56.8	see text	4	Y	ECHA (2023d)
BT	Platyhel-minthes	*Dugesia japonica*	96 h	EC_{50}	142.9	see text	2	Y	Li (2013)

CHRONIC									
Substance	**Taxon**	**Species**	**Duration**	**Criterion**	**Value [mg L^{-1}]**	**Remarks**	**R**	**New**	**Source**
BT	Algae	*Desmodesmus subspicatus*	72 h	EC_{10}	1.18	see text	3		ECHA (2022a) / ECHA (2023a) / Oekotoxzentrum (2015) / Seeland et al. (2012)
BT	Algae	*Desmodesmus subspicatus*	72 h	NOE_rC	18.9	see text	2		Baumann et al. (2013) / Oekotoxzentrum (2015)
BT	Algae	*Desmodesmus subspicatus*	72 h	E_rC_{10}	49.5	derived by authors, see text	2		Based on data from Baumann et al. (2013) /
BT	Algae	*Desmodesmus subspicatus*	72 h	E_rC_{10}	58	see text	4		ECHA (2022a) / ECHA (2023a) / Oekotoxzentrum (2015)
BT	Algae	*Raphidocelis subcapitata*	72 h	E_rC_{10}	10.5	see text	2		ECHA (2022a) / ECHA (2023a) / Oekotoxzentrum (2015)
NaBT	Algae	*Raphidocelis subcapitata*	72 h	E_rC_{10}	47.3	see text	2	Y	ECHA (2023d)
BT	Crustacea	*Daphnia galeata*	21 d	EC_{10}	0.97		2		ECHA (2022a) / ECHA (2023a) / Oekotoxzentrum (2015) / Seeland et al. (2012)
BT	Crustacea	*Daphnia magna*	21 d	NOEC	≥12.8		2		ECHA (2022a) /

CHRONIC									
Substance	**Taxon**	**Species**	**Duration**	**Criterion**	**Value [mg L^{-1}]**	**Remarks**	**R**	**New**	**Source**
									ECHA (2023a) / / Seeland et al. (2012)
BT	Crustacea	*Daphnia magna*	21 d	NOEC	≥20		2		Baumann et al. (2013) / Oekotoxzentrum (2015)
BT	Crustacea	*Daphnia magna*	21 d	NOEC	25.9		4		ECHA (2022a) / ECHA (2023a) / Oekotoxzentrum (2015)
BT	Crustacea	*Daphnia magna*	21 d	NOEC	≥2	see text	4	Y	Giraudo et al. (2017)
BT	Crustacea	*Daphnia magna*	21 d	NOEC	≥0.0043	see text	4	Y	Im et al. (2023)
BT	Crustacea	*Daphnia magna*	21 d	NOEC	≤15	see text	4	Y	Im et al. (2023)
BT	Macrophyta	*Lemna minor*	7 d	EC_{10}	3.94	new E_rC_{10} derived, see text	3		ECHA (2022a) / ECHA (2023a) / Oekotoxzentrum (2015) / Seeland et al. (2012)
BT	Macrophyta	*Lemna minor*	7 d	E_rC_{10}	7.4	newly derived, see text	2		Based on raw data from Seeland et al. (2012)
BT	Pisces	*Danio rerio*	35 dpf	NOEC	1.07	see text	2	Y	ECHA (2022a) / ECHA (2023a)
BT	Pisces	*Oryzias latipes*	13 d	NOEC	41	see text	2	Y	Shin et al. (2022)
BT	Pisces	*Oryzias latipes*	42 d	NOEC	4	see text	2	Y	Shin et al. (2022)

Discussion of acute ecotoxicity data

Acute toxicity studies were retrieved from the REACH registration dossiers for benzotriazole (BT; CAS no. 95-14-7) and sodium 1H-benzotriazolide (NaBT; CAS no. 15217-42-2) and the open literature. In addition, data from the Swiss EQS derivation was taken into account. A brief summary of relevant data and explanation on reliability scoring per taxa is presented below. The focus of this section is mainly on algae and fish, because of diverging assessments of reliability and/or the presence of additional data. For other taxa newly found studies did not contain lower effect concentrations than already available, these taxa are discussed less extensively.

Bacteria/protozoa
Reliable values were taken over from the Swiss EQS report, but not further discussed because they are not critical for EQS derivation and no new data was found for these taxa. Note that for *Vibrio fischeri* only the 5-min EC_{50} (41.13 mg L^{-1}) is given in the overview table, as the 15-min EC_{50} was higher (41.65 mg L^{-1}).

Algae
As mentioned in section 3.1.2, the lowest reliable effect concentration for algae in the Swiss evaluation is a 72-h E_rC_{50} of 189 mg L^{-1} for *Desmodesmus subspicatus* from a study by Baumann et al. (2013). This value is included as reliable without restrictions (R1) based on an evaluation of the German UBA. Since studies with lower E_rC_{50} (and E_rC_{10}) values were found, these and the study by Baumann et al. (2013) were consulted and assessed for reliability.

The E_rC_{50} of 189 mg L^{-1} from Baumann et al. (2013) originates from a 72-h algae test according to DIN EN ISO 8692 in 24-well microplates. Test concentrations were confirmed by chemical analysis. Cell density was measured as chlorophyll-fluorescence and the E_rC_{50} of 189 mg L^{-1} is explicitly referred to as based on growth rate. A growth-rate inhibition curve is included in the paper, but information on control performance and validity criteria was not presented. Therefore the authors of the paper were contacted for additional information. Unfortunately all authors were no longer working at Bayerisches Landesamt für Umwelt (LfU Bayern), and raw data could not be found by LfU Bayern. As the results could not be checked, we do not consider the reliability score R1 justified, at most a score of R2 (reliable with restrictions).

A more or less comparable 72-h E_rC_{50} of 231 mg L^{-1} for the same species is reported in the REACH registration dossier on BT (ECHA, 2023a). The study was performed according to DIN 34812-9 and cell counts are reported in the summary. However, the study is rated R4 (not assignable) by the registrant due to deficiencies in reporting (e.g., missing details on test conditions such as pH and water, number of replicates) and because a positive control is missing. Based on reported cell numbers, multiplication in the control was sufficient, but the registrant reports that it is not able to check the validity criteria of current guidelines. Therefore the result is rated as R4.

A lower 72-h E_rC_{50} of 75 mg L^{-1} (nominal) for *Raphidocelis subspicatus* is included in the REACH registration dossier on BT (ECHA, 2023a). This value originates from a test according to OECD TG 201. Since the test was carried out in 1993, presumably the 1984-version of this guideline was used. In the summary details on inhibition percentages are not given, however it is stated that control growth was exponential and cell density increased by a factor of 45 during the test. Abnormalities were not observed, and validity criteria are reported to be met. The results are considered R2 in this EQS-derivation. It is noted that the effect concentrations are also assigned R2 in the RAC opinion (ECHA, 2022a).

