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Bioaccumulation of 2,4,6-trinitrotoluene (TNT) and its metabolites leaking from corroded munition in transplanted blue mussels (*M. edulis*)

Daniel Appel*, Jennifer S. Strehse, Hans-Jörg Martin, Edmund Maser

Institute of Toxicology and Pharmacology for Natural Scientists, University Medical School Schleswig-Holstein, Brunswiker Str. 10, 24105 Kiel, Germany

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ABSTRACT

Bioaccumulation of 2,4,6-trinitrotoluene (TNT) and its main metabolites 2-amino-4,6-dinitrotoluene (2-ADNT) and 4-amino-2,6-dinitrotoluene (4-ADNT) leaking from corroded munitions at a munitions dumping site (Kolberger Heide, Germany) was evaluated in transplanted blue mussels (*Mytilus edulis*). Six moorings with mussel bags were placed east and west at varying positions near the mine mound. In order to monitor any differences resulting from changing seasons, three exposure times were chosen. First exposure period: April–July 2016 (106 days); second exposure period: July–December 2016 (146 days); third exposure period: December 2016–March 2017 (92 days). We found amounts of 4-ADNT in mussel tissue ranging from 2.40 ± 2.13 to 7.76 ± 1.97 ng/(g mussel wet weight). Neither TNT nor 2-ADNT could be detected. Considering seasonal differences, orientation and distances of the moorings to the mine mound no correlation between levels in mussel tissue was evident.

1. Introduction

Huge amounts of munitions dumped in the sea after World Wars I and II are a worldwide problem for shipping, installation of offshore wind farms and the aquatic ecosystem (Beddington et al., 2005; Beck et al., 2018). About 1.6 million tonnes of warfare material have been dumped mostly after World War II in the German part of the North and Baltic sea (Böttcher et al., 2011; BLANO et al., 2012–2015). More than 70 years later most of the casings are more or less corroded resulting in a constant release of toxic substances like nitroaromatic compounds in the marine environment (Lewis et al., 2009; Pennington et al., 2008; Gębka et al., 2016; Szarejko and Namieśnik, 2009). However, the exact state of corrosion varies widely and is unknown in many cases (Beddington et al., 2005). Aside from natural corrosion of sea-dumped munitions low-order detonations, occurring either spontaneously or during the clearing of contaminated sites, elevate the level of toxic explosives in the marine environment. Low-order detonations distribute a large variety of unexploded munitions material at the sea floor, which dissolves slowly over time (Juhasz and Naidu, 2007).

As one of the most abundantly produced explosives the nitroaromatic energetic compound 2,4,6-trinitrotoluene (TNT) can be found in most munition formulations. TNT is thermally and chemically stable with a low solubility in water of about 100 mg/L (Rosen and Lotufo, 2007). Munition constituents like TNT are known to degrade in the marine environment forming a variety of products. The nitro-groups of TNT are prone to reduction resulting mainly in 2-amino-4,6-dinitrotoluene (2-ADNT) and 4-amino-2,6-dinitrotoluene (4-ADNT) (Lotufo et al., 2016; Elovitz and Weber, 1999; Conder et al., 2004a). This is the main degradation pathway of TNT in the environment (Rosen and Lotufo, 2007).

Further reduction of the nitro-groups may result in diamino compounds and ultimately 2,4,6-triaminotoluene (Pennington and Brannon, 2002). The chemical structures, physical and chemical properties of TNT and its main metabolites, 2-ADNT and 4-ADNT, are given in Table 1.

Several studies have shown a correlation between explosive concentration and various toxicological endpoints in marine organisms, see for example Nipper et al. (2001). Bioaccumulation and toxicity of sediment-associated TNT and its metabolites have been reported for the fish sheepshead minnows (*Cyprinodon variegatus*) and freckled blennies (*Hypsoblennius ionthas*) (Lotufo et al., 2010).

As for human toxicity several endpoints including methemoglobinemia, cataracts, mutagenic metabolites and possible genotoxic effects have been reported (Sabbioni and Rumler, 2007; Richter-Torres et al., 1995).

People can be exposed to explosives through their workplace (e.g. production of warfare material) or by living near contaminated or military sites. Another route of exposure may be eating contaminated

* Corresponding author.
E-mail address: Appel@toxi.uni-kiel.de (D. Appel).

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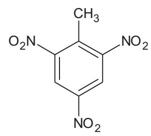
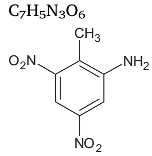
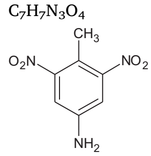
Table 1
Physical and chemical properties of 2,4,6-trinitrotoluene (TNT) and its main metabolites 2-amino-4,6-dinitrotoluene (2-ADNT) and 4-amino-2,6-dinitrotoluene (4-ADNT).

