Recommended Operating Procedure (ROP)

Aim of ROP (tick box)

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

7. Catalase activity

version 1.1

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Scope

This ROP describes the analysis method for assessing oxidative stress in biological samples exposed to hazardous substances, including substances derived from sea-dumped munitions. The method is based on Claiborne et al.[1], but has been modified for mussel and fish samples by SYKE. Elevated levels of CAT activity indicate activation of the cellular ADS 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 CAT 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). CAT is a non-specific stress indicator. It has been used in the monitoring and ecological risk assessment of dumped munitions in two EU-funded projects: Chemical Munitions, Search and Assessment (CHEMSEA) and Decision Aid for Marine Munitions (DAIMON)[5].

Summary of the method/ROP

Catalase activity is measured spectrophotometrically from tissue homogenates of mussel digestive gland or fish liver (see separate SOP ”Homogenisation of fish liver and mussel digestive gland tissues”[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. Hydrogen peroxide is harmful if swallowed and poses a risk of eye damage.

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 240 nm [OD/min]
Sample volume in the analysis [µl]
Protein concentration [mg/ml]
CAT activity [nmol mg^{-1} protein min^{-1}]

Methods

Matrix: Fish liver, mussel digestive gland and gill tissue homogenates (see also SOPs for homogenisation[6,7]).

Equipment: Spectrophotometer /microplate reader able to measure kinetics at 240 nm; UV microplates; basic laboratory equipment (pipettes, decanters), laboratory grade water purifying equipment. For reagents, see Table 1 and 2.

Table 1: Solutions used in the analysis.

reagent/ solution	details
100 mM potassium phosphate buffer	100mM K_2HPO_4 + 100mM KH_2PO_4 in purified water, pH 7.0
H_2O_2 -solution (shield from light)	30mM H_2O_2 in cold 100mM potassium phosphate buffer

Table 2: Reagents used in the analysis.

reagent	**CAS-number**
K_2HPO_4	7758-11-4
KH_2PO_4	7778-77-0
H_2O_2 hydrogen peroxide	7722-84-1

Measurements and units:

Reaction buffer should be room temperature for the measurements. Dilute the fish liver or mussel digestive gland homogenates 1:10 in the potassium buffer. Pipette 10 µl/well of the samples in 96-well plate in four replicates. Keep the plate chilled e.g. over ice. In the first column, pipette 10 µl /well of the buffer to be used as blank references. Add 270 µl of buffer to each well. Add 10 µl of 30 mM H_2O_2 -solution to each well and start measurement in the spectrophotometer at 240 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, 2 and 3. The activity of CAT (OD/min) is adjusted to the protein concentration of the sample, measured with, e.g., the Bradford method[4].

Calculate thechange in absorbance (OD/min). Subtract blank measurement values from the sample measurements. Calculate a mean OD/min value from the four replicates. CAT activity is calculated

with the formula

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

where molar attenuation factor of H_2O_2 is 0.04 $mM^{-1}\,cm^{-1}$.

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

Conclusions (if applicable)

Data evaluation:

Compare the CAT 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.

CAT is ubiquitous in the animal kingdom and can be measured also in other species than those mentioned in this ROP.

References

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

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

Change history

1.0	11.2.2020	First edition
1.1	8.6.2020	
1.2	18.05.2021	Definition of the document was changed from SOP to ROP

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