

TEXTE

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# Derivation of Environmental Quality Standards for 2,4,6- Trinitrotoluene

by:

Dieter Schudoma

German Environment Agency

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On behalf of the German Environment Agency

## Imprint

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## **Foreword**

The following EQS dossier was created according to the Technical Guidance for Deriving Environmental Quality Standards. Guidance Document No. 27 (European Commission, 2018) to support the project CONMAR (CONcepts for conventional MARine MUnition Remediation in the German North and Baltic Sea). The tentative Quality Standards derived in the dossier serve as reference values for the risk assessment of munition compounds released from the dumping areas of corroding munition.

**Abstract: Derivation of Environmental Quality Standards for 2,4,6-Trinitrotoluene**

To assess the risks posed by legacy munition contamination, preliminary quality standards for TNT (2,4,6-trinitrotoluene) were developed for the protected resources of pelagic communities in freshwater and seawater, for human health via fish consumption, and for drinking water. The quality standard for the pelagic community (freshwater and seawater) is the "critical quality standard" for deriving the proposed environmental quality standard (EQS).

To protect aquatic communities in freshwater and saltwater, an annual average environmental quality standard (AA-EQS) of 0.15 µg/L and a maximum acceptable concentration (MAC-EQS) of 1.8 µg/L are proposed for TNT.

Data for TNT degradation products (2-ADNT and 4-ADNT) show that, for many species, the toxicity is similar to that of TNT. Assuming concentration additivity, the proposed EQS for TNT can also be applied to the combined concentration of TNT, 2-ADNT, and 4-ADNT as a preliminary assessment. However, this requires further investigation.

**Kurzbeschreibung: Ableitung von Umweltqualitätsnormen für 2,4,6-Trinitrotoluol**

Zur Bewertung der Risiken von Rüstungsaltslasten wurden vorläufige Qualitätsstandards für TNT (2,4,6-Trinitrotoluol) für die Schutzgüter Pelagiale Lebensgemeinschaften in Süßwasser, Meerwasser, Gesundheit des Menschen via Fischkonsum und Trinkwasser erarbeitet. Der Qualitätsstandard für die pelagische Gemeinschaft (Süß- und Meerwasser) ist der „kritische QS“ für die Ableitung des vorgeschlagenen Umweltqualitätsstandards.

Zum Schutz der Aquatischen Lebensgemeinschaften in Süßwasser und Salzwasser wird für TNT eine Jahresdurchschnitt-Umweltqualitätsnorm (JD-UQN/AA-EQS) von 0.15 µg/L und eine Zulässige Höchstkonzentration (ZHK-UQN/MAC-EQS) von 1.8 µg/L vorgeschlagen.

Die Daten für Abbauprodukte von TNT (2-ADNT und 4-ADNT) zeigen, dass für viele Spezies eine ähnliche Toxizität wie für TNT besteht. Unter der Annahme einer Konzentrationsadditivität kann als vorläufige Bewertung die vorgeschlagene UQN für TNT auch auf die Konzentration der Summe von TNT, 2-ADNT und 4-ADNT angewendet werden. Dies muss jedoch durch weitere Untersuchungen bestätigt werden.

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## List of abbreviations

Abbreviation	Explanation
AA-EQS	Annual average environmental quality standard (long-term EQS)
AA-QS <sub>fw, eco</sub>	Annual average quality standard for freshwater community
AA-QS <sub>sw, eco</sub>	Annual average quality standard for saltwater pelagic community
ADI	Acceptable daily intake
2-ADNT	2-Amino-2,6-dinitrotoluene
4-ADNT	4-Amino-2,6-dinitrotoluene
AF	Assessment factor
BCF	Bioconcentration factor
bw	Body weight
C&L	Classification and Labelling
CMR	Carcinogenic, mutagenic and reprotoxic
2,6-DNT	2,6-Dinitrotoluene
dw	Dry weight
ECHA	European Chemicals Agency
EC <sub>x</sub>	Concentration at which x% effect is observed
EqP	Equilibrium partitioning
EQS	Environmental quality standard
E <sub>r</sub> C <sub>x</sub>	Concentration of the test substance which results in an x % (e.g. 50%) reduction in growth rate relative to the control within a stated exposure period
EU	European Union/ Europäische Union
HC <sub>5</sub>	Hazardous concentration for 5% of species in a SSD
hh	Human health
IRIS	Integrated Risk Information System   US EPA
IUPAC	International union of pure and applied chemistry
JD-UQN	Jahresdurchschnitt-Umweltqualitätsnorm (corresponds to AA-EQS)
K <sub>oc</sub>	Organic carbon-water partition coefficient
K <sub>ow</sub>	Octanol-water partition coefficient
LC <sub>x</sub>	Concentration at which x % mortality is observed
LOEC	Lowest observed effect concentration: lowest concentration tested at which the measured parameter shows significant inhibition relative to the control
LW <sub>tw</sub>	Trinkwasser-Leitwert (drinking water guidance value)
MAC-EQS	Maximum acceptable concentration (short-term EQS)

Abbreviation	Explanation
<b>NOAEL</b>	No observed adverse effect level
<b>NOEC</b>	No observed effect concentration: highest tested concentration for which the observed effect is not significantly different to the controls
<b>PBT</b>	Persistent, bioaccumulative and toxic substances
<b>PNEC</b>	Predicted no effect concentration
<b>POP</b>	Persistent organic pollutants
<b>PT</b>	Product type
<b>QS</b>	Quality standard
<b>QS<sub>biota, hh, food</sub></b>	Quality standard for humans for the protection against adverse health effects from consuming fisheries products
<b>QS<sub>biota, secpois</sub></b>	Quality standard for biota to protect against secondary poisoning of predators
<b>QS<sub>dw, hh</sub></b>	Quality standard for human health via consumption of drinking water
<b>SSD</b>	Species sensitivity distribution
<b>STOT-RE</b>	Specific target organ toxicity - repeat exposure
<b>T</b>	toxic
<b>TGD-EQS</b>	Technical guidance for deriving environmental quality standards No. 27
<b>TL<sub>hh</sub></b>	Threshold level for human health
<b>TNT</b>	2,4,6-trinitrotoluene
<b>UBA</b>	Umweltbundesamt Deutschland (German Environment Agency)
<b>uptake<sub>dw</sub></b>	Daily uptake of drinking water
<b>UQN</b>	Umweltqualitätsnorm (German expression for EQS)
<b>WGK</b>	Wassergefährdungsklasse (water hazard class)
<b>WHO</b>	World Health Organisation
<b>ZHK-UQN</b>	Zulässige Höchstkonzentration (corresponds to MAC-EQS)

## Summary

TNT (2,4,6-trinitrotoluene) is primarily used as an explosive. Enormous quantities are present in the North and Baltic Seas in unexploded and dumped munitions (Beck et al., 2018). Significant emissions of TNT into the marine environment are expected in the coming decades due to the corrosion of munition casings. TNT exhibits low water solubility and moderate mobility. Relevant inputs via volatilization and deposition are not anticipated due to TNT's physicochemical behavior. Because of its low octanol-water partition coefficient ( $\log K_{ow} < 3$ ), a high bioaccumulation potential is not expected. This is confirmed by the available bioaccumulation data. TNT is poorly biodegradable in water. The biodegradation of TNT in the water column occurs much more slowly than in sediments. The most important metabolites produced during the degradation of TNT are 2-amino-4,6-dinitrotoluene (2-ADNT), 4-amino-2,6-dinitrotoluene (4-ADNT), 2,4-diaminonitrotoluene (2,4-DANT), and 2,6-diaminonitrotoluene (2,6-DANT).

To assess the risks posed by legacy munition contamination, preliminary quality standards for TNT (2,4,6-trinitrotoluene) were developed for the protected resources of pelagic communities in freshwater and seawater, for human health via fish consumption, and for drinking water. The quality standard for the pelagic community (freshwater and seawater) is the "critical quality standard" for deriving the proposed environmental quality standard (EQS).

To protect aquatic communities in freshwater and saltwater, an annual average environmental quality standard (AA-EQS) of 0.15  $\mu\text{g/L}$  and a maximum acceptable concentration (MAC-EQS) of 1.8  $\mu\text{g/L}$  are proposed for TNT.

Data for TNT degradation products (2-ADNT and 4-ADNT) show that, for many species, the toxicity is similar to that of TNT. Assuming concentration additivity, the proposed EQS for TNT can also be applied to the combined concentration of TNT, 2-ADNT, and 4-ADNT as a preliminary assessment. However, this requires further investigation.

## Zusammenfassung

TNT (2,4,6-Trinitrotoluol) wird hauptsächlich als Sprengstoff verwendet. In Nord- und Ostsee sind enorme Mengen von nicht explodierter Munition und versenkter Munition vorhanden (Beck et al., 2018). In den kommenden Jahrzehnten ist aufgrund der Korrosion der Munitionshülsen mit erheblichen TNT-Emissionen in die Meeresumwelt zu rechnen. TNT weist eine geringe Wasserlöslichkeit und moderate Mobilität auf. Relevante Stoffeinträge via Verflüchtigung und Deposition sind aufgrund des physikalisch-chemischen Verhaltens von TNT nicht zu erwarten. Aufgrund seines geringen Octanol-Wasser-Verteilungskoeffizienten ( $\log K_{ow} < 3$ ), ist nicht mit einem hohen Bioakkumulationspotenzial zu rechnen. Dies wird durch die vorliegenden Daten zur Bioakkumulation bestätigt. TNT ist im Wasser schlecht biologisch abbaubar. Der biologische Abbau von TNT in der Wassersäule erfolgt viel langsamer als in Sedimenten. Die wichtigsten Metabolite, die beim Abbau von TNT entstehen, sind 2-Amino-4,6-dinitrotoluol (2-ADNT), 4-Amino-2,6-dinitrotoluol (4-ADNT), 2,4-Diaminonitrotoluol (2,4-DANT) und 2,6-Diaminonitrotoluol (2,6-DANT).

