In vitro effects of Trinitrotoluene on Baltic flatfish hepatocytes

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Introduction

Trinitrotoluene (TNT) in dumped ammunition on the seafloor is subject to various biotic and abiotic transformation processes. Understanding these conversion reactions is important when evaluating TNT-induced effects on fish, and along the food chain up to human consumers. Since TNT derivatives cause different biological effects than TNT, we here address the potential effects of TNT on biotransformation enzymes as well as the associated formation of TNT metabolites in fish. By using *in vitro* metabolism experiments, we found both known and new TNT metabolites formed in fish.

Methods

- *In vitro* experiments (see Figure 1) were carried out with pooled dab liver samples from a reference area close to Kiel Bight in the western Baltic Sea.
- Liver homogenates were prepared by differential centrifugation[1] and analysis was carried out on HPLC-QQQ-MS (for details see Poster A2.5_10).
- Enzyme activity - using the example of ethoxyresorufin-O-deethylase (EROD) - was determined photometrically[2].

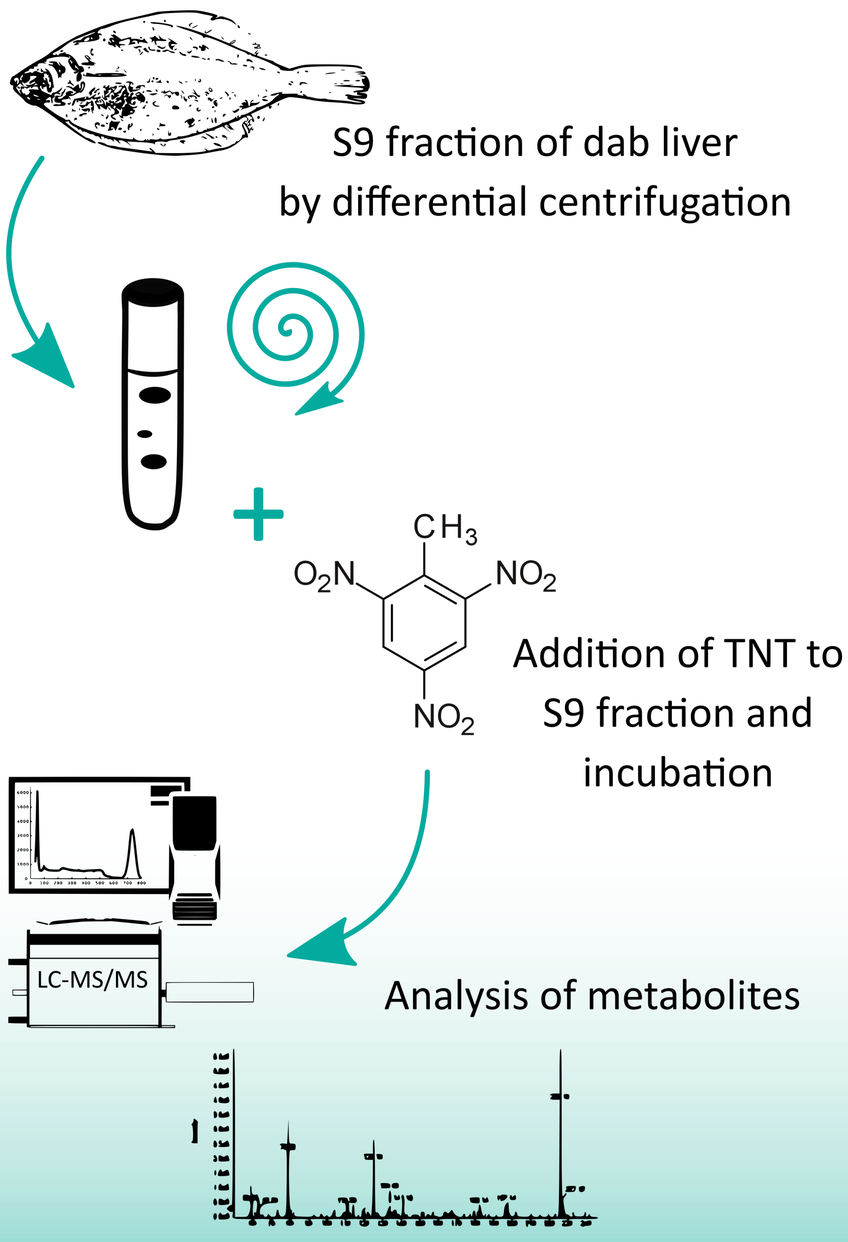

Figure 1: Scheme of *in vitro* experiments with dab livers.

Results & Discussion

