DAIMON Toolbox Fact Sheets:

Methods to Study the Impact of Dumped Munitions on Marine Biota

Assessment category 2: Hazardous Substances

Toolbox component: Biota Chemistry

Fact Sheet 2.10: Extraction of explosives and metabolites from fish bile

Authors: Nadine I. Goldenstein and Daniel Koske, Thünen Institute of Fisheries Ecology

What is it?

The gallbladder as storage and excretion organ accumulates bile, produced in the liver. Hence, investigating bile offers a representative picture of the uptake of xenobiotics that are being processed in the fish liver and the detoxification performed by the latter organ, which leads to production of metabolites of the respective toxicants.

What does it tell you?

Bioavailable explosives compounds accumulate in fish and are metabolized in the liver via enzymatic derivatisation (Koske et al., 2019, in prep.). These derivatives can be analysed in the fish bile and represent reliable biomarkers for the exposition of fish to dumped munitions.

Type of Indicator (tick box)

☐ non-specific stress indicator

☐ specific for groups of contaminants incl. CWA or explosives

☐ CWA-specific indicator

☒ specific for substances related to explosives (e.g. TNT)

How to conduct the procedure?

***Species*:** Analysis of explosives in bile was so far conducted in common dab (*Limands limanda*), European flounder (*Plathichthys flesus*) and plaice (*Pleuronectes platessa*).

***Matrix*:** Bile extracted from the gallbladder.

***Equipment*:** Eppendorf pipettes for quantitative transfer of liquid volumes of 25, 5 and 1000 µL. Eppendorf vials of 1.5 µL volume as well as screw-capped analytical brown glass vials (combusted) of 1.5 µL volume with and without insert. A 100 µL analytical syringe, a cooled Eppendorf centrifuge and a N_2 reduction unit.

Procedure: An aliquot of 25 µL of the original bile sample is transferred into a 1.5 mL Eppendorf vial using a 100 µL Eppendorf pipette with disposable tips. For every 20 samples one vial is filled with 25 µL of Milli Q water instead of bile as an extraction blank and treated as a sample for the following steps. The subsamples are amended with 5 µL of internal standard (1,4-DNB; [10 ng μL^{-1}]). Thereafter, 1 mL of cold (~4°C) Acetonitirle is added to each vial as extraction solvent and the solution is mixed vigorously for 30 sec. on a Vortex mixer. The samples are left to separate for 10 minutes at 4 °C in the dark and are subsequently centrifuged for 10 min at 6000 rpm at 4 °C. The supernatant from each sample is right away transferred into 1.5 mL brown glass vials and reduced to ~ 200 µL under a stream of nitrogen. The reduced sample is transferred into a new 1.5 mL brown glass injection vial with insert and again reduced to a total volume of ~ 50 µL. From this concentrated sample 5 µL are directly afterwards injected on the HPLC-MS for analysis of explosives and metabolites compounds. If samples have to be stored for short periods of time before analysis these are kept in the dark at -20 °C until further treatment.

How to analyse and assess the data?

Quantification: The amount of the analyte present in the sample is quantified via the internal standard 1,4-DNB, added to the sample before extraction. Analytical quantification as performed after analysis on the HPLC-MS is described in more detail in DAIMON Fact Sheet 2.11 (Goldenstein 2019).

References

Koske, D., Goldenstein, N.I., Rosenberger, T., Machulik, U., Hanel, R., Kammann, U., 2019. Dumped munition: New insights into the metabolization of 2,4,6-trinitrotoluene in Baltic flatfish. *in prep.*