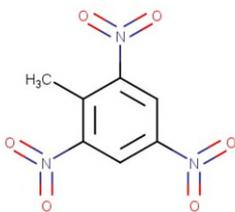
Zur Bewertung der Risiken von Rüstungsaltslasten wurden vorläufige Qualitätsstandards für TNT (2,4,6-Trinitrotoluol) für die Schutzgüter Pelagiale Lebensgemeinschaften in Süßwasser, Meerwasser, Gesundheit des Menschen via Fischkonsum und Trinkwasser erarbeitet. Der Qualitätsstandard für die pelagische Gemeinschaft (Süß- und Meerwasser) ist der „kritische QS“ für die Ableitung des vorgeschlagenen Umweltqualitätsstandards.

Zum Schutz der Aquatischen Lebensgemeinschaften in Süßwasser und Salzwasser wird für TNT eine Jahresdurchschnitt-Umweltqualitätsnorm (JD-UQN/AA-EQS) von 0.15 µg/L und eine Zulässige Höchstkonzentration (ZHK-UQN/MAC-EQS) von 1.8 µg/L vorgeschlagen.

Die Daten für Abbauprodukte von TNT (2-ADNT und 4-ADNT) zeigen, dass für viele Spezies eine ähnliche Toxizität wie für TNT besteht. Unter der Annahme einer Konzentrationsadditivität kann als vorläufige Bewertung die vorgeschlagene UQN für TNT auch auf die Konzentration der Summe von TNT, 2-ADNT und 4-ADNT angewendet werden. Dies muss jedoch durch weitere Untersuchungen bestätigt werden.

# 1 Chemical Identity

**Table 1: Chemical identity 2,4,6-Trinitrotoluol**

<b>Common name</b>	2,4,6-Trinitrotoluol
<b>Chemical name (IUPAC)</b>	2-methyl-1,3,5-trinitrobenzene
<b>Synonym(s)</b>	TNT
<b>Chemical class (when available/relevant)</b>	
<b>CAS number</b>	118-96-7
<b>EU number</b>	204-289-6
<b>Molecular formula</b>	C <sub>7</sub> H <sub>5</sub> N <sub>3</sub> O <sub>6</sub>
<b>Molecular structure</b>	 <p>(ECHA, 2024)</p>
<b>Molecular weight (g mol<sup>-1</sup>)</b>	227.13 (NCBI, 2021)

## 2 Existing Evaluations and Regulatory Information

**Table 2: Existing Evaluations and Regulatory Information for TNT**

<b>Annex II EQS Dir. (2013/39/EC)</b>	Not included
<b>Existing Substances Reg. (793/93/EC)</b>	Not applicable
<b>Plant protection products regulation (Regulation (EC) No 1107/2009)</b>	Not included in Annex I
<b>Biocides (Regulation (EU) No 528/2012)</b>	Not included in Annex I
<b>PBT substances</b>	Conclusions/Not investigated
<b>Substances of Very High Concern (1907/2006/EC)</b>	No
<b>POP regulation (EU) 2019/1021</b>	Not included
<b>Other relevant chemical regulation (veterinary products, medicament, ...)</b>	No
<b>Endocrine disrupter</b>	Not investigated
<b>CLP Classification (Regulation (EC) No. 1272/2008)</b>	C&L notifications submitted to (ECHA, 2024) Harmonised Classifications Expl. 1.1 H201 Acute Tox. 3 H301 Acute Tox. 3 H311 Acute Tox. 3 H331 Aquatic Chronic 2 H411 STOT RE 2 H373 Other REACH registration dossiers notifications Repr. 2 H361 Carc. 1B H350 Muta. 2 H341

For the hazard classification and labelling in Table 2, (ECHA, 2024) additionally provides the following information:

*“Danger!* According to the **harmonised classification and labelling** (CLP00) approved by the European Union, this substance is explosive (mass explosion hazard), is toxic if swallowed, is toxic in contact with skin, is toxic if inhaled, is toxic to aquatic life with long lasting effects and may cause damage to organs through prolonged or repeated exposure.

**Additionally**, the classification provided by companies to ECHA in **REACH registrations** identifies that this substance may cause cancer, is suspected of damaging fertility or the unborn child and is suspected of causing genetic defects.

**Additionally**, the classification provided by companies to ECHA in **CLP notifications** identifies that this substance is very toxic to aquatic life. “

### 3 Proposed Quality Standards (QS)

#### 3.1 Environmental Quality Standard (EQS)

QS for the pelagic community (freshwater and marine water) is the “critical QS” for derivation of the proposed Environmental Quality Standard (see Table 3 and 4).

In water bodies used for drinking water production, the drinking water guideline value can be used for a preliminary assessment. The drinking water guidance value was published in 2005. It has not been reviewed whether an update is necessary.

**Table 3: Proposed Environmental Quality Standards for TNT**

	Value	Comments
Proposed AA-EQS for freshwater	0.15 µg/L	See section 7.2
Proposed AA-EQS for saltwater	0.15 µg/L	
Proposed MAC-EQS for freshwater	1.8 µg/L	See section 7.2
Proposed MAC-EQS for saltwater	1.8 µg/L	

#### 3.2 Specific Quality Standard (QS)

**Table 4: Tentative Quality Standards for different protection objectives**

Protection Objective	Value	Comments
Pelagic community (freshwater)	AA-QS <sub>fw, eco</sub> = 0.15 µg/L MAC <sub>freshwater, eco</sub> = 1.8 µg/L	See section 7.2
Pelagic community (marine waters)	AA-QS <sub>sw, eco</sub> = 0.15 µg/L MAC <sub>saltwater, eco</sub> = 1.8 µg/L	
Benthic community (freshwater)	-	See section 7.3
Benthic community (marine)	-	
Predatores (secondary poisoning)	-	See section 7.4
Human health via consumption of fishery products	4.1 µg/kg <sub>biota ww</sub> corresponding water concentration (0.8 µg/L)	See section 7.5
Human health via consumption of water	LW <sub>TW</sub> = 0.2 µg/L	

## 4 Major Uses and Environmental Emissions

TNT is used in products as explosive. An enormous quantity of TNT in unexploded ordnance and munitions from intentional disposal is present in North and Baltic Sea. Coastal waters around the world are contaminated with munitions from the two world wars (World War I and World War II), with around 1.6 million tonnes of munitions alone in the German parts of the North and Baltic Seas (Beck et al., 2018). The emission to the environment from production and disposal is not assessed in the data sheet.

### 4.1 Uses and Quantities

Total tonnage band, Total range:  $\geq 1\ 000$  to  $< 10\ 000$  tonnes (ECHA, 2024),

### 4.2 Estimated Environmental Emissions

The emission of TNT to the environment is not estimated in the data sheet. The emission rates of munition dump sites in the North and Baltic Sea are unknown. Significant emissions of TNT into the marine environment can be expected in the coming decades due to corrosion of the munition shells.

## 5 Environmental Behaviour

### 5.1 Environmental Distribution

Information on the physicochemical behaviour and bioaccumulation of TNT is provided in Table 5. TNT has low water solubility and moderate mobility. Significant entry through volatilization/deposition is not expected. Due to the low octanol-water partition coefficient ( $\log K_{ow} < 3$ ), a high bioaccumulation potential is not expected. With a  $K_d$  value of approximately 10 l/kg, TNT exhibits only weak sorption to sediments.

**Table 5: Physico-chemical behaviour and bioaccumulation of TNT**

Parameter	Value	Master reference
Water solubility	127 mg/L at 20°C and pH 5.8. 115 mg/L at 23°C	(ECHA, 2024) (ECHA, 2021)
Volatilisation		
Vapour pressure (Pa)	0.004 Pa at 20°C	(ECHA, 2024)
Henry's Law constant (Pa.m <sup>3</sup> .mol <sup>-1</sup> )	0.01 Pa.m <sup>3</sup> .mol <sup>-1</sup> at 20 °C and 101.325 kPa	(ECHA, 2024)
Adsorption		
Organic carbon – water partition coefficient (KOC)	KOC = 44.29 at 20°C	(ECHA, 2023)
sediment– water partition coefficient (K <sub>sed-water</sub> )	around 10 l/kg	(Beck et al., 2018)
Bioaccumulation	The BCF value 4.97 L/kg on fish is used for derivation of quality standards.	
Octanol-water partition coefficient (Log K <sub>ow</sub> )	1.65 ± 0.01 at 20°C	Dossier (ECHA, 2023)
BCF (measured)	<i>Pimephales promelas</i> 4.97 L/kg ww	(Yoo et al., 2006)

#### Bioaccumulation

Via aqueous exposure, for the marine mussel, *Mytilus galloprovincialis* BCF values of 0.3 and 1.37 L/kg were found by (Gunther Rosen & Guilherme R. Lotufo, 2007) and (G. Rosen & G. R. Lotufo, 2007). An BCF of 14,2 L/kg was determined for the freshwater amphipod *Hyaella azteca* by (Sims & Steevens, 2008). For two benthic invertebrates (*Chironomus tentans* and *Lumbriculus variegatus*) the bioconcentration factors for parent TNT ranged from 3 to 4 L/kg as reported by (Belden et al., 2005). TNT accumulation in through trophic transfer would be negligible compared to aqueous exposure. As indicated by a small bioaccumulation factor via oral exposure of food pellets of channel catfish (*Ictalurus punctatus*), the TNT accumulation in through trophic transfer is negligible compared to aqueous exposure. The study of (Belden et al., 2005) showed that TNT was rapidly bio-transformed resulting in minimal accumulation and that TNT extractable biotransformation products accumulated to a greater degree than parent TNT for all three species.