The REACH registration dossier on NaBT presents a 72-h E_rC_{50} of 150 mg L^{-1} (nominal), equivalent to 126.6 mg L^{-1} as BT for *R. subspicatus* (ECHA, 2023d). This value originates from a test according to OECD TG 201/US EPA OPPTS 850.5400. The summary states that chemical analysis showed that the test item was stable over the test duration. Validity criteria were reported to be met, but details are not presented. Tables with detailed information on cell counts, daily specific growth rate and inhibition values, and time-dependent growth curves are referred to in the summary, but these are not disclosed on the public ECHA website. Because of this, the reliability of the study cannot be assessed when strictly based on the summary. However, the extensive description of results, including mentioning concentrations with significant effects, gives confidence that the summary adequately reflects the results of the study (R2).

From the open literature, another study was retrieved with lower acute values than used in former EQS derivations. In a growth inhibition test according to OECD TG 201 with the green microalga *Chlorella sorokiniana*, Gatidou et al. (2019) derived a 72-h E_rC_{50} of 8.3 mg L^{-1} for BT. *C. sorokiniana* is not a species recommended by OECD TG 201, however the species is extensively used as model system. Therefore, results are considered relevant, and all validity criteria of OECD 201 were met. Exposure concentrations were not measured in the test. In the same research, concurrent hydrolysis experiments at 0.2 mg L^{-1} showed that BT was more or less stable in the dark. In the presence of light, BT was stable for about 2 days, after which a gradual decline was observed with a half-life time of 12 days, which decreased to 6.5 days in the presence of *C. sorokiniana*. Addition of sodium acetate slowed down the loss of BT, indicating a preference of micro-organisms for sodium acetate over BT. It is difficult to extrapolate these results to the toxicity test, since the latter experiment was performed at a low concentration and lasted for 16 days. Since test concentrations in the ecotoxicity test were at least 15 times higher, the results of the fate test were not used for interpretation of the ecotoxicity results. Therefore, the nominal E_rC_{50} of 8.3 mg L^{-1} is accepted as reliable with restrictions (R2) and as lowest value for algae used for EQS derivation.

Crustacea

The lowest valid and relevant acute effect concentration for crustacea is the 48-h EC_{50} of 15.8 mg L^{-1} for *Daphnia galeata* from Seeland et al. (2012) that was already used in the existing EQS derivations by RIVM and Switzerland. In the open literature, two new studies were found with *Daphnia magna*. Although not critical, the studies were assessed for

reliability. Also some (new) studies from the REACH registration dossiers of BT and NaBT are discussed below.

Giraudo et al. (2017) reported a 48-h EC_{50} of 93.3 mg L^{-1} for *Daphnia magna* exposed to BT, while Durjava et al. (2013) reported a 48-h EC_{50} of 155.4 mg L^{-1} for the same species. As important information is missing in both studies (for example nominal and measured test concentrations and control performance), the studies are assigned a score R4.

For *D. magna*, three studies in the REACH registration dossier of BT (ECHA, 2023a), with 48-h EC_{50} values of 91, 107 and 137 mg L^{-1}, were rated R4 in the Swiss evaluation, but are considered R2 in the present report because sufficient details are presented in the summaries to assess the reliability (e.g., study design, test conditions, and tabulated results).

In the REACH dossier on NaBT (ECHA, 2023d) multiple studies with NaBT and *Americamysis bahia* are available, and one study with NaBT and *D. magna*. For *A. bahia* 96-h LC_{50} values of 113.57 mg L^{-1} and 141.04 mg L^{-1} NaBT, equal to 95.9 and 119.1 mg L^{-1} BT, are given. For *D. magna* a 48-h LC_{50} of 195.57 mg L^{-1} NaBT, equal to 165.1 mg L^{-1} BT, is given. A broad spectrum of information is provided in most dossiers, however, in all summaries important information on the test material is missing. In all studies the LC_{50} for the active ingredient is ≥50% lower than for the formulation, however, no information is provided on the product formulations. The studies are therefore regarded R4 (reliability cannot be assessed).

Fish
Acute toxicity studies with fish were retrieved from the REACH registration dossiers for BT and NaBT, and from the open literature. In the Swiss derivation no reliable acute data for fish were available. Therefore, all new data are discussed below.

The nominal 96-h LC_{50} of 180 mg L^{-1} for *Danio rerio* from the REACH registration dossier for BT (ECHA, 2023a) was used by RIVM for the indicative EQS, but considered R4 (not assignable) in the Swiss evaluation (see section 3.1.2). According to the study summary, the test was performed according to OECD TG 203. Data on control performance and test conditions are provided in the summary and since daily renewal was applied, the absence of measurements is not considered as a crucial deficiency. Although the original report is not available for review, the information in the summary is considered sufficient to assign the LC_{50} as reliable for use in EQS derivation (R2). Moreover, the test was accepted with a rating of R1 in the CLH-report. However, lower LC_{50} values for this and other fish species were found in the registration dossier for NaBT and the open literature, and checked for reliability and relevance.

Damalas et al. (2018) exposed *D. rerio* embyros to BT in a test according to OECD TG 236 Fish Embryo Toxicity (FET). 3 hpf (hours post fertilisation) embryos were exposed for 96-h after which mortality was assessed. A LC_{50} of 170 mg L^{-1} was reported. While the study report and supporting information provide some details on the study design, most

information to assess the reliability is missing, for example test concentrations and conditions are missing and insufficient information is available to assess whether validity criteria were met. Therefore the study is scored R4.

Also Durjava et al. (2013) determined *D. rerio* embryo mortality according to the then draft OECD TG 236 Fish Embryo Toxicity (FET). Fertilised eggs were exposed to BT for 72 hours and embryo mortality was assessed. Tests were repeated three times, with reported LC_{50} values of 16, 2.63 and 0.67 mg L^{-1}. The large variation between repeated tests indicates that the test system and/or exposure conditions were not adequate in producing consistent results and results are thus considered not reliable (R3).

Shin et al. (2022) generated a 96-h LC_{50} value of 143.47 mg L^{-1} for BT for *Oryzias latipes* in a Fish Acute Toxicity Test according to OECD TG 203. While the chronic toxicity tests of these authors were assigned an R2 score (see below), the limited information provided for the acute test (e.g., mortality data for each test concentration is missing and it cannot be judged whether validity criteria were met) makes that the reliability could not be assessed. Therefore, a reliability score R4 (not assignable) was assigned.

Pillard et al. (2001) determined the toxicity of BT (and derivatives) to 5-7 d old *Pimephales promelas* and reported a 96-h LC_{50} of 65 mg L^{-1}. The study was rated R3 in the Swiss evaluation without further details, a reason might be that animals were fed during the experiment. Details on control performance and other test characteristics are not presented and therefore the study is scored R4.

The REACH registration dossier on NaBT (ECHA, 2023d) lists multiple studies with NaBT and fish. Two studies are available for the marine species *Menidia beryllina.* In the first, a 96-h LC_{50} of 30.75 mg L^{-1}, equivalent to 26 mg L^{-1} as BT (nominal), is reported. The test was performed according to EPA guidelines and validity criteria are reported to be met. The test was performed under static conditions, without analytical measurements. Details on performance in control and treatment groups are not given. Results based on tested formulation differ more than a factor of 2 from those based on the active ingredient, but no information is given on the formulation that was tested. The reliability of the study cannot be assessed on the basis of the information in the summary (R4). The same assessment and reliability score applies to another dossier study with NaBT and *M. beryllina*, for which a 96-h LC_{50} of 39.36 mg L^{-1}, equivalent to 33.2 mg L^{-1} BT, was found.

