

DAIMON Toolbox Fact Sheets:

Methods to Study the Impact of Dumped Munitions on Marine Biota

Assessment category 2: Hazardous substances

Toolbox component: Biota chemistry

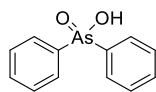
Fact Sheet 2.6: Chemical analysis of CWA-related phenylarsenic chemicals in fish cut fillet

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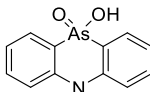
What is it?

Chemical analysis of chemical warfare agent-related phenylarsenic chemicals in fish cut fillet is based on liquid chromatography-tandem mass spectrometric technique (LC-MS/MS) in multiple reaction mode (MRM). Identification criteria of detected chemicals are based on European Union guidelines.

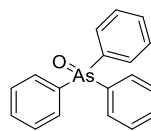
Target chemicals are diphenylarsinic acid DPA[ox], 10-hydroxy-5H-phenarsazine 10-oxide DM[ox] and triphenylarsine oxide TPA[ox].



DPA[ox]



DM[ox]



TPA[ox]

What does it tell you?

Chemical analysis of CWAs in fish cut fillet indicates the case if fish is exposed to CWA-related chemicals and the chemicals have been accumulated in fish cut fillet. A positive analysis result is a specific indicator for exposure of CWAs.

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?

Detailed sample preparation and analytical techniques are available in the DAIMON Report on Chemical Analysis of Sea-Dumped Chemical Warfare Agents in Marine Biota.¹

Species: Existence of CWA-related chemicals in fish cut fillet can be measured in all fish species

Matrix: fish cut fillet

Equipment: For measurement of CWAs in fish cut fillet samples special laboratory equipment are required. This includes sample homogenizer, shaker and evaporator. Instrumental analysis shall be carried out with liquid chromatography combined with triple mass spectrometry to achieve required sensitivity and specificity.

Measurements and units: After gutting of fish, fillet should be immediately frozen. 5 g of fish cut fillet is prepared and analyzed according to validated method.¹ Sample preparation includes extraction, purification, concentration and filtration steps prior to instrumental analysis. Samples are analysed by LC-MS/MS in MRM mode using positive ionization technique. Chromatographic and mass spectrometric parameters are available in previously published documents.^{1,2} Calibration curve (0.2-5 ng/g) are run at the same batch as samples. The results are presented as ng/g in the original fish sample. If concentration range of detected CWA is below limit of quantification (LOQ), the existence of detected chemical should be evaluated using sophisticated MS-technique, *eg.* high-resolution MS.

How to analyse and assess the data?

The qualitative identification of the detected chemicals are based on EU guidelines³. In LC analysis, retention time of the identified compound shall not differ more than ± 0.2 min from calibration standard sample. For MS/MS technique used in this work, the maximum permitted tolerance for the ratio of the areas of the q and Q ion is ± 20 % when relative peak intensity is more than 50 % of Q peak.

There are no assessment criteria for CWA-related phenyl arsenic chemicals in fish cut fillet.

Limit of quantification (LOQ) values for target chemicals:

- DPA[ox]: 0.8 ng/g
- DM[ox]: 0.6 ng/g
- TPA[ox]: 0.9 ng/g

Contact information of the analyzing laboratory

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References

¹ DAIMON report Chemical analysis of chemical warfare agent-related phenylarsenic chemicals from various fish tissue samples

- ² Niemikoski, H., Söderström, M., Vanninen P., (2017). Detection of Detection of Chemical Warfare Agent-Related Phenylarsenic Compounds in Marine Biota Samples by LC-HESI/MS/MS. *Anal. Chem.* 89 (20), 11129–11134
- ³ COMMISSION DECISION of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Official Journal of the European Communities, 2002, 2002/657/E, pp. 36