The bioaccumulation of TNT is low and environmental samples have shown that there is no accumulation along the food chain (Schick et al., 2022) and (Strehse et al., 2024). Therefore, the derivation of a biota quality standard to protect wildlife secondary poisoning is not indicated.

## 5.2 Abiotic and Biotic Degradations

The degradation of 2,4,6-trinitrotoluenes (TNT) is determined by both the environment (e.g., temperature and salinity) and its physical-chemical properties such as water solubility, vapor pressure, etc. (Ariyaratna et al., 2017) and is accordingly situation-dependent.

During the degradation of TNT, formation of metabolites occurs. The major transformation products are 2-amino-4,6-dinitrotoluene (2-ADNT), 4-amino-2,6-dinitrotoluene (4-ADNT), 2,4-diaminonitrotoluene (2,4-DANT), and 2,6-diaminonitrotoluene (2,6-DANT) (Yost et al., 2007).

Studies indicate that biodegradation of TNT occurs much more slowly in the water column than in sediments ((Harrison & Vane, 2010); (Smith et al., 2013)). For example, in a study by Smith et al. a half-life of 3.8 days occurred for TNT in artificial seawater under aerobic conditions. In contrast, seawater in close proximity to fine-grained sediment yielded a half-life of only 0.4 days (Smith et al., 2013). Another study using seawater from the North Sea and under much lower temperatures (7°C), as well as without any contact to sediment, yielded a half-life of 1900 days (Harrison & Vane, 2010). These results show that TNT is poorly biodegradable in water.

This is also confirmed by OECD Test 301 D, that TNT is not readily biodegradable (ECHA, 2023).

**Table 6: Abiotic and biotic degradation of TNT**

Degradation by	Value	Master reference
Hydrolysis	DT <sub>50</sub> = d at °C (distilled water) DT <sub>50</sub> = d at °C (salt water)	
Photolysis	DT <sub>50</sub> =	
Biodegradation	OECD 301 D, 17.52% after 28 d (not readily biodegradable)	(ECHA, 2023)
	DT <sub>50sed</sub> = 24 d	(ECHA, 2023)
	DT <sub>50water</sub> = 1900 d DT <sub>50sed</sub> = 130 d (clay-sediment)	(Harrison & Vane, 2010)
	DT <sub>50water</sub> = 3.8 d DT <sub>50sed</sub> = 0.4 d (fine-grained sediment)	(Smith et al., 2013)

## 6 Aquatic Environmental Concentrations

### 6.1 Estimated Concentrations

Concentrations of TNT in the environment is not estimated in the data sheet.

### 6.2 Measured Concentrations

**Table 7: Measured concentration of TNT in different environmental compartments**

Compartment	Measured environmental concentration (MEC)	Master reference
Freshwater		
Marine waters (coastal and/or transitional)	Wrack V 1302 0.001 -0.016 µg/L Munition dump area Kolberger Heide 0.0001 – 0.081 µg/L Southwestern Baltic Sea, average year 2018, dissolved 0.0039 µg/L	(Maser et al., 2023) (Greinert, 2019)  (Beck et al., 2025)
WWTP effluent		
Sediment	Munition dump area Kolberger Heide 0 – 42 µg/kg dry or wet	
Biota	Flatfish filet 3 – 8 µg/kg dry wt 0.6 – 1.6 µg/kg ww	(Strehse et al., 2019)
	Blue mussel 31 µg/kg ww TNT	(Schick et al., 2022) (Strehse et al., 2017)
	Common Eiders < LoD	(Schick et al., 2022)
Biota (marine predators)	< LoD	(Strehse et al., 2024)

Contamination of the aquatic environment with TNT is essentially limited to contaminated sites (warship wrecks, etc.) and munition dumping areas. A selection of measured concentrations of TNT in different environmental compartments is shown in Table 7. The contamination in the munition dumping ground in the Baltic Sea at Kolberger Heide was monitored in the scientific project (UDEMM) between 2016 and 2019 (Greinert, 2019), and TNT concentrations between 0.0001 – 0.081 µg/L could be determined in open water. Only directly on a piece of exposed explosive were TNT concentrations of up to 3100 µg/L measured, which dropped to 3.3 µg/L at 50 cm distance (Beck et al., 2019).

TNT concentrations have been mapped in the Aarhus Bay area of the Baltic Sea. The highest concentration measured is 0.00123 ng/L in the water close to the seabed. The measured levels are generally lower than those found in German coastal waters, which typically range between 0.001 and 0.025 µg/L (Sanderson & Fauser, 2023).

Trends and data on the distribution of munitions remnants (TNT and metabolites) in the marine environment of the North and Baltic Seas are recently published by (Strehse et al., 2024):

“Analyses of blue mussels from the annual collections of the German Environmental Specimen Bank sampled over the last 30 years at three different locations along the coastline of the Baltic and North Seas showed that only the metabolites 2-ADNT and 4-ADNT could be detected”. The highest concentrations were measured just below the limits of quantification (0.14 ng/g dry weight for 2-ADNT and 0.17 ng/g dry weight for 4-ADNT) in Eckwarderhörne in 2019 and 2020 (Strehse et al., 2023).

Investigations into the possible accumulation of TNT and its metabolites in the marine food web of the Baltic Sea examined the possible occurrence in marine organisms from different trophic levels. With the exception of the flatfish examined, no TNT was detected in the eelpout, eider ducks, porpoises and seals examined (Strehse et al., 2024). Based on the available study results, there is no evidence of an accumulation of TNT along the food chain.

## 7 Effects and Quality Standards

### 7.1 Acute and Chronic Aquatic Ecotoxicity

The relevant studies were evaluated and assessed according to Moermond et al. (Moermond et al., 2016). This assessment includes a set of 20 reliability and 13 relevance criteria, whereby the classes assigned (R1-4) match those of Klimisch (Klimisch et al., 1997):

R1 Reliable without restrictions: All critical reliability criteria for this study are fulfilled. The study is well designed and performed, and it does not contain flaws that affect the reliability of the study.

R2 Reliable with restrictions: The study is generally well designed and performed, but some minor flaws in the documentation or setup may be present.

R3 Not reliable: Not all critical reliability criteria for this study are fulfilled. The study has clear flaws in study design and/or how it was performed.

R4 Not assignable: Information needed to assess the study is missing. This concerns studies that do not give sufficient experimental details and that are only listed in abstracts or secondary literature (books, reviews, etc.) or studies of which the documentation is not sufficient for assessment of reliability for one or more vital parameters.

The key acute and ecotoxicity data of TNT for freshwater and marine water organisms are reported in the table 9 and 10 below. The effect data for TNT and metabolites were already summarized by (Lotufo et al., 2017) and are supplemented by current research and investigations within the CONMAR project. A difference in the sensitivity of freshwater and marine is not observed. The ratio of acute/chronic effects for the most species is low. For example, for the marine algae species *Phaeodactylum tricornutum* and the acute/chronic ratio is < 2. The marine diatom *Skeletonema marinoi* is the most sensitive algae species in the data set for TNT and its major metabolites 2-ADNT and 4-ADNT. The data obtained in the CONMAR project showed, that for the diatom *Skeletonema marinoi* the toxicity TNT and 2-ADNT and 4-ADNT is comparable (see table 8).

