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 3.13: Glutathione peroxidase activity (GPx)

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What is it?

Glutathione peroxidase enzyme (GPx) catalyzes the reduction of hydrogen peroxide to water and oxygen, as well as the reduction of peroxide radicals to alcohols and oxygen. GPx is a key enzyme in the antioxidant defence system (ADS).

What does it tell you?

Elevated levels of GPx indicate that the cellular ADS has been activated due to an accelerated production of reactive oxygen species (ROS) that need to be neutralized to prevent oxidative damage to macromolecules. On the other hand, lowered GPx levels may indicate that the ADS is already overloaded and the organism cannot properly cope with the excessive amounts of ROS (the so-called bell-shape response).

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

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

***Equipment*:** Spectrophotometer/microplate reader able to measure at 340 nm in intervals; 96-well UV microplate or 96-well half area microplates; basic laboratory equipment (pipettes, decanters). For reagents, see, e.g., Sigma-Aldrich (2017) and Vuori et al. (2015).

***Measurements and units*:** Measurements is recommended to be perfomed using a commercial kit, e.g., Glutathione Peroxidase Cellular Activity Assay Kit (Sigma CGP1-1KT), modified for liver samples. Briefly, the activity of GPx is measured as the change in absorption at 340 nm in a mixture containing 0.5 mM NADPH, 4.2 mM GSH (reduced glutathione) and 1 unit/ml GR (glutathione reductase) at a final concentration in a NaN_3 (sodium azide) supplemented reaction

buffer (NaN_3 is used to inhibit CAT activity that may disturb the assay; not provided in the kit). The activity of GPx (OD/min) is adjusted to the protein concentration of the sample, measured with, e.g., the Bradford method (Bradford, 1976).

***Calculations*:** change in absorbance (OD/min). Subtract blank measurement values from the sample measurements.

GPx activity is calculated with the formula

$$activity = \frac{-absorbance\ change * analysis\ volume}{molar\ attenuation\ factor\ of\ NADPH * light\ path * sample\ volume * sample\ protein\ concentration}$$

where molar attenuation factor of NADPH is 6.22 $mM^{-1}\,cm^{-1}$.

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

How to analyze and assess the data?

Compare the GPx activity 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

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*. 72:248-254.

Sigma-Aldrich. 2017. Glutathione Peroxidase Cellular Activity Assay Kit Technical Bulletin. *Sigma-Aldrich Co, St. Louis, USA.*

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.