Standard Operating Procedure (SOP)

Aim of SOP (tick box)

☐ Munition detection or identification	☒ Toxicity
☐ Sampling	☒ In situ exposure studies
☒ Sample preparation	☐ Bioassays
☐ Chemical analysis	
☐ Bioindicators/biomarkers	

8. Homogenisation of fish liver and mussel digestive gland tissues

version 1.1

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Scope

This SOP describes the method of preparing samples for biochemical biomarker analysis in biological samples exposed to dumped munitions. The method is based on references 1 and 2 and has been modified for mussel and fish samples by SYKE. Related to dumped munitions, it has been used two EU-funded projects: Chemical Munitions, Search and Assessment (CHEMSEA) and Decision Aid for Marine Munitions (DAIMON).

Summary of the method/SOP

Tissue and cells are broken mechanically in a buffer solution, the sample is centrifuged and the resulting supernatant is used in the assays.

Safety aspects

Normal laboratory safety rules should be applied.

Documentation

All samples should have a unique code and label. Pipetting errors or other anomalies that could affect the interpretation of the results should be recorded.

Methods

Matrix: Fish liver and mussel digestive gland tissue.

Equipment: Weighing balance (1 mg accuracy); homogenizer (e.g., Qiagen TissueLyser Eppendorf-tube centrifuge with cooling possibility and 10 000 x g; basic laboratory equipment (2 ml eppendorf tubes, decanters).

Table 1: Solutions used in the analysis.

solution	

100 mM potassium phosphate buffer	100mM K_2HPO_4 + 100mM KH_2PO_4 in water, pH 7.0

reagent	**CAS-number**
K_2HPO_4	7758-11-4
KH_2PO_4	7778-77-0

Procedure: Tissues are homogenized in individual 2 ml eppendorf tubes in potassium phosphate buffer. The volume of the buffer (in µl) is calculated as ten times the weight (for fish liver) and three times the weight (for mussel dg) of the tissue sample in mg. After homogenization in the TissueLyser the samples are centrifuged for 20 min in 10,000 g at 4°C and aliquots of the supernatant are taken in new tubes and stored immediately at -80°C.

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

Conclusions (if applicable)

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References

[1] Turja, R., Höher, N., Snoeijs, P., Baršienė, J., Butriavičienė, L., Kuznetsova, T., Kholodkevich, S.V., Devier, M.H., Budzinski, H., Lehtonen., K.K., *Sci Total Environ 473-474,* **2014**, 398-409.

Change history

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