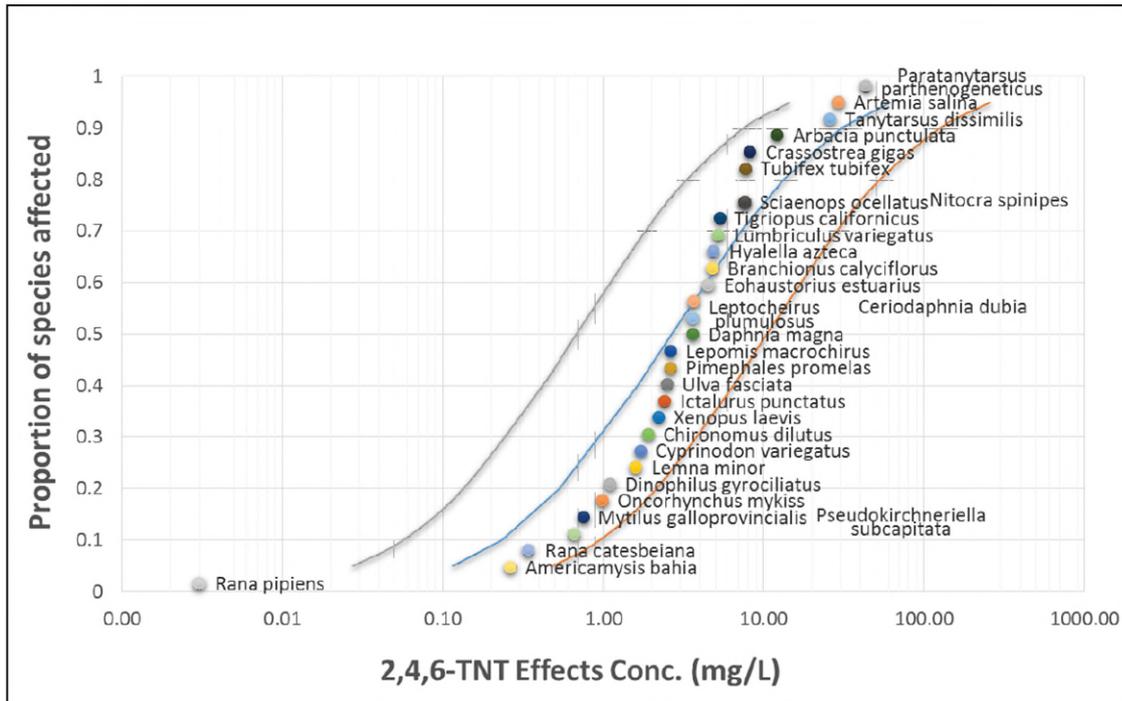
**Table 8: Results for TNT, 2-ADNT and 4-ADNT of the marine algal growth inhibition test with *Skeletonema marinoi* and *Phaeodactylum tricornutum* performed in the CONMAR project**

TNT	TNT	2-ADNT	4-ADNT	4-ADNT
Phaeodactylum tricornutum	Skeletonema marinoi	Skeletonema marinoi	Skeletonema marinoi	Skeletonema marinoi
ISO 10253 (2016)	ISO 10253 (2016)	ISO 10253 (2016)	ISO 10253 (2016)	ISO 10253 (2016)
Growth rate	Growth rate	Growth rate	Growth rate	Growth rate
72h	72h	72h	72h	72h
ErC10	ErC10	ErC10	ErC10	ErC10
1321 µg/L	130 µg/L	231 µg/L	237 µg/L	297 µg/L
ErC50	ErC50	ErC50	ErC50	ErC50
1468 µg/L	182 µg/L	259 µg/L	351 µg/L	485 mg/l
real	real	real	real	real
(Schelzig, 2022)	(Schelzig, 2023d)	(Schelzig, 2023a)	(Schelzig, 2023b)	(Schelzig, 2023c)
Prüfung der Toxizität von TNT auf marine Kieselalgen der Art <i>Phaeodactylum tricornutum</i> (Test no.C07Pt)	Prüfung der Toxizität von TNT auf marine Kieselalgen der Art <i>Skeletonema marinoi</i> (Test no.C135m)	Prüfung der Toxizität von 2-ADNT auf marine Kieselalgen der Art <i>Skeletonema marinoi</i> (Test no.C225m)	Prüfung der Toxizität von 4-ADNT auf marine Kieselalgen der Art <i>Skeletonema marinoi</i> (Test no.C255m)	Prüfung der Toxizität von 4-ADNT auf marine Kieselalgen der Art <i>Skeletonema marinoi</i> (Test no.C275m)

For the TNT metabolite 2,6-Dinitrotoluol (2,6-DNT) a 72h-ErC10 of 5.260 µg/L and 72h-ErC50 was determined for *Skeletonema marinoi* by (Schelzig, 2024). Compared to the toxicity of TNT, 2-ADNT and 4-ADNT, the metabolite 2,6-DNT was significantly less toxic in the algae test with the diatom *Skeletonema marinoi*.

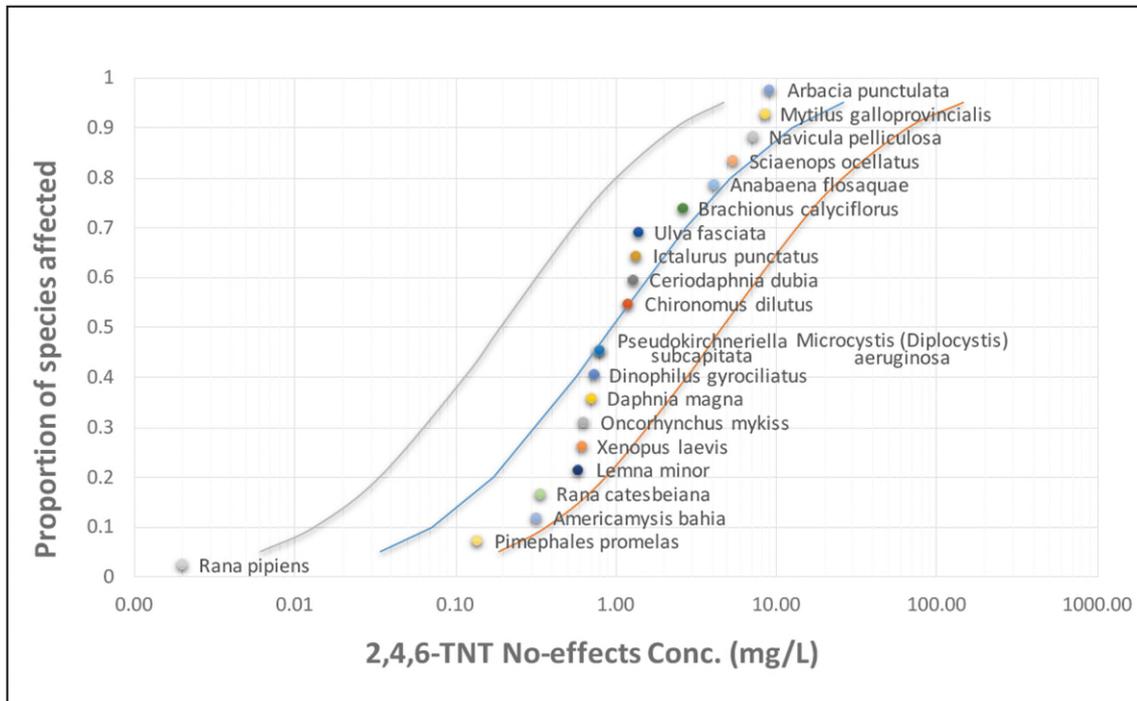
For the environmental risk assessment of munition compounds, data on the acute and chronic aquatic ecotoxicity of TNT and metabolites were already summarized and analysed by (Lotufo et al., 2017). In Fig 1 species sensitivity distribution for 2,4,6-TNT using effects data and in Fig. 2 the distribution for 2,4,6-TNT using no-effects data created by (Lotufo et al., 2017) is shown. The data include data for effects on fresh and sea water species as well.

**Figure 1: Species sensitivity distribution for 2,4,6-TNT using effects data. The outer lines represent 95% confidence intervals**



Source: Lotufo et al. (2017), figure 71

**Figure 2: Species sensitivity distribution for 2,4,6-TNT using no-effects data. The outer lines represent 95% confidence intervals**



Source: Lotufo et al. (2017), figure 81

In the tables below the most relevant data are summarised, which are used to derive quality standards to protect aquatic life.

The data for acute effects of the most sensitive freshwater and marine water species in table 9 show, that the sensitivity of freshwater and marine species is comparable. For acute effects algae species seemed to be the most sensitive group. The data for chronic effects of the most sensitive freshwater and marine species in table 10 show, that the sensitivity of freshwater and saltwater species is in general comparable, but the tad poles of the frog *Rana pipiens* is the most sensitive species.

Detailed acute and chronic ecotoxicity datasets for TNT, the metabolites 2-ADNT and 4-ADNT are include in the appendices.

