

DAIMON Toolbox Fact Sheets:

Methods to Study the Impact of Dumped Munitions on Marine Biota

Assessment category 3: Biological effects

Toolbox component: Neurotoxicity

Fact Sheet 3.24: Acetylcholinesterase inhibition (AChE)

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What is it?

Acetylcholinesterase (AChE) is a key neurotransmitter enzyme functioning especially in neuromuscular junctions. Inhibition of AChE can lead to serious neuromuscular disorders, leading to tetanus and death.

What does it tell you?

Originally used for the detection of neurotoxic effects of organophosphates and carbamates AChE has in later studies been shown to be affected also by many other ubiquitous pollutant groups.

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

Matrix: Fish muscle and mussel gill tissue homogenates (see Indicator Leaflets 2.4.1 and 2.4.2 for tissue homogenization procedures)

Equipment: Spectrophotometer/ microplate reader able to measure at 412 nm intervals; basic laboratory equipment (pipettes, microplates, decanters). For reagents, see, e.g., Bocquené et al., 1998.

Measurements and units: For a detailed description, see, e.g., Bocquené et al. (1998). Briefly, the activity of AChE is measured as the colour change at 412 nm in a mixture containing 0.5 mM DTNB (5,5'-dithiobis 2-nitrobenzoic acid) and 2.6 mM ACTC (acetylthiocholine iodide) at a final concentration in phosphate buffer. The activity of AChE (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.

AChE activity is calculated with the formula

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

where molar attenuation coefficient of DTNB is $1.36 * 10^4 \text{ M}^{-1} \text{ cm}^{-1}$

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 AChE activity levels measured from organisms collected from the target area to those from the reference area. A lowered AChE activity level indicates a negative health effect. If the mean activity level is lower for more than one standard deviation (SD) of the mean values measured in the reference area, stress is considered moderate. If the level is more than two SDs lower, stress is severe.

References

- Bocquené, G., F. Galgani, 1998. Biological effects of contaminants: cholinesterase inhibition by organophosphate and carbamate compounds. *International Council for the Exploration of the Sea Copenhagen, Denmark.*
- 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.