Also a study with NaBT and *Lepomis macrochirus* is available in the dossier. A 96-h LC_{50} of 75.85 mg L^{-1}, equivalent to 64 mg L^{-1} BT (nominal), is reported. The test was performed in accordance with the same EPA guideline and methods as described above for *M. beryllina*, and the same information is missing to assess reliability (R4).

For NaBT and *Pimephales promelas* a 96-h LC_{50} of 67.24 mg L^{-1}, equivalent to 56.8 mg L^{-1} BT (nominal), is reported in the REACH

registration dossier. Also for this study the reliability could not be assessed (R4), due to the same reasons as described above.

Lastly, the REACH dossier on NaBT reports a 96-h LC_{50} of 14.84 mg L^{-1} for the freshwater species *Oncorhynchus mykiss*, equivalent to 12.5 mg L^{-1} as BT (nominal). Also this test was performed according to EPA guidelines and validity criteria are reported to be met. The test was performed under static conditions, without analytical measurements. However, details on composition of the test substance and performance in control and treatment groups are not given. The reliability of the study cannot be assessed on the basis of the information in the summary (R4).

For *O. mykiss* and BT, the Swiss dossier (Oekotoxzentrum, 2015) gives a geometric mean LC_{50} of 21.6 mg L^{-1}, based on LC_{50} values of 39 and 12 mg L^{-1} from the IUCLID dossiers. Further information is not available and the individual studies could not be traced back to the current REACH dossiers (R4).

Platyhelminthes
Li (2013) performed an acute toxicity study with the freshwater planarian *Dugesia japonica* exposed to BT. No guideline was followed, but the test design is described in the article. *D. japonica* were exposed to BT for 96 h under static conditions (a series of five concentrations with five replicates per concentrations and five individuals per replicate). A 96-h LC_{50} of 142.9 mg L^{-1} (nominal) was reported for BT, no analytical measurements were performed. No toxicity was observed in the controls. The results were not considered in the Swiss EQS derivation for BT, however results for 5-methyl-1H-benzotriazole from the same study were considered in the EQS derivation for methylbenzotriazoles (R2). Based on the available information from the study report, the study is considered reliability with restrictions (R2). This is the only study available for this taxon and it is not critical for the EQS derivation.

Discussion of chronic ecotoxicity data

New chronic toxicity studies were retrieved from the REACH registration dossiers and the open literature, a brief summary of relevant information on algae, macrophytes and fish is presented below. For other taxa, no additional potentially critical effect concentrations were found. The REACH registration dossiers for BT and NaBT refer to the existing 21-d EC_{10} for *D. galatea* of 0.97 mg L^{-1} as key value.

Algae
As mentioned in section 3.1.2, the Swiss EQS-report (Oekotoxzentrum, 2015) uses a NOEC for *Desmodesmus subspicatus* of 4.76 mg L^{-1}. This is a geometric mean of 18.9 and 1.2 mg L^{-1}, which, according to the Swiss evaluation, are both based on growth rate. Because of the >10-fold difference between the two values, the underlying studies were consulted.

The higher NOEC of 18.9 mg L^{-1} originates from the study by Baumann et al. (2013) which is described above in the discussion of acute data. This concerns a 72-h algae test according to DIN EN ISO 8692 in 24-well

microplates with BT. The authors report the NOEC as 19 mg L^{-1}. Because of incomplete reporting on control performance and validity criteria, we consider the result to be at most reliable with restrictions (R2). It is also noted that no E_rC_{10} is reported by the authors. As mentioned in the section on acute data, the authors were contacted but raw data could not be obtained. To estimate the E_rC_{10} datapoints from the dose-response curve in the paper were retrieved (TechDig version 2.0d), concentrations were log-transformed, and plotted (GraphPad Prism 10.2.2). The E_rC_{10} was 49.5 mg L^{-1} (95% CI: 34.3-67.8) with an R^2 of 0.9926, and is considered R2.

The lower value of 1.2 mg L^{-1} originates from a test according to OECD TG 201 (version 2002) and BT by Seeland et al. (2012). Validity criteria were reported to be met, but from the publication it remains unclear if the NOEC is given for growth rate. Since the results section only specifies '80% lower cell numbers' and 'a complete stagnation of cell growth at the highest concentration', it may be that cell density (yield) was used as parameter. On the other hand, the REACH registration dossier (ECHA, 2023a) and the RAC-opinion (ECHA, 2022a) both refer to the corresponding E_rC_{10} of 1.18 mg L^{-1} from this study as being for growth rate (E_rC_{10}). As also no E_rC_{50} was given in the study report, the authors of the article were contacted. The raw data for the alga tests with BT (and 5-MeBT) were retrieved, and the data was examined before (re-)calculating E_rC_{10} and E_rC_{50}. The raw data showed that results were based on growth rate, but also that not all OECD TG 201 validity criteria were met. The mean coefficient of variation for section-by-section specific growth rates in the ten controls (pooled) was almost 70% and must not exceed 35%. Between 0-24 h the average specific growth rate of the controls was around 2.6 d^{-1}, between 24-48 h 0.4 d^{-1} and between 48-72 h 2.1 d^{-1}. Also the variation between the controls was 8.2%, and therefore higher than maximum 7% recommended by OECD TG 201. As there is uncertainty regarding the validity of the test, the results are considered R3.

Based on above assessment it also not considered justified to take the geometric mean of both *Desmodesmus*-studies, and the studies are listed separately in the overview table.

Besides the 72-h E_rC_{10} of 1.18 mg L^{-1} from Seeland et al. (2012), the REACH registration dossier on BT reports another 72-h E_rC_{10} of 58 mg L^{-1} for *D. subspicatus* exposed to BT. This value originates from the same study as the E_rC_{50} of 231 mg L^{-1} discussed earlier. In line with the acute endpoint, the E_rC_{10} is considered R4.

The 72-h E_rC_{10} of 10.5 mg L^{-1} (nominal) for *Raphidocelis subcapitata* and BT is included in the REACH registration dossier on BT. This value originates from the same test as the acute 72-h E_rC_{50} of 75 mg L^{-1} discussed above, and is also considered reliable with restrictions (R2).

The REACH registration dossier on NaBT (ECHA, 2023d) includes a 72-h E_rC_{10} of 56 mg L^{-1} (nominal), equivalent to 47.3 mg L^{-1} as BT. This value originates from the same test as the acute 72-h E_rC_{50} of 150 mg L^{-1} (126.6 mg L^{-1} as BT) discussed above, and is also considered reliable with restrictions (R2).

The new study with the green microalgae *C. sorokiniana* (Gatidou et al., 2019) is discussed above in the section on acute toxicity. It was not possible to derive a reliable chronic effect concentration from this study. From the supplementary information it can be deduced that <10% inhibition was recorded at exposure concentrations of 0.02 and 0.2 mg L^{-1} in a range-finding test, and ~12% at 2 mg L^{-1}. This indicates that the EC_{10} is somewhere between 0.2 and 2 mg L^{-1}. However, only three concentrations were tested, and insufficient information is available to derive a chronic effect concentration. In addition, an attempt to acquire additional information from the authors failed. Therefore, the study is assigned a score R4.