**Table 9: Acute effects of TNT on the most sensitive freshwater and marine water species**

Taxonomic groups	Habitat	Species / Effect	Reference
Algae & aquatic plants	Freshwater	<i>Pseudokirchneriella subcapitata</i> / 72 h EyC <sub>50</sub> : 0.19 mg/L Reliability : 1	(ECHA, 2023)
	Marine	<i>Phaeodactylum tricornutum</i> / 72 h ErC <sub>50</sub> : 1.468 mg/L (real), Reliability: 1 <i>Skeletonema marinoi</i> / 72 h ErC <sub>50</sub> : 0.182 mg/L (real) Reliability: 1	(Schelzig, 2022)  (Schelzig, 2023d)
Invertebrates	Freshwater	<i>Daphnia magna</i> / 48 h EC <sub>50</sub> : 9.49 mg/L Reliability: 1 <i>Daphnia magna</i> / 48 h NOEC : 6.25 mg/L LOEC: 12.5 mg/L Reliability: 1	(ECHA, 2023)
	Marine	<i>Tigriopus californicus</i> / 3 d Mortality LC <sub>50</sub> : 5.300 mg/L (nom) <i>Americamysis bahia</i> / 4 d Mortality EC <sub>50</sub> : 0.260 mg/L Reliability: 3	(Won et al., 1976)  (Nipper et al., 2001)
	Sediment	<i>Eohaustorius estuarius</i> / 10 d Mortality LC <sub>50</sub> : 23 mg/kg dw Reliability: 3	(Rosen & Lotufo, 2005)
Fish	Freshwater	<i>Pimephales promelas</i> / 96 h LC <sub>50</sub> : 2,7 mg/L Reliability: 1	(ECHA, 2023)
	Marine	<i>Sciaenops ocellatus</i> / 2 d Mortality LC <sub>50</sub> : 7.600 mg/L	(Nipper et al., 2001)
Other taxonomic groups			
Amphibia	Freshwater	<i>Xenopus laevis</i> / 4 d Embryotoxicity LC <sub>50</sub> : 3.79 mg/L	(Saka, 2004)
Mussels	Marine	<i>Mytilus galloprovincialis</i> / 2 d Survival Embryos EC <sub>50</sub> : 0.750 mg/L	(G. Rosen & G. R. Lotufo, 2007)

**Table 10: Chronic effects of TNT on the most sensitive freshwater and marine water species**

Taxonomic groups	Habitat	Effect	Reference
Algae & aquatic plants	Freshwater	<i>Pseudokirchneriella subcapitata</i> / (growth rate) 72 h NOEC: 0.1 mg/L / LOEC: <= 0.14 mg/L based on geometric mean test item concentrations Reliability : 1	(ECHA, 2023)
	Marine	<i>Phaeodactylum tricornutum</i> / (growth rate) 72 h ErC10: 1.321 mg/L (real) <i>Skeletonema marinoi</i> / (growth rate) 72 h ErC10: 0.130 mg/L (real)	(Schelzig, 2022)
Invertebrates	Freshwater	<i>Daphnia magna</i> / Reproduction 21 d NOEC = 0.480 mg/l (real)	(Bailey et al., 1985)
	Marine		
	Sediment	<i>Eohaustorius estuarius</i> / survival 10 d NOEC: 20 mg/kg dw Reliability : 3	(Rosen & Lotufo, 2005)
Fish	Freshwater	<i>Pimephales promelas</i> / Reproduction 60 d LOEC: 0.04 mg/L	(Bailey et al., 1985)
Other taxonomic groups			
Amphibia	Freshwater	<i>Rana pipiens</i> / Mortality / 28 d LOEC: 0.003 mg/L	(Stanley et al., 2015)

## 7.2 Tentative QSwater

According to TGD No. 27 (EC 2018), “two Qs are required for the water compartment to cover both long-term and short-term exposures to a chemical. Whilst derivation of the QS typically employs chronic toxicity data, the MAC-QS always relies on acute data”.

To derive a  $MAC_{\text{freshwater, eco}}$  acute effect data are available for bacteria, algae, invertebrates, fish and other taxonomic groups. The lowest acute test result exists for the marine algae *Skeletonema marinoi* (growth rate 72 h;  $ErC_{50} = 182 \mu\text{g/L}$ ). An assessment factor (AF) = 100 is used to derive the EQS for the maximum concentration.

$$MAC_{\text{freshwater, eco}} = 182 \mu\text{g/L} * 0.01 = 1.8 \mu\text{g/L}$$

To derive an  $AA-QS_{\text{freshwater, eco}}$  data of test with long-term exposure are available for bacteria, algae, invertebrates, fish and amphibia. The lowest test result for fish was the LOEC of  $40 \mu\text{g/L}$  for the survival (60 d) in the F1 generation of the fish *Pimephales promelas*. The LOEC is divided by 2 to calculate a NOEC of  $20 \mu\text{g/L}$  for fish *Pimephales promelas*. The lowest test result for amphibia was determined for tadpoles of frog *Rana pipiens* (28 d / survival / LOEC =  $3 \mu\text{g/L}$ ). For *Rana pipiens* a NOEC is extrapolated  $LOEC/2 = 1.5 \mu\text{g/L}$ .

As NOEC values with long-term test are existing, an AF = 10 is used to calculate  $AA-QS_{\text{freshwater, eco}}$

$$AA-QS_{\text{freshwater, eco}} = 1.5 \mu\text{g/L} * 0.1 = 0.15 \mu\text{g/L}$$

Since there are no differences in the sensitivity of freshwater and marine organisms, and the lowest test result comes from a freshwater species, the  $MAC_{\text{freshwater, eco}}$  and the  $AA-QS_{\text{freshwater, eco}}$  can also be adopted for the marine area.

**Table 11: Tentative Quality Standards for TNT in water**

Tentative $QS_{\text{water}}$	Relevant study for derivation of EQS	Assessment factor	Tentative QS
$MAC_{\text{freshwater, eco}}$	<i>Skeletonema marinoi</i> 72 h growth rate / ErC50 = 182 µg/L	100	1.8 µg/L
$MAC_{\text{saltwater, eco}}$		100	1.8 µg/L
$AA-QS_{\text{freshwater, eco}}$	<i>Rana pipiens</i> 28 d / survival / NOEC = 1.5 µg/L	10	0.15 µg/L
$AA-QS_{\text{saltwater, eco}}$		10	0.15 µg/L

### 7.3 Tentative $QS_{\text{sediment}}$

TNT exhibits weak sorption to sediments.  $K_d$  values on the order of 10 l/kg were reported by (Beck et al., 2018). A trigger value of  $K_{oc} \geq 1000$  is given in TGD EQS to perform a sediment effects assessment.

The Equilibrium Partitioning (EqP) method is therefore not used to estimate the  $QS_{\text{sediment}}$  (EC, 2018). Nevertheless, TNT and its metabolites were found in sediments and some results from sediment ecotoxicity tests exist.

For TNT and its transformation products, rapid transformation, disappearance of transformation products and distribution into overlying water after initiation of whole sediment toxicity testing were reported. The rapidly fluctuating concentrations and the transformation products in sediment pose unique challenges for the development of accurate toxicity data to assess risks to biota at sites with contaminated sediments. The toxicity of TNT-enriched sediment to marine polychaete, estuarine amphipod, freshwater midge, amphipod, oligochaete and estuarine fish occurred over a wide concentration range 37 to 508 mg/kg (Lotufo et al., 2021). Due to the very heterogeneous data situation, it is currently not possible to derive a reliable quality standard for the protection of the benthic community.

### 7.4 Secondary Poisoning

The bioaccumulation of TNT is low. The reliable BCF values for fish or other aquatic organisms are below the trigger value of 100 l/kg in TGD EQS to derive a  $QS_{\text{biota}}$ , secondary poisoning, therefore no assessment of secondary poisoning of top predators is made. Table 12 shows information on the oral toxicity of TNT in mammals and birds. In the Appendix relevant BCF are listed.

**Table 12: Information on the oral toxicity of TNT in mammals and birds**

Secondary poisoning of top predators		Master reference
Mammalian oral toxicity	Maus / Oral / duration / Endpoint LD50 bei 660 mg/kg	(Haas & Thieme, 1996)
	<i>Rattus rattus</i> / Oral / duration / Endpoint LD50 bei 765 mg/kg	(Haas & Thieme, 1996)
Avian oral toxicity	<i>Colinus virginianus</i> / Oral / 90 d LOAEL: 178 mg/kg bw/day NOAEL: >= 7 mg/kg bw/day EC 50: mg.kg <sup>-1</sup> bw.d <sup>-1</sup> NOEC: mg.kg <sup>-1</sup> feed ww (CF=)	(Gogal Jr. et al., 2002)

## 7.5 Human Health

To protect human health via consumption the information published by (IRIS) is used to calculate a tentative QS<sub>biota, hh</sub> for toxic and carcinogen effects of TNT (see table 13).

**Table 13: Relevant endpoints for the toxic and carcinogen effect of TNT**

Human health via consumption of fishery products		Master reference
Mammalian oral toxicity	26-Week Dog Feeding Study; LOAEL: 0.5 mg.kg <sup>-1</sup> bw.d <sup>-1</sup>	(IRIS)
CMR	Oral Slope Factor: 3.0 10 <sup>-2</sup> per mg.kg <sup>-1</sup> bw.d <sup>-1</sup>	(IRIS)

According to TGD-EQS the QS<sub>biota, hh</sub> can be calculated with the formular and data given below:

$$QS_{biota, hh, food} = 0.2 * TL * 70 / 0.115 = 0.2 * TL / 0.00163$$

$$\text{Allocation factor} = 0.2$$

TL = threshold level, human health (ADI, MTR, TDI [µg/kg bw/d])

$$\text{Body weight (bw)} = 70 \text{ [kg]}$$

$$\text{Fish consumption} = 0.115 \text{ [kg/d]}$$

$$\text{Fish consumption / Body weight} = 0.00163 \text{ [kg/kg/d]}$$

The results for calculated QS<sub>biota, hh</sub> based on toxic and carcinogenic effects of TNT are shown in table 14 and 15.