- EROD activity in dab livers is reduced by competitive inhibition of the enzyme after *in vitro* exposure to TNT. Approximately 35 % inhibition of EROD activity is observed at 7 mg/l TNT (see Figure 2).
- Since EROD is a representative biotransformation enzyme, the inhibition of such enzymes could reduce the fish's ability to cope with other pollutants.

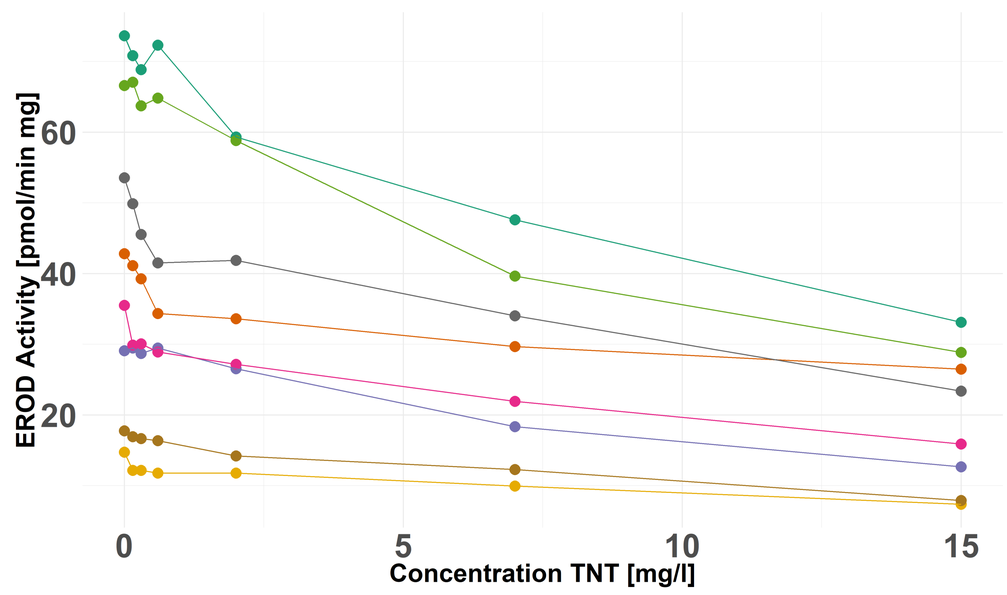

Figure 2: *In vitro* inhibition of ethoxyresorufin-O-deethylase (EROD) in dab liver samples after exposure to TNT. Eight independent liver samples were used and incubated with TNT concentrations ranging from 0.15 mg/l to 15 mg/l.

- The experiments revealed a time-dependent transformation of TNT into specific metabolites via liver-enzyme catalysed *in vitro* conversions (see Figure 3). The detection of primary metabolites – such as ADNTs – proves the functionality of the method since they were also found *in vivo*[3].
- In addition, we found so far unidentified TNT metabolites for fish like dinitrobenzoic acid (DNBA) which is only known as photodegradation product of TNT[4].
- The metabolites found can serve as target compounds for munition monitoring in the future since rapid degradation processes and low concentrations make it necessary to focus on several compounds.