Concluding, the 72-h E_rC_{10} of 10.5 mg L^{-1} for *R. subcapitata* is considered the lowest reliable chronic effect concentration for the taxon algae.

Crustacea
The lowest valid and relevant chronic effect concentration is the 21-d EC_{10} of 0.97 mg L^{-1} for *Daphnia galeata* from Seeland et al. (2012) that was already used in the existing EQS derivations by RIVM and Switzerland (R2). A few new toxicity studies were found with *Daphnia magna*, which were assigned a reliability score R4. Giraudo et al. (2017) and Im et al. (2023) studied the chronic toxicity to *D. magna* and did not observe effects on offspring numbers at the highest concentrations tested (2 and 0.0043 mg L^{-1}, respectively). In another experiment of Im et al. (2023) effects were found at all test concentrations (NOEC < 15 mg L^{-1}). Both studies are not well reported, and the number of test concentrations is too low (R4).

Fish
Two new chronic toxicity studies with fish were retrieved. The lowest chronic effect concentration was found for *Danio rerio*. A NOEC of 1.07 mg L^{-1} for post-hatch survival 35 days after fertilization (35 dpf) was generated in a Fish Sexual Development Test with BT according to OECD TG 234 (ECHA, 2023a). Based on a CRED assessment, the study is given a reliability score of R2. In total, 5 concentrations were tested (0.10, 0.33, 1.07, 3.34 and 11.0 mg L^{-1}, mean measured). For the two highest test concentrations a reduced post-hatch survival was observed. In the REACH registration dossier, these effects are considered not relevant as post-hatch survival in the highest treatment (11.0 mg L^{-1}) reached 83.3%, which is higher than the validity criterion set for controls in OECD TG 234 (≥70%). We do not agree with this conclusion as the results from the test concentrations should be assessed in comparison with the results from the control(s), whereas the validity criteria of the test guideline serve to judge the control performance. At 35 dpf 15.8% fish of the died when exposed to 3.34 mg L^{-1} of BT and 16.3% when exposed to 11 mg L^{-1}, compared to 4% in the control. When exposed to 0.1, 0.33 and 1.07 mg L^{-1}, 7.5, 10 and 5.8% mortality was observed, respectively. These results indicate a concentration-dependent effect. It is noted that at 21 dpf and 60 dpf mortality was approximately equal in all test concentrations, except for the control and the lowest test concentration. However, for the endpoint post-hatch survival the results at 35 dpf are considered relevant. Therefore, the

NOEC is considered to be 1.07 mg L^{-1}, which is consistent with the RAC-opinion (ECHA, 2022a).

Shin et al. (2022) performed two (semi-)chronic fish toxicity tests with *Oryzias latipes*. As a preliminary range-finding test, a Fish Short-term Toxicity Test on Embryo and Sac-fry Stages according to OECD TG 212 was performed. In this test, a 13-d NOEC of 41 mg L^{-1} for development was found. Based on these results, a 42-d Fish Early-Life Stage Toxicity Test (ELS test) according to OECD TG 210 was performed. In this study a NOEC of 4 mg L^{-1} was derived for multiple endpoints (survival 30 dph, abnormality, length, and weight). From the OECD TG 210 study, also a 42-d LOEC of ≤0.04 mg L^{-1} was reported for condition index, calculated as the ratio between total weight and total length. In contrast to growth itself, this is not a regular endpoint for EQS derivation, since the population relevance of the ratio is not clear, therefore the endpoint is not taken into account for EQS derivation. Based on the CRED assessment, a reliability score of R2 (reliable with restrictions) was assigned to both studies, but the relevant effect concentrations (41 and 4 mg L^{-1}) are higher than the above derived NOEC of 1.07 mg L^{-1} for *D. rerio*.

Macrophytes
Both the REACH dossier and the RAC-opinion on BT (ECHA, 2022b, 2023a) refer to an EC_{10} of 3.94 mg L^{-1} for *Lemna minor* (duckweed) from Seeland et al. (2012), which was also used by RIVM for the indicative EQS. This study is rated R1 in the RAC-opinion, and considered reliable with restrictions in the Swiss EQS report. As for the results with algae (see above), no E_rC_{50} was provided in the study report and it was unclear whether results were reported for growth rate. Raw data was retrieved from the authors and examined. The data showed that validity criteria of OECD TG 221 were met, and that results were based on growth rate. E_rC_{50} and E_rC_{10} were (re-)calculated after log-transformation of concentrations by non-linear regression (GraphPad Prism 10.2.2). Recalculation led to an E_rC_{10} of 7.4 mg L^{-1} (95% CI: 2.4-12.2), which is around twice higher than the EC_{10} derived by the authors of the study report. Visual inspection showed that the E_rC_{10} of 7.4 mg L^{-1} better fits the data, and therefore the newly derived E_rC_{10} is considered reliable with restrictions (R2). Insufficient effects were recorded to derive an E_rC_{50}.

Appendix 4 Toxicity data methylbenzotriazoles

Table A4.1 Overview of ecotoxicity data for methylbenzotriazoles, including data for Methyl-1H-benzotriazole (MeBT; CAS no. 29385-43-1), 4-Methyl-1H-benzotriazole (4-MeBT; CAS no. 29878-31-7), 5-Methyl-1H-benzotriazole (5-MeBT; CAS no. 136-85-6) and Sodium 4(or 5)-methyl-1H-benzotriazolide (Na-4/5-MeBT; CAS no. 64665-57-2). Presented are the relevant reliable studies and studies of which we regard the reliability differently from the Swiss EQS derivation, supplemented with new and relevant data from the REACH registration dossiers and open literature. Results are expressed in mg L^{-1} of the respective benzotriazole compound (Na-4/5-MeBT is recalculated to MeBT). Lowest, or if relevant geometric mean, reliable and relevant effect concentrations per taxon are indicated on a grey background. The results are ordered by taxon and alphabetically by species.

ACUTE									
Substance	**Taxon**	**Species**	**Duration**	**Criterion**	**Value [mg L^{-1}]**	**Remarks**	**R**	**New**	**Source**
4-MeBT	Bacteria	*Vibrio fischeri*	15 min	EC_{50}	21	see text	2		Pillard et al. (2001) / Oekotoxzentrum (2016)
MeBT	Bacteria	*Vibrio fischeri*	15 min	EC_{50}	6.08	see text	2		Corsi et al. (2006) / Oekotoxzentrum (2016)
MeBT	Bacteria	*Vibrio fischeri*	15 min	EC_{50}	7.3	see text	2		Pillard et al. (2001) / Oekotoxzentrum (2016)
5-MeBT	Bacteria	*Vibrio fischeri*	5 min	EC_{50}	5.69	see text	2		Cancilla et al. (2003) / Oekotoxzentrum (2016)
5-MeBT	Bacteria	*Vibrio fischeri*	15 min	EC_{50}	5.91	see text	2		Cancilla et al. (2003) /