**Table 14: Tentative QS<sub>biota, hh</sub> for the toxic effects of TNT**

Tentative QS <sub>biota, hh</sub>	Relevant study for derivation of QS <sub>biota, hh</sub>	Assessment Factor	Tentative QS <sub>biota, hh</sub>
Human health	TL = 0.5 µg/kg bw /d (RfD, IRIS)	1000	60 µg/kg biota ww

**Table 15: Tentative  $QS_{biota, hh}$  for the toxic effects of TNT**

Tentative $QS_{biota, hh}$	Relevant study for derivation of $QS_{biota, hh}$	Risk Level	Tentative $QS_{biota, hh}$
Human health	Oral Slope Factor: $3.0 \cdot 10^{-2}$ per $mg \cdot kg^{-1} \cdot bw \cdot d^{-1}$ TL = $0.0333 \mu g/kg \text{ bw } /d$	$10^{-6}$	$4.1 \mu g \cdot kg^{-1} \text{ biota } ww$ ( $0.8 \mu g/L$ )

The TL of  $0.0333 \mu g/kg \text{ bw}/d$  was calculated based on the Oral Slope Factor for a  $10^{-6}$  Risk Level.

The BCF value for *Pimephales promelas*  $4.97 \text{ L}/kg \text{ ww}$  is used to calculate a corresponding water concentration for  $QS_{water \text{ biota, hh}}$  of  $0.8 \mu g/L$ . This value should only be used to compare the **Tentative  $QS_{biota, hh}$**  with other **Tentative  $QS_{water}$** .

For TNT no drinking water standard is set in the EU directive 2020/2184. In Table 16 proposed guidance values are listed.

**Table 16: Quality standards and guidance for drinking water to protect human health**

Human health via consumption of drinking water	Master reference
Existing drinking water standard(s)	- Directive (EU) 2020/2184
Any guidance	(IRIS) Drinking Water Unit Risk: $9.0E^{-7}$ per $\mu g/L$ Extrapolation Method — Linearized multistage procedure, extra risk Drinking Water Concentrations at Specified Risk Level: E-6 (1 in 1,000,000) = $1 \mu g/L$
	(Wollin & Dieter, 2005) Drinking water guidance value Trinkwasserleitwert ( $LW_{TW}$ ) = $0.2 \mu g/L$

The drinking water guidance value published by (Wollin & Dieter, 2005) has not been reviewed since 2005.

Using a human body weight (bw) of 70 kg, a daily uptake of drinking water ( $uptake_{dw}$ ) of 2 L and as threshold level for human health ( $TL_{hh}$ ) for of  $0.033 \text{ mg}/kg \text{ bw}/d$  (Unit risk +) (Wollin & Dieter, 2005) a  **$QS_{dw, hh}$  of  $0.23 \mu g/L$**  can be calculated, according to the formular given in the TGD-EQS.

$$QS_{dw, hh} = \frac{0.2 \cdot TL_{hh} \cdot bw}{uptake_{dw}}$$

## 8 Identification of issues relating to uncertainty in relation to the Qs derived

A derivation of a **MAC**<sub>freshwater, eco</sub> and an **AA-QS**<sub>freshwater, eco</sub> using the species sensitivity distribution method was not yet performed. This would require the reliability of all studies and compliance with the minimum requirements of the dataset in the TGD EQS are achieved. The HC<sub>5</sub> values for TNT (Effects, HC<sub>5</sub> = 116 µg/L and No effects HC<sub>5</sub> = 34 µg/L), which were calculated using the SSD method by (Lotufo et al., 2017) with the data shown in Fig. 1 and 2 for the sensitivity distribution of species, indicate that the Quality standards (**MAC**<sub>freshwater, eco</sub> = 1.8 mg/L and **AA-QS**<sub>freshwater, eco</sub> = 0.15 µg/L) calculated with the assessment factor method are conservative values.

It should also be noted that the lowest test result in the data set was determined for tad poles of the frog *Rana pipiens* (28 d / survival / LOEC = 3 µg/L) by (Stanley et al., 2015).

Data for TNT degradation products (2-ADNT and 4-ADNT) indicate that, for many species, toxicity is similar to that of TNT. Assuming concentration additivity, the proposed EQS for TNT can also be applied to the concentration of the sum of TNT, 2-ADNT, and 4-ADNT as a preliminary assessment. However, this would need to be confirmed by further studies.

Since there are great uncertainties regarding the derivation of a reliable quality standard to protect the benthic community, no value (**AA-QS**<sub>freshwater, sed.</sub> or **AA-QS**<sub>marine water, sed.</sub>) has been established at this time.

## **9 Identification of any potential implementation issues in relation to the Qs derived**

The quality standards derived in this dossier serve as a benchmark for assessing the munition compound deposits in the dumping areas or other contaminated sites. Establishing a quality standard under the German Surface Water Ordinance is currently unlikely to be necessary, as no exceedance of the conservatively derived quality standard have been detected to date and the pollution is essentially limited to the munitions dumping areas.

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## A Appendix

### Abbreviations used in the Appendix

**Table 17: Column “Analytic”**

Abbreviation	Definition
ana	Analytically monitored test concentrations
nom	The statistical endpoint is based on nominal values
real	The statistical endpoint is based mean measured concentration

**Table 18: Column “Validity”**

Abbreviation	Definition
CRED	Criteria for reporting and evaluating ecotoxicity data: "The CRED evaluation method is accompanied by reporting recommendations for aquatic ecotoxicity studies, with 50 specific criteria divided into 6 categories: general information, test design, test substance, test organism, exposure conditions, and statistical design and biological response." (Environ Toxicol Chem. 2016 May;35(5):1297-309. doi: 10.1002/etc.3259
Klimisch	Study quality assessed according to the scheme developed by Klimisch, H.J., Andreae, M. and Tillmann, U. (1997)
UBA-R+D	Test Evaluation Code used by research and development projects of the German Environment Agency (UBA)
RI-1 / CRED	reliable
RI-2 / CRED	reliable with some restriction
RI-3 / CRED	not reliable
RI-4 / CRED	not assessable

Abbreviation	Definition
1 / Klimisch	reliable without restrictions: studies or data generated according to generally valid and/or internationally accepted testing guidelines
2 / Klimisch	reliable with restrictions
3 / Klimisch	not reliable
4 / Klimisch	not assignable: "studies or data which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature (books, reviews, etc.)"
1 / UBA-R+D	Reliable: test well preformed according to a standard test procedure
2 / UBA-R+D	reliable with restrictions: test based on a standard test procedure
3 / UBA-R+D	Not reliable: test not well performed
4 / UBA-R+D	not assessable: the implementation and documentation of the biotest are insufficient in the literature source

### A.1 ETOX aquatic effect data 2,4,6-trinitrotoluene (TNT)

Type	Substance Number
CAS Number	118-96-7
EC Number	204-289-6

The data on 2,4,6-trinitrotoluene from ETOX have been migrated to ChemInfo and will only be hosted there in the future (ChemInfo-ETOX, 2024; ChemInfo, 2024). The information for **TNT** can be accessed at the following link: <https://recherche.chemikalieninfo.de/etox/stoff/6575>

Data available at ECHA on TNT have not been included in ChemInfo. The data from the briefprofile and the REACH dossiers can be accessed via the following links:

<https://echa.europa.eu/brief-profile/-/briefprofile/100.003.900>

<https://echa.europa.eu/de/registration-dossier/-/registered-dossier/16165>

<https://chem.echa.europa.eu/100.003.900/dossier-view>

**Table 19: Aquatic effect data TNT**

Taxonomic Group	Species	Effect	Effect Measurement	Duration	Value	Analytic	Exposure Type	pH	Temperature	Validity	Reference
Algae	<i>Chlorophyceae</i>	Growth		3 d	EC50 = 2,300 µg/L						(Neuwoehner et al., 2007)
Algae	<i>Phaeodactylum tricornutum</i>	Cell Proliferation	Growth rate	3 d	ErC10 = 1,321 µg/L	real	static	7.9 – 8.1	20 °C	RI-1 / CRED	(Schelzig, 2022a)
Algae	<i>Phaeodactylum tricornutum</i>	Cell Proliferation	Growth rate	3 d	ErC50 = 1,468 µg/L	real	static	7.9 - 8.1	20 °C	RI-1 / CRED	(Schelzig, 2022a)
Algae	<i>Phaeodactylum tricornutum</i>	Cell Proliferation	Growth rate	3 d	ErC50 = 1,508 µg/L	nom	static	7.9 - 8.1	20 °C	RI-2 / CRED	(Schelzig, 2022b)
Algae	<i>Selenastrum capricornutum</i>	Cell Proliferation	Cell Count / Yield	7 d	NOEC = 1,000 µg/L	nom					(Won et al., 1976)
Algae	<i>Skeletonema marinoi</i>	Cell Proliferation	Growth rate	3 d	ErC10 = 188 µg/L	nom	static	7.9 - 8.2	20 °C		(Schelzig, 2023d)
Algae	<i>Skeletonema marinoi</i>	Cell Proliferation	Growth rate	3 d	ErC10 = 130 µg/L	real	static	7.9 - 8.2	20 °C	RI-1 / CRED	(Schelzig, 2023d)
Algae	<i>Skeletonema marinoi</i>	Cell Proliferation	Growth rate	3 d	ErC50 = 182 µg/L	real	static	7.9 - 8.2	20 °C	RI-1 / CRED	(Schelzig, 2023d)
Algae	<i>Skeletonema marinoi</i>	Cell Proliferation	Growth rate	3 d	ErC50 = 252 µg/L	nom	static	7.9 - 8.2	20 °C		(Schelzig, 2023d)

