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 3.15: Glutathione *S*-transferase activity (GST)

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What is it?

Glutathione *S*-transferase (GST) catalyzes the conjugation of reduced glutathione (GSH) to a xenobiotic molecule to make it more water soluble and thus excretable. GST also participates in the functioning of the antioxidant defence system (ADS).

What does it tell you?

Elevated levels of GST activity indicate indicate exposure to xenobiotics involving detoxification by GSH conjugation. A lowered GST activity level may indicate toxic effects caused by the incapability to respond to xenobiotic exposure (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*:** GST can be measured in a large variety of organisms, including fish and mussels.

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

***Equipment*:** Spectrophotometer/microplate reader able to measure at 340 nm in intervals; basic laboratory equipment (pipettes, microplates, decanters). For reagents, see, e.g., Turja et al. (2014).

***Measurements and units*:** For a detailed desicription, see, e.g., Habig et al. (1974) and Turja et al. (2014). Briefly, the activity of GST is measured as the colour change in 340 nm in a mixture containing 2 mM GSH (reduced glutathione) and 1 mM CDNB (1-chloro-2,4 dinitrobenzene) at final concentration in Dulbecco's buffer. The activity of GST (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.

GST activity is calculated with the formula

$$activity = \frac{absorbance\ change * analysis\ volume}{molar\ attenuation\ coefficient * light\ path * sample\ volume * sample\ protein\ concentration}$$

where the molar attenuation coefficient for the CDNB conjugate is 9.6 $M^{-1}\,cm^{-1}$.

***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 GST 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.

Habig, W.H., Pabst, M.J., Jakoby, W.B. 1974. Glutathione *S*-transferases - first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry* 249: 7130-7139.

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