ACUTE									
Substance	**Taxon**	**Species**	**Duration**	**Criterion**	**Value [mg L^{-1}]**	**Remarks**	**R**	**New**	**Source**
									Oekotoxzentrum (2016)
5-MeBT	Bacteria	*Vibrio fischeri*	15 min	EC_{50}	4.25	see text	2		Cancilla et al. (2003) / Oekotoxzentrum (2016) / Cited in Corsi et al. (2006)
5-MeBT	Bacteria	*Vibrio fischeri*	15 min	EC_{50}	8.7	see text	2		Pillard et al. (2001) / Oekotoxzentrum (2016)
MeBT/ 4-MeBT/ 5-MeBT	Bacteria	*Vibrio fischeri*	15 min	EC_{50}	7.67	see text	2		Geometric mean of all reliable 15 min EC_{50}
5-MeBT	Algae	*Chlorella sorokiniana*	72 h	E_rC_{50}	22	see text	3	Y	Gatidou et al. (2019)
4-MeBT	Algae	*Desmodesmus subspicatus*	72 h	E_rC_{50}	58		2		Baumann et al. (2013) / Oekotoxzentrum (2016)
5-MeBT	Algae	*Desmodesmus subspicatus*	72 h	E_rC_{50}	149		2	Y	Baumann et al. (2013)
5-MeBT	Algae	*Desmodesmus subspicatus*	72 h	E_rC_{50}	71.9	see text	4	Y	ECHA (2023g)
MeBT	Algae	*Skeletonema costatum* (saltwater)	72 h	E_rC_{50}	53	see text	4	Y	ECHA (2023b)
MeBT	Crustacea	*Acartia tonsa* (saltwater)	48 h	LC_{50}	55	see text	4	Y	ECHA (2023b)

ACUTE									
Substance	**Taxon**	**Species**	**Duration**	**Criterion**	**Value [mg L^{-1}]**	**Remarks**	**R**	**New**	**Source**
Na-4/5-MeBT	Crustacea	*Americamysis bahia* (saltwater)	96 h	LC_{50}	38.5	see text	4	Y	ECHA (2023d)
MeBT	Crustacea	*Ceriodaphnia dubia*	48 h	EC_{50}	80.7		4		Corsi et al. (2006) / Oekotoxzentrum (2016)
5-MeBT	Crustacea	*Ceriodaphnia dubia*	48 h	EC_{50}	81.3	See text	4		Cancilla et al. (2003) / Oekotoxzentrum (2016) / Cited in Corsi et al. (2006)
5-MeBT	Crustacea	*Daphnia galeata*	48 h	EC_{50}	8.58		2		Seeland et al. (2012) / Oekotoxzentrum (2016) / ECHA (2022b)
5-MeBT	Crustacea	*Daphnia magna*	48 h	EC_{50}	51.6		2		ECHA (2022b) / Oekotoxzentrum (2016) / Seeland et al. (2012)
4-MeBT	Crustacea	*Daphnia magna*	48 h	EC_{50}	44		2		Baumann et al. (2013) / Oekotoxzentrum (2016)
5-MeBT	Crustacea	*Daphnia magna*	48 h	EC_{50}	49		2		Baumann et al. (2013) / Oekotoxzentrum (2016)

ACUTE									
Substance	**Taxon**	**Species**	**Duration**	**Criterion**	**Value [mg L^{-1}]**	**Remarks**	**R**	**New**	**Source**
5-MeBT	Crustacea	*Daphnia magna*	48 h	EC_{50}	50.89	see text	4	Y	Giraudo et al. (2017)
5-MeBT	Crustacea	*Daphnia magna*	48 h	EC_{50}	51.57	see text	2	Y	ECHA (2023g)
Na-4/5-MeBT	Crustacea	*Daphnia magna*	48 h	LC_{50}	180.2	see text	4	Y	ECHA (2023d)
MeBT	Pisces	*Cyprinodon variegatus* (saltwater)	96 h	LC_{50}	55	see text	4	Y	ECHA (2023b)
4-MeBT	Pisces	*Danio rerio*	96 h	LC_{50}	59	see text	4	Y	Damalas et al. (2018)
4-MeBT	Pisces	*Danio rerio* (embryo's)	48 h	EC_{50}	53	see text	4		Baumann et al. (2013) / Oekotoxzentrum (2016)
5-MeBT	Pisces	*Danio rerio* (embryo's)	48 h	EC_{50}	68	see text	4		Baumann et al. (2013) / Oekotoxzentrum (2016)
5-MeBT	Pisces	*Danio rerio*	96 h	LC_{50}	128	see text	4	Y	Damalas et al. (2018)
Na-4/5-MeBT	Pisces	*Lepomis macrochirus*	96 h	LC_{50}	64.4	see text	4	Y	ECHA (2023d)
Na-4/5-MeBT	Pisces	*Menidia beryllina* (saltwater)	96 h	LC_{50}	40	see text	4	Y	ECHA (2023d)
Na-4/5-MeBT	Pisces	*Oncorhynchus mykiss*	96 h	LC_{50}	14.6	see text	4	Y	ECHA (2023d)
5-MeBT	Pisces	*Pimephales promelas*	96 h	LC_{50}	22	see text	4	Y	Cancilla et al. (2003) / Oekotoxzentrum (2016) / cited in

ACUTE									
Substance	**Taxon**	**Species**	**Duration**	**Criterion**	**Value [mg L^{-1}]**	**Remarks**	**R**	**New**	**Source**
									Corsi et al. (2006)
MeBT	Pisces	*Pimephales promelas*	96 h	LC_{50}	30.1	see text	4		Corsi et al. (2006) / Oekotoxzentrum (2016)
Na-4/5-MeBT	Pisces	*Pimephales promelas*	96 h	LC_{50}	55.8	see text	4	Y	ECHA (2023d)
5-MeBT	Platyhelminthes	*Dugesia japonica*	96 h	EC_{50}	74.0	see text	2	Y	Li (2013) / Oekotoxzentrum (2016)

Chronic									
Substance	**Taxon**	**Species**	**Duration**	**Criterion**	**Value [mg L^{-1}]**	**Remarks**	**R**	**New**	**Source**
4-MeBT	Algae	Desmodesmus subspicatus	72 h	NOE_rC	7		2		Baumann et al. (2013) / ECHA (2022b) / Oekotoxzentrum (2016)
5-MeBT	Algae	Desmodesmus subspicatus	72 h	NOE_rC	7.5		2		Baumann et al. (2013)
5-MeBT	Algae	*Desmodesmus subspicatus*	72h	E_rC_{10}	2.86	see text	3		Seeland et al. (2012) / ECHA (2022b) / Oekotoxzentrum (2016)
5-MeBT	Algae	*Desmodesmus subspicatus*	72h	E_rC_{10}	11.73	see text	4	Y	ECHA (2023g)
MeBT	Algae	*Skeletonema costatum* (saltwater)	72 h	E_rC_{10}	36	see text	4	Y	ECHA (2023b)
5-MeBT	Crustacea	Daphnia galeata	21 d	EC_{10}	0.4	see text	2		Seeland et al. (2012) / ECHA (2022b) / Oekotoxzentrum (2016)
MeBT	Crustacea	Daphnia magna	21 d	NOEC	18.4		1		ECHA (2023e) / ECHA (2022b) / Oekotoxzentrum (2016)
4-MeBT	Crustacea	*Daphnia magna*	21 d	NOEC	7.5		2		Baumann et al. (2013) / Oekotoxzentrum (2016)