Taxonomic Group	Species	Effect	Effect Measurement	Duration	Value	Analytic	Exposure Type	pH	Temperature	Validity	Reference
Algae	<i>Ulva fasciata</i>	Growth	Cell Count / Yield	4 d	EC50 = 1,100 µg/L	real	static	8.0	20 °C		(Nipper et al., 2001)
Algae	<i>Ulva fasciata</i>	Growth	Cell Count / Yield	4 d	NOEC ≤ 180 µg/L	real	static	8.0	20 °C		(Nipper et al., 2001)
Algae	<i>Ulva fasciata</i>	Growth	Cell Count / Yield	4 d	LOEC = 180 µg/L	real	static	8.0	20 °C		(Nipper et al., 2001)
Amphibia	<i>Rana catesbeiana</i>	Mortality	Survival	90 d	LOEC ≤ 120 µg/L	real	semi-static	6.5 - 7.1	21.0 - 23.5 °C	2 / Klimisch	(Paden et al., 2011)
Amphibia	<i>Rana pipiens</i>	Mortality	Survival	28 d	LOEC = 3 µg/L	real	semi-static		23 °C	2 / Klimisch	(Stanley et al., 2015)
Bacteria	<i>Pseudomonas putida</i>	Growth inhibition			EC50 > 19,000 µg/L						(Neuwoehner et al., 2007)
Bacteria	<i>Vibrio fischeri</i>	Luminescence		0,020833 319 d	EC50 510 µg/L						(Neuwoehner et al., 2007)
Bacteria	<i>Vibrio fischeri</i>	Growth inhibition			EC50 1,400 µg/L						(Neuwoehner et al., 2007)
Monocotyledoneae	<i>Lemna minor</i>	Growth inhibition	Growth rate	4 d	EC50 = 1,590 µg/L	real	semi-static	7.7 (7.3 - 8.1)	25 °C		(Burton et al., 1993)
Monocotyledoneae	<i>Lemna minor</i>	Growth inhibition	Growth rate	4 d	NOEC = 590 µg/L	real	semi-static	7.7 (7.3 - 8.1)	25 °C		(Burton et al., 1993)

Taxonomic Group	Species	Effect	Effect Measurement	Duration	Value	Analytic	Exposure Type	pH	Temperature	Validity	Reference
Monocotyledoneae	<i>Lemna minor</i>	Growth inhibition	Growth rate	4 d	LOEC = 1,210 µg/L	real	semi-static	7.7 (7.3 - 8.1)	25 °C		(Burton et al., 1993)
Fishes	<i>Danio rerio</i>	Mortality		5 d	LC50 = 4,500 µg/L		semi-static		26 °C		(Koske et al., 2019)
Fishes	<i>Ictalurus punctatus</i>	Bioconcentration Factor	Accumulation, whole body	1 d	BCF = 0.79 l/kg						(Ownby et al., 2005)
Fishes	<i>Ictalurus punctatus</i>	Mortality	Deceased	4 d	LC50 = 2,400 µg/L						(Liu et al., 1983)
Fishes	<i>Lepomis macrochirus</i>	Mortality	Deceased	4 d	LC50 = 2,600 µg/L	real					(Liu et al., 1983)
Fishes	<i>Oncorhynchus mykiss</i>	Mortality	Deceased	4 d	LC50 = 800 µg/L	real					(Liu et al., 1983)
Fishes	<i>Oncorhynchus mykiss</i>	Reproduction	Survival	60 d	NOEC = 420 µg/L					2 / UBA-R+D	(Bailey et al., 1985)
Fishes	<i>Pimephales promelas</i>	Bioconcentration Factor	Accumulation, whole body	0,5 d	BCF = 4.97 l/kg	ana	static	n.D.	23 ± 1	2 / UBA-R+D	(Yoo et al., 2006)
Fishes	<i>Pimephales promelas</i>	Mortality	Deceased	4 d	LC50 = 2,700 µg/L	real	static	6-9	23 ± 1 °C	2 / UBA-R+D	(Yoo et al., 2006)
Fishes	<i>Pimephales promelas</i>	Mortality	Survival	10 d	LC50 = 2,200 µg/L	real	semi-static	6-9	23 ± 1 °C		(Yoo et al., 2006)
Fishes	<i>Pimephales promelas</i>	Mortality	Deceased	4 d	LC50 = 2,660 µg/L	real	flow-through	7.9	25±1 °C		(Burton et al., 1993)

Taxonomic Group	Species	Effect	Effect Measurement	Duration	Value	Analytic	Exposure Type	pH	Temperature	Validity	Reference
Fishes	<i>Pimephales promelas</i>	Mortality	Deceased	4 d	LC50 = 2,900 µg/L	ana	static	7.7	20±1 °C		(Liu et al., 1983)
Fishes	<i>Pimephales promelas</i>	Reproduction	Survival	60 d	LOEC = 40 µg/L						(Bailey et al., 1985)
Fishes	<i>Salmo salar</i>	Bioconcentration Factor	Akmus	0,25 d	BCF = 14 l/kg	ana	static	6.7 - 7.5	10 °C	2 / UBA-R+D	(Mariussen et al., 2018)
Fishes	<i>Sciaenops ocellatus</i>	Mortality	Survival Embryos	2 d	EC50 = 7,600 µg/L	real	static		25 °C		(Nipper et al., 2001)
Insecta	<i>Chironomus tentans</i>	Bioconcentration Factor	Accumulation, whole body	1 d	BCF = 2.62 l/kg	ana	static				(Belden et al., 2005)
Insecta	<i>Paratanytarsus parthenogenetica</i>	Mortality		2 d	LC50 = 42,900 µg/L	real	semi-static	7.4	22.2 °C		(Burton et al., 1993)
Crustacea	<i>Americamysis bahia</i>	Mortality	Survival	4 d	EC50 = 260 µg/L	real	static			3 / UBA-R+D	(Nipper et al., 2001)
Crustacea	<i>Americamysis bahia</i>	Mortality	Survival	4 d	NOEC = 320 µg/L	real	static			3 / Klimisch	(Nipper et al., 2001)
Crustacea	<i>Americamysis bahia</i>	Mortality	Survival	4 d	LOEC = 720 µg/L	real	static			3 / UBA-R+D	(Nipper et al., 2001)
Crustacea	<i>Ceriodaphnia dubia</i>	Mortality	Motility	2 d	LC50 = 4,030 µg/L	real	semi-static	8.1 (8.0 - 8.2)	24.9		(Burton et al., 1993)

Taxonomic Group	Species	Effect	Effect Measurement	Duration	Value	Analytic	Exposure Type	pH	Temperature	Validity	Reference
Crustacea	<i>Ceriodaphnia dubia</i>	Reproduction	Number of Juveniles	7 d	NOEC = 1,640 µg/L	real	semi-static	8.1	25±1 °C		(Burton et al., 1993)
Crustacea	<i>Ceriodaphnia dubia</i>	Reproduction	Number of Juveniles	7 d	LOEC = 2,710 µg/L	ana	semi-static	8.1	25±1 °C		(Burton et al., 1993)
Crustacea	<i>Daphnia magna</i>	Mobility		2 d	EC50 7,700 µg/L						(Neuwoehner et al., 2007)
Crustacea	<i>Daphnia magna</i>	Reproduction		21 d	NOEC = 480 µg/L	real					(Bailey et al., 1985)
Crustacea	<i>Daphnia magna</i>	Reproduction		21 d	LOEC = 1,030 µg/L	real				2 / UBA-R+D	(Bailey et al., 1985)
Crustacea	<i>Daphnia magna</i>	Swimming ability		1 d	ECO = 6,000 µg/L						(Bringmann & Kühn, 1977)
Crustacea	<i>Daphnia magna</i>	Swimming ability		1 d	ECO = 9,000 µg/L						(Bringmann & Kühn, 1982)
Crustacea	<i>Daphnia magna</i>	Swimming ability		2 d	LC50 = 12,000 µg/L				20±2 °C		(Dave et al., 2000)
Crustacea	<i>Hyalella azteca</i>	Bioconcentration Factor	Accumulation, whole body	6 d	BCF =	ana	semi-static	6.5-9	23 ±2°C	2 / UBA-R+D	(Sims & Steevens, 2008)
Crustacea	<i>Hyalella azteca</i>	Mortality		4 d	LC50 = 3,620 µg/L						(Sims & Steevens, 2008)