Compound name	Chemical structure and formula	Molar mass $\left[\frac{g}{mol}\right]$	Melting point [°C]	Solubility in water (Juhasz and Naidu, 2007) $\left[\frac{g}{L}\right]$	Octanol-water partitioning coefficient (Juhasz and Naidu, 2007)	Henry's law constant (Juhasz and Naidu, 2007) $\left[\frac{mol}{m^3\,Pa}\right]$
2,4,6-Trinitrotoluene (TNT)	 $C_7H_5N_3O_6$	227.13	80.4	0.13 (20 °C)	1.86	22–840
2-Amino-4,6-dinitrotoluene (2-ADNT)	 $C_7H_7N_3O_4$	197.15	176	0.017	2.8	80
4-Amino-2,6-dinitrotoluene (4-ADNT)	 $C_7H_7N_3O_4$	197.15	171	0.036	2.62	60

food (Richter-Torres et al., 1995; Voie and Mariussen, 2016).

Recently, we could show that the blue mussel (*Mytilus edulis*) accumulates TNT and its main metabolites 2-ADNT and 4-ADNT when transplanted near a bulk of explosive material lying on the sea ground in a field experiment at Kolberger Heide in the German part of the Baltic Sea (Strehse et al., 2017).

As benthic filter feeders, mussels filter around 24 L of water and suspended material per day and thus accumulate chemicals in their tissue. Mussels have been robust and useful bioindicators for environmental contamination assessments for > 40 years (Farrington et al., 1983; Salazar and Salazar, 1995).

Several studies under laboratory conditions have shown that TNT and its metabolites impair the performance of mussels (Rosen and Lotufo, 2007; Lotufo et al., 2016; Won et al., 1976). However, although negative effects on mussel survival could be clearly documented, most of these experiments were run for a rather short time span ranging from a few hours to a few days. Therefore, they do not reflect any long-term exposure which mussels normally face in their natural habitats. Furthermore, the concentrations of explosives used in some of the studies exceeded open water pollution levels by far. For these reasons we assume that their ecological relevance to chronic effects is limited.

In this field study we transplanted several bags of blue mussels in the study area Kolberger Heide near a mound of moored mines dumped after World War II. In this area no pieces of explosive material are lying on the seafloor and lower concentrations of TNT and its metabolites can be anticipated. Therefore, we prolonged the incubation period from days to months. We investigated whether *M. edulis* can accumulate TNT and its metabolites 2-ADNT and 4-ADNT leaking from corroded munitions after exposure times of several months and if accumulation is seasonally dependent.

2. Material and methods

2.1. Study site and mussel collection

Individuals of the blue mussel *Mytilus edulis* were obtained from a commercial mussel farm located in Kiel Bay, Germany. Mussels were transported in cooled insulation boxes with seawater to the shipping vessel and transferred to the study site Kolberger Heide (54,46°N, 10,34°E). In this area of approximately 1.260 ha up to 18.000 torpedo heads and mines of various types have once been dumped (BLANO et al., 2011). Due to the fact that the study site is a sandy area which is covered with patches of brown algae *Mytilus edulis* can be found rarely and isolated. Visual inspection of the study site by divers showed no explosive material lying on the sea ground, contrary to the study site of a recent study (Strehse et al., 2017). Mussels were transplanted by divers in the vicinity of a mine mound composed of approximately 100 moored mines from World War II in different states of corrosion (see Fig. 1). The extent of explosives inside the dumped munition at the study site is poorly documented (BLANO et al., 2011). In 2009 several 250–500 kg mines were detonated underwater next to our study site (Beck et al., 2018). We therefore assume at least 25.000 kg of explosive material at the study site.

Six moorings with two mussel bags each (see Fig. 2), one directly on the ground (P0) and one 1 m above ground level (P1), were deployed at different distances (closest distance 0 m) from the mine mound. Each mussel bag was filled with 20 mussels. Mussels were deployed at three different exposure periods in order to monitor any differences resulting from changing seasons. First exposure period: April–July 2016 (106 days); second exposure period: July–December 2016 (146 days); third exposure period: December 2016–March 2017 (92 days). Sampling was done by the explosive ordnance disposal unit (state office of criminal investigation) and/or Kiel University research divers. After recovery the mussels were immediately placed on dry ice and stored at −80 °C until further use.

2.2. Chemicals

2,4,6-trinitrotoluene (TNT), 2-amino-4,6-dinitrotoluene (2-ADNT) and 4-amino-2,6-dinitrotoluene (4-ADNT) were purchased from AccuStandard (New Haven, CT, USA) with a chemical purity of 100% for all compounds. Gradient grade acetonitrile was purchased from Th. Geyer GmbH & Co. KG (Renningen, Germany).

2.3. Sample preparation

Extraction and analysis of TNT, 2-ADNT and 4-ADNT was carried out as described previously (Strehse et al., 2017). In brief, mussels