5-MeBT	Crustacea	*Daphnia magna*	21 d	NOEC	10		2		Baumann et al. (2013) / Oekotoxzentrum (2016)
5-MeBT	Crustacea	*Daphnia magna*	21 d	EC_{10}	5.93		2		Seeland et al. (2012) / ECHA (2022b) / Oekotoxzentrum (2016)
5-MeBT	Crustacea	*Daphnia magna*	21 d	NOEC	0.002	see text	3	Y	Giraudo et al. (2017)
5-MeBT	Macrophyta	*Lemna minor*	7 d	EC_{10}	2.11	new E_rC_{10} derived, see text	3		Seeland et al. (2012) / ECHA (2022b) / Oekotoxzentrum (2016)
5-MeBT	Macrophyta	*Lemna minor*	7 d	EC_{10}	11.0	newly derived, see text	2		Based on raw data from Seeland et al. (2012)

Discussion of acute toxicity data

Acute toxicity studies were retrieved from the REACH registration dossiers and the open literature for Methyl-1H-benzotriazole (MeBT; CAS no. 29385-43-1), 4-methylbenzotriazole (4-MeBT; CAS no. 29878-31-7), 5-Methyl-1H benzotriazole (5-MeBT; CAS no. 136-85-6) and Sodium 4(or 5)-methyl-1H-benzotriazolide (NaMeBT; CAS no. 64665-57-2). In addition, data from the Swiss EQS derivation was taken into account. A brief summary of relevant data per taxa is presented below. Most information is given for bacteria as this taxon is critical for the EQS derivation.

Bacteria
The most critical effect concentration for the EQS derivation in the Swiss report (Oekotoxzentrum, 2016) is the 15 min EC_{50} of 4.25 mg L^{-1} for V*ibrio fischeri*, which received a reliability score of R2. For that reason, the underlying studies by Cancilla et al. (2003) and Corsi et al. (2006) were retrieved and re-assessed for reliability.

The results section of Cancilla et al. (2003) mentions that the 5-MeBT EC_{50} of 4.25 mg L^{-1} for *V. fischeri* is determined in a laboratory toxicity study, but the methods section describing the toxicity tests with 5-MeBT does not specify any information that such test with the compound is performed. Execution of a Microtox assay with *V. fischeri* is only briefly mentioned as a side note under the methods section on toxicity testing with field-collected water samples. Based on the similarity in results for *V. fischeri* (see brief discussion of other results below), the results are expected to be for 5-MeBT and are considered to be reliable with restrictions (R2).

For 5-MeBT, Corsi et al. (2006) report exactly the same $L(E)_{50}$ values and 95% confidence intervals for the Microtox-test, *P. promelas* and *C. dubia* as Cancilla et al. (2003). Cancilla et al. (2003) are not cited as the source of these results, but since the same co-authors are involved in both studies and the study area is the same, it is concluded that both references refer to the same experiments. Corsi et al. (2006) does not give much additional information either. These authors also report a 15 min EC_{50} of 6.08 mg L^{-1} for the mixture of 4-MeBT and 5-MeBT, and the result is considered R2 as it is a highly standardised test.

There is another toxicity study with V*. fischeri* which was scored R2 in the Swiss report. In an earlier study, (Cancilla et al., 1997) report results of a triplicate Microtox assay with 5-MeBT, with an average 5 min EC_{50} of 5.69 mg L^{-1} (average 15 min EC_{50} of 5.91 mg L^{-1}). Also here limited information is provided to check the reliability, but the results are considered R2.

One study with *V. fischeri* toxicity data was scored R4 by the Swiss. Pillard et al. (2001) tested different benzotriazole compounds on *V. fischeri* with the microtox assay, with average 15 min EC_{50} of 21 mg L^{-1} for 4-MeBT, 8.7 mg L^{-1} for 5-MeBT and 7.3 mg L^{-1} for a 1:1 mixture of 4-MeBT and 5-MeBT. Results are based on measured concentrations, which were within 8% of the nominal concentrations. In view of the comparable lack of detail in reporting, it is not clear why this study was

rated R4 instead of R2 in the Swiss evaluation. We consider the study results as R2.

As multiple reliable results are available for *V. fischeri* the geometric mean of the 15-min EC_{50} was determined and used for EQS-derivation. The geometric EC_{50} is 7.67 mg L^{-1}.

Algae
None of the algae studies are critical for acute EQS derivation, however for the newly found studies a brief reliability assessment is given below.

Gatidou et al. (2019) report an 72-h E_rC_{50} of 22 mg L^{-1} for growth rate of the green alga *C. sorokiniana* after exposure to 5-MeBT. This study is considered reliable with respect to the results for benzotriazole (BT, see Appendix 3). However, effect concentrations for 5-MeBT are less certain in view of the faster degradation observed in the parallel fate experiment. After incubation of 0.2 mg L^{-1} for 16 days in the dark or under light, decline of 5-MeBT was comparable with BT (stable for about 2 days, followed by gradual decline with a half-life time of 12 days). However, a half-life time of 2.4 days was estimated in the presence of algae (and light). It is noted that the test concentration in the fate experiment is much lower than the observed E_rC_{50}, and degradation may have been lower at the level of the E_rC_{50} due to the toxic effect of the test compound. Still, actual concentrations at the level of the E_rC_{50} were likely lower than the reported 22 mg L^{-1} nominal, because of which the study is rated R3. However, it is unlikely that the E_rC_{50} would be lower than the existing most sensitive acute endpoint of 4.25 mg L^{-1} for *Vibrio fischeri* used by Oekotoxzentrum (2016).

An OECD 201 study with *Desmodesmus subspicatus* is available in the REACH registration dossier on 5-MeBT (ECHA, 2023g). The algae are exposed to 5-MeBT for 72 hours and the 72-h E_rC_{50} of 71.9 mg L^{-1} was based on measured concentrations. All validity criteria were met according to the dossiers, and the deviations specified in the dossier are not critical for reliability. Results per test concentration are not given, therefore the endpoint cannot be assessed for reliability (R4).

The REACH registration dossier on MeBT (ECHA, 2023b) contains a 72-h study with the marine species *Skeletonema costatum*. A 72-h E_rC_{50} of 53 mg L^{-1} (nominal) for the whole product was found in a test according to ISO 10253. Granules with MeBT were prepared in line with a method for water insoluble test substances and the water accommodated fraction was used for testing. Validity criteria were met. No analytical measurements were performed, in combination with the insolubility of the test material and missing information on compositional properties of the granules, the results cannot be assessed for reliability (R4). It is however noted that the study is scored R1 in the RAC-opinion (ECHA, 2022b). Unfortunately, the authors were not able to gain additional information on the reliability assessment by the RAC.

Crustacea
None of the crustacea studies are critical for the acute EQS derivation, however, for the newly found studies a short reliability assessment is given below.

The REACH registration dossier on MeBT (ECHA, 2023b) contains a 48-h study with the marine species *Acartia tonsa*, with as result a 48-h LC_{50} of 55 mg L^{-1}. ISO guideline 14669 to determine toxicity to marine copepods was followed and according to the dossier validity criteria were met. Results are presented per test container and concentration. Information on the purity of the tested granules is missing and concentrations in water were not measured. As the substance is insoluble in water, it is not clear whether the nominal concentrations are reliable. Therefore, the study is scored R4. It is however noted that the study is scored R1 in the RAC-opinion (ECHA, 2022b). Also for this study the authors could not retrieve additional information on the reliability assessment by the RAC.