Taxonomic Group	Species	Effect	Effect Measurement	Duration	Value	Analytic	Exposure Type	pH	Temperature	Validity	Reference
Crustacea	<i>Nitocra spinipes</i>	Mortality		2 d	LC50 = 7,600 µg/L				20±2 °C		(Dave et al., 2000)
Crustacea	<i>Tigriopus californicus</i>	Mortality	Deceased	3 d	LC50 5,300 µg/L	nom	static		20 °C		(Won et al., 1976)
Mussels	<i>Crassostrea gigas</i>	Mortality		4 d	LC50 = 8,100 µg/L	nom	static		20 °C		(Won et al., 1976)
Mussels	<i>Crassostrea gigas</i>	Mortality		4 d	NOEC = 5,000 µg/L	nom	static		20 °C		(Won et al., 1976)
Mussels	<i>Mytilus galloprovincialis</i>	Bioconcentration Factor	Accumulation, whole body	0.25 d	BCF =	real	static	7.4 - 8.1	15 °C		(Rosen & Lotufo, 2007b)
Mussels	<i>Mytilus galloprovincialis</i>	Bioconcentration Factor	Accumulation, whole body	0.916666 7 d	BCF =	ana	static	7.94-7.97	15 °C	1 / UBA-R+D	(Rosen & Lotufo, 2007a)
Mussels	<i>Mytilus galloprovincialis</i>	Larval Toxicity	Survival Embryos	2 d	EC50 = 750 µg/L	real	static	7.4 - 8.1	15 °C		(Rosen & Lotufo, 2007b)
Mussels	<i>Mytilus galloprovincialis</i>	Larval Toxicity	Survival Embryos	2 d	NOEC = 440 µg/L	real	static	7.4 - 8.1	15 °C		(Rosen & Lotufo, 2007b)
Mussels	<i>Mytilus galloprovincialis</i>	Mortality	Survival	4 d	LC50 = 19,500 µg/L	real	semi-static	7.4 - 8.1	15 °C		(Rosen & Lotufo, 2007b)
Mussels	<i>Mytilus sp.</i>	Mortality		14 d	LC0 10,000 µg/L	nom			9 °C	3 / Klimisch	(Schuster et al., 2021)

Taxonomic Group	Species	Effect	Effect Measurement	Duration	Value	Analytic	Exposure Type	pH	Temperature	Validity	Reference
Mussels	<i>Mytilus sp.</i>	Mortality		14 d	LC100 30,000 µg/L	nom				3 / Klimisch	(Schuster et al., 2021)
Annelids	<i>Dinophilus gyrociliatus</i>	Mortality	Survival	7 d	EC50 = 5,600 µg/L	real	static		20 °C	4 / Klimisch	(Nipper et al., 2001)
Annelids	<i>Dinophilus gyrociliatus</i>	Reproduction	Number of Eggs	7 d	EC50 = 1,000 µg/L	real	static		20 °C		(Nipper et al., 2001)
Annelids	<i>Lumbriculus variegatus</i>	Bioconcentration Factor		1 d	BCF = 3.97 l/kg						(Belden et al., 2005)

## A.2 ETOX benthic effect data 2,4,6-trinitrotoluene (TNT)

Type	Substance Number
CAS Number	118-96-7
EC Number	204-289-6

**Table 20: Benthic effect data TNT**

Taxonomic Group	Species	Effect	Effect Measurement	Duration	Value	Reference weight	Analytic	C <sub>org</sub> [%]	Clay [%]	pH	Temperature	Validity	Reference
Crustacea	Eohaustorius estuarius	Mortality		10 d	LC50 = 23 mg/kg	TG	real	0.36	23	7.6 - 8.2	14.8 - 16.1 °C	RI-3 / CRED	(Rosen & Lotufo, 2005)
Crustacea	Eohaustorius estuarius	Mortality		10 d	LR50 = 2,497 mg/kg	FG	real	0.36	23	7.6 - 8.2	14.8 - 16.1 °C	RI-3 / CRED	(Rosen & Lotufo, 2005)
Crustacea	Leptocheirus plumulosus	Mortality	Survival	28 d	LC50 = 202,7 mg/kg	TG		2.66		7.8	21.3 °C		(Green et al., 1999)
Annelids	Neanthes arenaceodentata	Mortality	Survival	28 d	LC50 = 320 mg/kg	TG							(Green et al., 1999)

### A.3 ETOX aquatic effect data 2-amino-4,6-dinitrotoluene (2-ADNT)

The data on 2-amino-4,6-dinitrotoluene from ETOX have been migrated to ChemInfo and will only be hosted there in the future. The information for 2-ADNT can be accessed at the following link: <https://recherche.chemikalieninfo.de/etox/stoff/207167>

Type	Substance Number
CAS Number	35572-78-2
EC Number	690-132-0

**Table 21: Aquatic effect data 2-ADNT**

Taxonomic Group	Species	Effect	Effect Measurement	Duration	Value	Analytic	Exposure Type	Water hardness	pH	Temperature	Validity	Reference
Algae	Chlorophyceae	Growth		3 d	EC50 > 11,200 µg/L							(Neuwoehner et al., 2007)
Algae	<i>Skeletonema marinoi</i>	Cell Proliferation	Growth rate	3 d	EC50 = 259 µg/L	real	static		7.7 - 8.7	20 °C	RI-1 / CRED	(Schelzig, 2023a)
Algae	<i>Skeletonema marinoi</i>	Cell Proliferation	Growth rate	3 d	ErC10 = 231 µg/L	real	static		7.7 - 8.7	20 °C	RI-1 / CRED	(Schelzig, 2023a)
Amphibia	<i>Xenopus laevis</i>	Embryo toxicity	Embryo development	4 d	EC50 = 16.9 µmol/l	nom	semi-static					(Saka, 2004)
Amphibia	<i>Xenopus laevis</i>	Embryo toxicity	Mortality, Behaviour	4 d	LC50 = 166 µmol/l	nom	semi-static					(Saka, 2004)
Bacteria	<i>Pseudomonas putida</i>	Growth inhibition			EC50 > 9,400 µg/L							(Neuwoehner et al., 2007)
Bacteria	<i>Vibrio fischeri</i>	Luminescence			EC50 > 9,400 µg/L							(Neuwoehner et al., 2007)
Bacteria	<i>Vibrio fischeri</i>	Growth inhibition			EC50 > 9,400 µg/L							(Neuwoehner et al., 2007)

Taxonomic Group	Species	Effect	Effect Measurement	Duration	Value	Analytic	Exposure Type	Water hardness	pH	Temperature	Validity	Reference
Fishes	<i>Cyprinodon variegatus</i>	Mortality		5 d	LC50 = 8,600 µg/L	ana				23 °C		(Lotufo et al., 2010)
Crustacea	<i>Daphnia magna</i>	Mobility		2 d	EC50 > 11,200 µg/L							(Neuwoehner et al., 2007)
Crustacea	<i>Hyalella azteca</i>	Mortality	Mortality, Behaviour	4 d	LC50 = 3,830 µg/L	ana	semi-static			23 ± 2 °C		(Sims & Steevens, 2008)

#### A.4 ETOX aquatic effect data 4-amino-2,6-dinitrotoluene (4-ADNT)

The data on 4-amino-2,6-dinitrotoluene from ETOX have been migrated to ChemInfo and will only be hosted there in the future. The information for 4-ADNT can be accessed at the following link: <https://recherche.chemikalieninfo.de/etox/stoff/207168>

Typ	Stoffnummer
CAS Number	19406-51-0
EC Number	621-857-2

**Table 22: Aquatic effect data 4-ADNT**

Taxonomic Group	Species	Effect	Effect Measurement	Duration	Value	Analytic	Exposure Type	pH	Temperature	Validity	Reference
Algae	Chlorophyceae	Growth		3 d	EC50 = 4,800 µg/L						(Neuwoehner et al., 2007)
Algae	<i>Skeletonema marinoi</i>	Cell Proliferation	Growth rate	3 d	ErC10 = 297 µg/L	real	static	8,0 - 8,3	20 °C	RI-1 / CRED	(Schelzig, 2023b)
Algae	<i>Skeletonema marinoi</i>	Cell Proliferation	Growth rate	3 d	ErC10 = 237 µg/L	real	static	7,8-8,7	20 °C	RI-1 / CRED	(Schelzig, 2023c)
Algae	<i>Skeletonema marinoi</i>	Cell Proliferation	Growth rate	3 d	ErC50 = 351 µg/L	real	static	7,8-8,7	20 °C	RI-1 / CRED	(Schelzig, 2023c)
Algae	<i>Skeletonema marinoi</i>	Cell Proliferation	Growth rate	3 d	ErC50 = 485 µg/L	real	static	8.0 - 8.3	20 °C	RI-1 / CRED	(Schelzig, 2023b)
Amphibia	<i>Xenopus laevis</i>	Embryotoxicity	Embryo development	4 d	EC50 = 85.8 µmol/l	nom	semi-static	6.5 to 8.5			(Saka, 2004)
Amphibia	<i>Xenopus laevis</i>	Embryotoxicity	Mortality, Behaviour	4 d	LC50 = 115 µmol/l	nom	semi-static	6.5 to 8.5			(Saka, 2004)
Bacteria	<i>Pseudomonas putida</i>	Growth inhibition			EC50 > 7,000 µg/L						(Neuwoehner et al., 2007)

Taxonomic Group	Species	Effect	Effect Measurement	Duration	Value	Analytic	Exposure Type	pH	Temperature	Validity	Reference
Bacteria	<i>Vibrio fischeri</i>	Luminescence			EC50 > 7,000 µg/L						(Neuwoehner et al., 2007)
Bacteria	<i>Vibrio fischeri</i>	Growth inhibition			EC50 > 7,000 µg/L						(Neuwoehner et al., 2007)
Crustacea	<i>Daphnia magna</i>	Mobility		2 d	EC50 4,700 µg/L						(Neuwoehner et al., 2007)
Crustacea	<i>Hyalella azteca</i>	Mortality		4 d	LC50 = 9,170 µg/L	ana			23 ± 2 °C		(Sims & Steevens, 2008)