DAIMON Toolbox Fact Sheets:

Methods to Study the Impact of Dumped Munitions on Marine Biota

Assessment category 3: Biological effects

Toolbox component: Biota sampling

Fact Sheet 3.3: Homogenisation of fish liver and mussel digestive gland tissues

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What is it?

Homogenisation of the target tissue is required prior to the measurement of enzymatic biomarkers and lipid peroxidation (LPX). Tissue and cells are broken mechanically in a buffer solution, the sample is centrifuged and the resulting supernatant is used in the assays.

How to do it?

***Species*:** A generic method used for most species, including fish and mussels.

***Matrix*:** Fish liver and mussel digestive gland tissue.

***Equipment*:** Weighing balance (1 mg accuracy); homogenizer (e.g., Qiagen TissueLyser); centrifuge; basic laboratory equipment (2 ml eppendorf tubes, decanters). For details, see, e.g., Turja et al. (2014).

***Procedure*:** Tissues are homogenized in individual 2 ml eppendorf tubes in phosphate buffer 100 mM K-PO_4, pH 7.4. The samples are then centrifuged for 20 min in 10,000 *g* and aliquots of the supernatant are taken in new tubes and stored immediately at -80°C. For details, see, e.g., Turja et al. (2014).

Samples for the measurement of LPX are taken before homogenization by dissecting a piece of tissue (20-30 mg) into a separate tube.

References

Turja, R., Höher, N., Snoeijs, P., Baršienė, J., Butriavičienė, L., Kuznetsova, T., Kholodkevich, S.V., Devier, M.H., Budzinski, H., Lehtonen., K.K. 2014. A multibiomarker approach to the assessment of pollution impacts in two Baltic Sea coastal areas in Sweden using caged mussels (*Mytilus trossulus*). *Science of The Total Environment*. 473-474:398-409.