The REACH registration dossier on NaBT contains two invertebrate studies on Na-4/5-MeBT, while these studies are not available in the dossier of Na-4/5-MeBT. The first one is a study with *Americamysis bahia*, for which a 96-h LC_{50} of 44.9 mg L^{-1}, equivalent with 38.5 mg L^{-1} MeBT, is calculated. The other study is performed with *Daphnia magna*, for which a 96-h LC_{50} of 210 mg L^{-1}, equivalent with 180.2 mg L^{-1} MeBT, is found. The summaries of both studies are too brief to assess the reliability (R4).

In the REACH registration dossier on 5-MeBT (ECHA, 2023g) a 48-h EC_{50} of 51.57 mg L^{-1} is available for *Daphnia magna* exposed to 5-MeBT. The 2019 study was performed in accordance with OECD TG 202 and all validity criteria were met. Information on test organism, test conditions and measured concentrations is given. Test concentrations were within 102-105% of nominal concentrations. Although results per test concentration are missing, in the details it is specified that at the two highest concentrations between 20 and 100% effects were found. Based on this information and the calculated EC_{50} is considered reliable with restrictions (R2).

Lastly a 48-h EC_{50} of 50.89 for 5-MeBT is available from Giraudo et al. (2017). As for the test with BT (see Appendix 3), the results are considered R4.

As the results for the V. *fischeri* from Cancilla et al. (2003) were reassessed, also the toxicity test with 5-MeBT and *Ceriodaphnia dubia* was evaluated, even though the study is not critical for EQS derivation. For *C. dubia* some information on test conditions is given for the toxicity test with field-collected water, but it is not clear whether the same conditions apply to the test with 5-MeBT. As very limited information is given on test design and results, the result for *C. dubia* (EC_{50} of 81.3 mg L^{-1}) is considered R4. It is noted that in the Swiss EQS-derivation the result is considered R2, however, insufficient information is available to compare the differences in scoring.

Fish
None of the fish studies are critical for the acute EQS derivation, however, for the completeness of acute data the fish studies are (re)assessed for reliability.

In the Swiss derivation multiple acute studies with fish are considered reliable.
The lowest, reliable effect concentrations for fish recorded in the report of the Swiss is the 96-h LC_{50} of 22 mg L^{-1} for *Pimephales promelas* and 5-MeBT from Cancilla et al. (2003). The study was therefore reassessed for reliability. Cancilla et al. (2003) conducted a 28-d study with laboratory-fortified water containing 5-MeBT to assess the accumulation of 5-MeBT in fish tissue. In the study design of the test it is mentioned that survival was recorded and that tissue of the surviving fish were analysed for 5-MeBT. The information seems to imply that survival was recorded at 28-d. Also a 96-h test with field-collected water was performed by the authors, and the recorded LC_{50} may have been derived based on these test results. No raw data is available to check the endpoint. As the 96-h test is considered unreliable and as it is unclear for which study the 96-h LC_{50} is reported, the study is scored R4.

As with the data for bacteria and crustacea (see above), the 96-h LC_{50} for *Pimephales promelas* and 5-MeBT is also reported by Corsi et al. (2006). In addition a 96-h LC_{50} of 30.1 mg L^{-1} for exposure to MeBT is given in Corsi et al. (2006). Corsi et al. (2006) performed acute toxicity tests in line with an US EPA Test Method 2002. Initial environmental conditions are reported and it is stated that environmental conditions were measured daily. However, the authors do not report test concentrations, measured environmental conditions and results per test concentration. Therefore insufficient information is available to assess the results (R4). The Swiss consider the results as R2, also for this study insufficient information is available to explain the differences in the reliable assessment.

Another study considered reliable by the Swiss report is the study of Baumann et al. (2013). In this study *Danio rerio* embryos were exposed for 48-h to 4-MeBT and 5-MeBT, with 48-h LC_{50} values of 53 and 68 mg L^{-1} respectively. It is reported that the study is conducted according to DIN EN ISO 15088, but also here no information on test concentrations, environmental conditions and results per test concentration is given. Therefore the results cannot be assessed for reliability, and the results are scored R4 in this report.

The Swiss report further considers multiple studies from IUCLID as reliable. Therefore the studies available in the REACH registration dossier were also (re-)assessed.

In the REACH registration dossier for MeBT (ECHA, 2023b) only one experiment with MeBT is available. An 96-h LC_{50} of 55 mg L^{-1} for *Cyprinodon variegatus* was derived, but this study result is not given in the Swiss EQS-derivation. The test with *C. variegatus* was performed in accordance with the modified OECD 203 test guideline and validity criteria were met according to the dossier. Purity of the tested granules is not given. As the test substance was insoluble in water, the water accommodated fraction (WAF) was used for testing, and the water was renewed after 48 hours. The result is expressed as nominal whole test substance. Although the registration dossier contains sufficient information on test conditions and results, the study is considered R4 due to insufficient information on the test material. It is not clear why

the granules are insoluble, while the substance itself is sufficiently soluble for testing. Preparation of the nominal concentrations is only described briefly, and no information is available to verify these nominal concentrations. Also no analytical measurements were performed, therefore exposure concentrations cannot be verified. It is, however, noted that the study is scored R1 in the RAC-opinion (ECHA, 2022b), and also for this study the authors could not gain additional information that explains the difference in the reliability score.

The REACH registration dossier on NaBT contains four fish studies on Na-4/5-MeBT, which are not recorded in the dossier of Na-4/5-MeBT. All fish studies are also not included in the Swiss EQS-derivation. Studies are available with the freshwater species *Lepomis macrochirus*, *Oncorhynchus mykiss* and *Pimephales promelas*, and the marine species *Menidia beryllina.* For all studies details are missing to assess reliability (e.g. results for test material and active ingredient without information on purity of the test material, whether validity criteria were met, and results per tested concentrations), therefore all studies are assigned a reliability score R4.

Lastly, a new endpoint is available from Damalas et al. (2018). The study of Damalas et al. (2018) was also discussed in Appendix 3 as *D. rerio* were exposed to different benzotriazoles. The same reliability score (R4) applies to the results for 4-MeBT (96-h LC_{50} of 59 mg L^{-1}) and 5-MeBT (96-h LC_{50} of 128 mg L^{-1}).

Although none of the acute fish studies is assessed as reliable (R1) or reliable with restrictions (R2), the range of available effect concentrations indicates that fish are less sensitive to methylbenzotriazoles than bacteria. This information is used to support the selection of the assessment factor in the EQS-derivation.

Platyhelminthes
Li (2013) performed an acute toxicity study with the freshwater planarian *Dugesia japonica* and 5-MeBT and a 96-h LC_{50} of 74.0 mg L^{-1} (nominal) was found. In the same study, also a test was performed with BT, and therefore this study is also discussed in Appendix 3. Based on the available information the study is rated as R2. This study is the only study available with platyhelminthes and is not critical for EQS derivation.

