

DAIMON Toolbox Fact Sheets:

Methods to Study the Impact of Dumped Munitions on Marine Biota

Assessment category 3: Biological effects

Toolbox component: General stress

Fact Sheet 3.10: Lipid peroxidation (LPX)

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What is it?

An overload of intracellular reactive oxygen species (ROS) can occur due to exposure to different groups of pollutants. If the antioxidant defence system (ADS) cannot effectively neutralize ROS, damage can occur to various cellular components and structures. Lipids are macromolecules readily damaged by ROS peroxidation, and in cell membranes this can cause serious cellular dysfunctions.

What does it tell you?

Elevated levels of lipid peroxidation (LPX) indicate a breakdown of the ADS and damage to cellular membranes that may cause various negative effects on the individual's health status.

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 measure it?

Species: LPX can be measured in a large variety of organisms, including fish and mussels.

Matrix: Fish liver and mussel digestive gland tissue.

Equipment: Spectrophotometer/microplate reader able to measure at 570 nm and 590 nm; microplates; basic laboratory equipment (pipettes, decanters). For reagents, see, e.g., Ohkawa et al. (1979) and Vuori et al. (2015).

Measurements and units: For a detailed description, see, e.g., Ohkawa et al. (1979) and Vuori et al. (2015). Briefly, the tissue is homogenized in methanol and centrifuged. A mixture containing sulphuric acid, ammoniumferrosulphate hexahydrate and xylenol orange in methanol is added and the absorbance is measured after incubation. Samples are prepared in duplicate with the other

sample being treated with TPP (triphenyl phosphate) that blocks lipid hydroperoxides interfering with the analysis. A standard curve is prepared with cumene.

Calculations: Absorbance difference between TPP-treated and untreated samples. LPX concentration is adjusted with tissue wet weight (mg).

Sample size: Measurements are made from at least 15-20 individual specimens from each study site.

How to analyse and assess the data?

Compare the LPX levels measured from organisms collected from the target area to those from the reference area. An elevated or lowered activity level (bell-shape response) compared to the reference area indicate a negative effect. If the difference in mean activity level is more than one standard deviation (SD) of the mean values measured in the reference area, stress is considered moderate. If the level differs more than two SDs, stress is severe.

References

Ohkawa, H., Ohishi, N., Yagi, K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry* 95: 351-359.

Vuori, K.A., Lehtonen, K.K., Kanerva, M., Peltonen, H., Nikinmaa, M., Berezina, N.A., Boikova, E. 2015. Oxidative stress biomarkers in the copepod *Limnocalanus macrurus* from the northern Baltic Sea: effects of hydrographic factors and chemical contamination. *Marine Ecology Progress Series* 538:131-144.