DAIMON Toolbox Fact Sheets:

Methods to Study the Impact of Dumped Munitions on Marine Biota

Assessment category: Other approaches

Toolbox component: Lab toxicity studies

Fact Sheet 4.4: Comet Assay (*applied to zebrafish embryos*)

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What is it?

Ecotoxicological test system to quantify genotoxicity in single cells after exposure to chemicals. The Comet Assay can be performed with different cell types, cell cultures or whole fish embryos and detects single strand breaks and double strand breaks under alkaline conditions (Singh et al. 1988). The test can be used both *in vivo* and *in vitro* (Tice et al. 2000) and was also applied to flatfish for marine biomonitoring (Akcha et al. 2003; Bean and Akcha 2016).

What does it tell you?

The Comet Assay applied to zebrafish (*Danio rerio*) embryos is used to quantify DNA damage in newly fertilized zebrafish eggs caused by a specific chemical. In comparison with the control, the genotoxic risk of a chemical can thus be determined. Effects of chemicals on DNA that may lead to genotoxicity can cause severe damage such as mutations or carcinogenesis. In addition, genotoxicity can ultimately affect individual fitness and populations if it leads to reproductive stress or altered genotypic diversity (Anderson et al. 1994; Hylland et al. 2017). Heavily damaged DNA migrates further in the electric field than less damaged DNA, resulting in a fluorescent 'comet' behind the nucleus.

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?

According to DAIMON Fact Sheet 4.4 (Koske 2019) zebrafish eggs are exposed to the test substance for 48 hours. The test concentrations should be chosen so that they are below the acute toxic threshold. Test solutions should be renewed after 24 hours and eggs should be checked for sublethal effects.

After 48 hour of exposure zebrafish embryos are used for the Comet Assay under alkaline conditions (Singh et al. 1988) according to the procedure described by Kosmehl et al. (2008).

***Matrix*:** Whole zebrafish embryos

***Equipment*:** Electrophoresis unit including power supply, troughs for horizontal and vertical storage of the slides, heating block or microwave, refrigerated centrifuge for reaction tubes, vortex mixer, ice machine, fluorescence microscope (100x/200x magnification) with possibility of excitation at 490 nm and emission at 530 nm, microscope camera, hemocytometer, reaction tubes, frosted microscope slides and cover glasses, 70 µm filter mesh and 2 ml syringes, pipettes, various glassware including 1 l bottles.

***Sample size*:** To determine the genotoxicity of a chemical, the Comet Assay should be performed in triplicate with twenty zebrafish embryos per replicate to ensure sufficient amount of DNA in the sample.

Additional notes:

If necessary, the exposure time of embryos can be prolonged up to 120 hours after fertilization as they are not subject to animal testing regulations until then.

Filtering the homogenized embryos is important to ensure that single cells are present in the samples for later analysis.

Midori Green (Biozym) can be used as an alternative to ethidium bromide as DNA fluorescent dye.

For information on how the comet assay is applied to flatfish species and bivalve molluscs, see ICES Technique No. 58 (Bean and Akcha 2016).

How to analyse and assess the data?

Image analysis of the comets to determine DNA damage can be performed with the open source program CASP (Końca et al. 2003), proprietary software solution are also available. Several parameters to quantify the DNA migration are given by the software and can be used for evaluation. Usually, at least 50 comets per gel should be analyzed (Møller and Loft 2014). For instance, the percentage of DNA in the tail of each comet is plotted and used for statistical analysis. In order to assess the genotoxicity of a given chemical, the control is used as a reference for statistical comparisons. Parametric as well as non-parametric can be applied to the Comet Assay data and the experimental design determines the type of statistical analysis (Møller and Loft 2014).

References

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