Recommended Operating Procedure (ROP)

Aim of ROP (tick box)

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

12. Glutathione-S-transferase activity

version 1.0

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Scope

This ROP describes the analysis method for assessing xenobiotic detoxification in biological samples hazardous substances, including substances derived from sea-dumped munitions. 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). 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). The method is based on Habig et al.[1], but has been modified for mussel and fish samples by SYKE. It has been used two EU-funded projects: Chemical Munitions, Search and Assessment (CHEMSEA) and Decision Aid for Marine Munitions (DAIMON)[4].

Summary of the method/ROP

Glutathione-S-transferase activity is measured spectrophotometrically from tissue homogenates of mussel digestive gland or fish liver (see separate SOP document for homogenization[5,6]). Measurements need to be done in samples from both the suspected dumping site and a reference area.

Safety aspects

Normal laboratory safety rules should be applied. CDNB (1-chloro-2,4 dinitrobenzene) is very hazardous to humans and the environment.

Documentation

All samples should have a unique code and label. Pipetting schemes should be used to record the analysis procedure and order of samples, as well as possible pipetting errors or other anomalies that could affect the interpretation of the results.

The test report should include the following results for each sample:

Absorption per minute of each sample at 340 nm [OD/min]
Sample volume in the analysis [µl]
Protein concentration [mg/ml]
GST activity [nmol mg^{-1} protein min^{-1}]

Methods

Matrix: Fish liver, mussel digestive gland and gill tissue homogenates (see SOPs for homogenization[5,6]).

Equipment: Spectrophotometer/microplate reader able to measure at 340 nm in intervals; basic laboratory equipment (pipettes, microplates, decanters). For reagents, see Table 1 and 2.

Table 1: Solutions used in the analysis.

solution	**details**
CDNB-solution	100 mM dissolved in 99% EtOH
GSH-solution (keep on ice)	100 mM GSH in Dulbecco's buffer
Dulbecco's buffer	commercial buffer, e.g. Merck product # D8662

Table 2: Reagents used in the analysis.

reagent	**CAS-number**
CDNB	97-00-7
GSH (reduced glutathione)	70-18-8
99% ethanol	64-17-5

Measurements and units: Reaction buffer should be room temperature for the measurements. Pipette 2µl/well of samples and blank (buffer) in four replicates in 96-well half-area plate. Mix 11,64 ml of Dulbecco's buffer with 240µl of GSH-solution and 120 µl of CDNB-solution. Prepare a new mix for each plate. Pipette 98µl of the mix in each well and start measurement on the spectrophotometer at 340 nm. Measure the change in absorbation for 5 minutes, in ca. 25 second cycles. Sample volumes in the analysis can and should be adjusted to achieve a satisfactory result i.e. measurable activity, defined as OD/min values over 0.05. If the variance between sample replicate absorbances is too high, measurements for that sample should be repeated.

For more information, see references 1 and 2. The activity of GST is adjusted to the protein concentration of the sample, measured with, e.g., the Bradford method[3]. Calculate the change in absorbance (OD/min). Subtract blank measurement values from the sample measurements. Calculate a mean OD/min value from the four replicates.

GST activity is calculated with the formula:

$$= \frac{absorbance\ change\ [\frac{1}{min}] * analysis\ volume[ml]}{molar\ attenuation\ coefficient\ \left[\frac{1}{(\frac{mol}{l}) * cm}\right] * light\ path[cm] * sample\ volume[ml] * sample\ protein\ concentrat}$$

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.

Conclusions (if applicable)

Sample volumes in the analysis can and should be adjusted to achieve a satisfactory result (i.e. measurable activity). GST is ubiquitous in the animal kingdom and can be measured also in other species than those mentioned in this ROP.

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

[1]Habig, W.H., Pabst, M.J., Jakoby, W.B., *J Biol Chem 294,* **1974,** 7130-7139.

[2]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.

[3]Bradford, M.M., *Anal Biochem 72, **1976**, 248-254.*

[4] Bełdowski, J., Fabisiak, J., Popiel, S., Östin, A., Olsson, U., Vanninen, P., Lastumaki, A., Lang, T., Fricke, N., Brenner, M., Berglind, R., Baršiene, J., Klusek, Z., Pączek, B., Söderström, M., Lehtonen, K., Szubska, M., Malejevas, V., Koskela, H., Michalak, J., Turja, R., Bickmeyer, U., Broeg, K., Olejnik, A. and Fidler, J. *Institute of Oceanology Polish Academy of Sciences, Gdańsk, Poland,* **2014**, ISBN: 978-83-936609-1-9, 86 pp

[5]Ahvo, A. *DAIMON 2 PROJECT SOPs: Homogenization of fish muscle and mussel gill tissues,* **2020.**

[6]Ahvo, A. *DAIMON 2 PROJECT SOPs:* Homogenisation of fish liver and mussel digestive gland tissues **2020.**

Change history

1.0	11.2.2020	First edition
1.1	10.6.2020	
1.2	18.5.2021	Definition of the document was changed from SOP to ROP

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