Discussion of chronic ecotoxicity data

No chronic toxicity studies with fish were retrieved. Recent chronic data were found for algae, a crustacean and a macrophyte. The effect concentrations from these studies are all higher than the most sensitive chronic effect concentration for 5-MeBT used by Oekotoxzentrum (2016), but some discrepancies were noted when consulting the same studies for benzotriazole. Therefore, relevant studies are briefly described.

Algae
For algae, the Swiss used a NOEC of 4.2 mg L^{-1} for *D. subspicatus* (Oekotoxzentrum, 2016). This is a geometric mean of the NOECs of 2.5

mg L^{-1} (5-MeBT) derived by Seeland et al. (2012) and 7.5 mg L^{-1} (4-MeBT) from Baumann et al. (2013). Both studies were already discussed in detail for benzotriazole (BT, see Appendix 3), and because of the concerns with control validity the results for BT from Seeland et al. (2012) were considered unreliable (R3). As the same concerns apply to the results for 5-MeBT, these are also considered R3. The results are therefore split in our EQS-derivation, and since preference is given to an EC_{10} over a NOEC, the E_rC_{10} of 2.86 mg L^{-1} is given in the overview table.

As for BT, the raw data for the alga tests with 4-MeBT and 5-MeBT could not be obtained, and therefore insufficient data is available to check the reliability of the effect concentrations. However, the visual check of the results for BT and *D. subspicatus* in figure 2 in the scientific article shows that the results for BT are plausible. Therefore the results for the other two compounds are also considered R2. In absence of raw data no E_rC_{10} could be derived.
Another chronic effect concentration for *D. subspicatus*, a 72-h E_rC_{10} of 11.73 mg L^{-1}, is recorded in the REACH registration dossier on 5-MeBT (ECHA, 2023g). However, as for the acute effect concentration (see section above), the chronic effect concenration cannot be assessed for reliability (R4). The same applies to the 72-h E_rC_{10} of 36 mg L^{-1} for MeBT and *Skeletonema costatum* from the registration dossier; also here the assessment is in line with the assessment for the acute endpoint, and considered R4.

Crustaceans
With respect to the lowest NOEC of 1 mg L^{-1} for *D. galeata* reported by Oekotoxzentrum (2016), it was already noted that the underlying study of Seeland et al. (2012) reports an EC_{10} of 0.4 mg L^{-1} (see section 4.1). It is recognised that the EC_{10} is less reliable due to the fact that effects were observed at the highest concentration only. However, from the graphs it appears that the NOEC of 1 mg L^{-1} corresponds with ~25% reduction in neonates as compared to the control. Using the EC_{10} of 0.4 mg L^{-1} would be more appropriate, especially since this was also done for BT tested in the same study. Moreover, the REACH registration dossier and RAC-opinion for MeBT also use the EC_{10} (ECHA, 2022b; 2023b). The study is assessed as reliable with restrictions (R2).

For the crustacean *Daphnia magna*, Giraudo et al. (2017) found a significant increase in molting frequency after exposure for 21 days to 5-MeBT at 2 mg L^{-1}. A slight but not significant increase was found at the next lower concentration of 0.002 mg L^{-1}. The spacing between the concentrations is very large and the implications of increased molting frequency are not clear (R3). Since no effects on growth (body length) and number of neonates were found, it is considered that effect concentrations for this species are not lower than those already included for *D. galeata* in the existing dataset.

Macrophyta
It is noted that the REACH registration dossier on MeBT (ECHA, 2023b) refers to a 7-d E_rC_{10} of 2.11 mg L^{-1} for *L. minor* from Seeland et al. (2012). This effect concentration was disregarded in the Swiss evaluation because of uncertainty about the maintenance of test

concentrations in the static test. It was anticipated that photodegradation could have occurred. It is noted, however, that the E_rC_{10} for *L. minor* is lower than the lowest available EC_{10} for algae of 2.86 mg L^{-1} obtained by the same authors and also based on nominal concentrations. As for BT (see Appendix 3) raw data was obtained and E_rC_{50} and E_rC_{10} were (re-)calculated after log-transformation of concentrations by non-linear regression (GraphPad Prism 10.2.2). Recalculation led to an E_rC_{10} of 11.0 mg L^{-1} (95% CI: 6.3-29.6), which is around 5 times higher. As for BT the recalculated value is considered reliable with restrictions (R2), and replaces the original value (R3). Insufficient effects were recorded to derive an E_rC_{50}. Replacing the E_rC_{10} has no effect on the EQS derivation, since a lower EC_{10}-value was obtained for *D. galeata*. It is noted that the original study result is considered reliable with restrictions (R2) in the RAC-opinion on MeBT (ECHA, 2022b).

Appendix 5 Toxicity data 1-hydroxybenzotriazole

Table A5.1 Overview of ecotoxicity data for 1-hydroxybenzotriazole. The results are ordered by taxon and alphabetically by species.

ACUTE data					
Species	**Duration**	**Parameter**	**Value [mg L^{-1}]**	**Remarks**	**Reference**
Algae					
Desmodesmus subspicatus	72 h	E_rC_{50}	87.3	OECD TG 201 study	ECHA (2023e)
Crustacea					
Daphnia magna	48 h	EC_{50}	75.5	OECD TG 202 study	ECHA (2023e)
Pisces					
Danio rerio	96 h	LC_{50}	>100	OECD TG 203 study	ECHA (2023e)

CHRONIC data					
Species	**Duration**	**Parameter**	**Value [mg L^{-1}]**	**Remarks**	**Reference**
No Data					

Appendix 6 Toxicity data sodium 5-N-butylbenzotriazole

Table A6.1 Overview of ecotoxicity data for sodium 5-N-butylbenzotriazole. The results are ordered by taxon and alphabetically by species. Results are expressed in mg L^{-1} for the non-salt form 5-butyl-1H-benzotriazole.

ACUTE data					
Species	**Duration**	**Parameter**	**Value [mg L^{-1}]**	**Remarks**	**Reference**
Bacteria					
Vibrio fischeri	15 min	EC_{50}	0.88		Pillard et al. (2001)
Algae					
Raphidocelis subcapitata	72 h	E_rC_{50}	5.04	72h E_rC_{50} salt: 5.7 mg L^{-1} OECD TG 201 study	ECHA (2023f)
Crustacea					
Daphnia magna	48 h	EC_{50}	5.65	48h EC_{50} salt: 6.4 mg L^{-1} OECD TG 202 study	ECHA (2023f)
Ceriodaphnia dubia	48 h	LC_{50}	1.1		Pillard et al. (2001)
Pisces					
Oncorhynchus mykiss	96 h	LC_{50}	2.47	96h LC_{50} salt: 2.8 mg L^{-1} OECD TG 203 study	ECHA (2023f)
Pimephales promelas	96 h	LC_{50}	3.3		Pillard et al. (2001)

CHRONIC data					
Species	**Duration**	**Parameter**	**Value [mg L^{-1}]**	**Remarks**	**Reference**
Algae					
Raphidocelis subcapitata	72h	NOE_rC	0.24	OECD TG 201 study 72h E_rC_{10}: 0.72-1.3 mg L^{-1} 72h NOEC salt: 0.27 mg L^{-1}	ECHA (2023f)

Published by

National Institute for Public Health and the Environment, RIVM
P.O. Box 1 | 3720 BA Bilthoven
The Netherlands
www.rivm.nl/en

August 2025

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