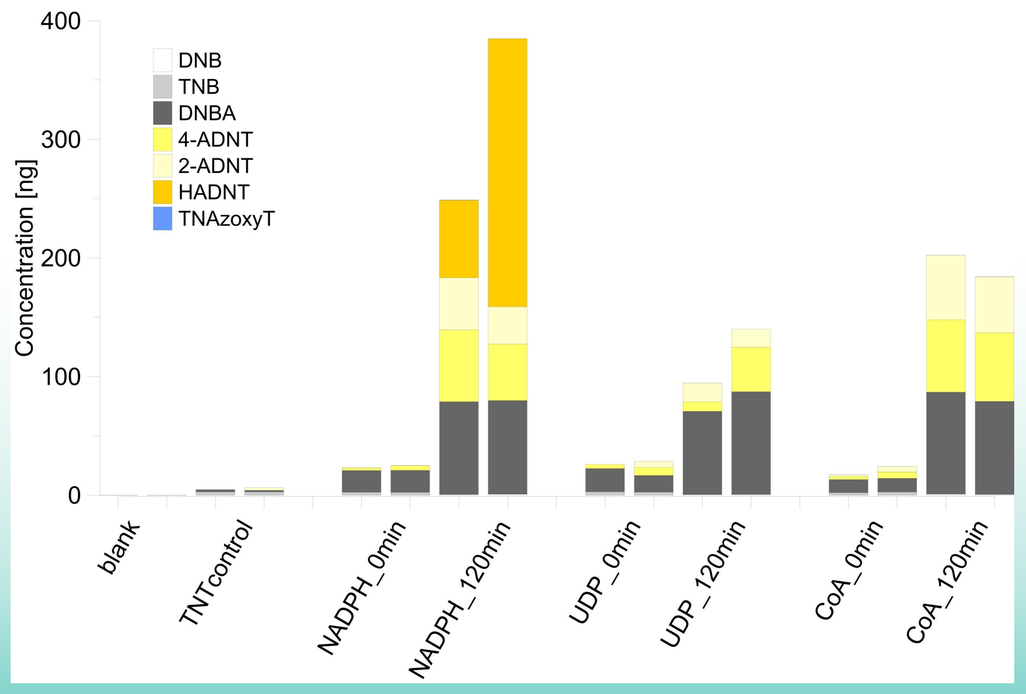

Figure 3: Detected nitroaromatic compounds after *in vitro* metabolism experiments with TNT and dab liver hepatocytes. Incubation was carried out with different cofactors (NADPH, UDP and Coenzyme A) and different incubation times (0, 120 min). Blank samples contained no TNT, TNT control were prepared without S9 fraction. All samples were prepared in duplicate.

[1] Ronisz D, Förlin L (1998), Interaction of isosafrole, β-naphthoflavone and other CYP1A inducers in liver of rainbow trout (Oncorhynchus mykiss) and eelpout (Zoarces viviparus), *Comparative Biochemistry and Physiology Part C Pharmacology, Toxicology and Endocrinology*, 121, 289–296.

[2] Burke DM, Mayer RT (1974), Ethoxyresorufin: Direct Fluorimetric Assay of a Microsomal O-Dealkylation which is Preferentially Inducible by 3-Methylcholanthrene, *Drug Metabolism and Disposition*, 2, 583–588.

[3] Mariussen, E., Stornes, S. M., Bøifot, K. O., Rosseland, B. O., Salbu, B., & Heier, L. S. (2018), Uptake and effects of 2, 4, 6-trinitrotoluene (TNT) in juvenile Atlantic salmon (*Salmo salar*), *Aquatic Toxicology*, *194*, 176-184.

[4] Prak, D. J. L., Breuer, J. E., Rios, E. A., Jedlicka, E. E., & O'Sullivan, D. W. (2017), Photolysis of 2, 4, 6-trinitrotoluene in seawater and estuary water: Impact of pH, temperature, salinity, and dissolved organic matter, *Marine pollution bulletin*, *114*(2), 977-986.