

Guidance on the Assessment of the Toxicity of Substances used and discharged offshore under the Harmonised Pre-Screening Scheme for offshore chemicals of OSPAR Recommendation 2017/01, as amended by OSPAR Recommendation 2019/04

OSPAR Agreement 2021-07<sup>1</sup> (Source: OIC 21/15/1, Annex 4)

**New and Existing offshore chemicals**

1. Toxicity testing for all offshore chemicals should be carried out on at the substance level, with each substance tested separately. This document provides guidance for test laboratories in order to assist them in conducting tests that are acceptable under the Harmonised Mandatory Control System.

**2. Sample preparation**

**2.1 Preliminary**

2.1.1. The supplier must present the testing laboratory with an accurate scientific name and full description/characterisation of the chemical to be tested. In addition, the following data and if available also the data suggested in Table 2 of OECD Guidance document on aquatic toxicity testing of difficult substances and mixtures (OECD No. 23, 2019<sup>2</sup>) should be presented.

Can be downloaded at:

<http://www.oecd.org/chemicalsafety/testing/seriesontestingandassessmentecotoxicitytesting.htm>

**2.2 Physical Properties**

2.2.1. State whether the substance is a liquid: .....solid: .....multi-component substance: .....polymer.....

2.2.2. If a multi-component substance or polymer, state whether it is:

an emulsion: .....

a suspension: .....

other (please describe): .....

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2.2.3 Provide the following information, or justify non-provision. This is necessary not only to ensure that the Test Laboratory has sufficient information to be able to perform the required test(s), but also to ensure that there is no ambiguity in the test report concerning the identity of test substance, as required by Section 6.2 of the GLP Regulations. Chemical Suppliers and Test laboratories should be aware that the unavailability of relevant information on the test substance may cause the resulting laboratory studies to be deemed inadmissible by the regulator.

a. Molecular weight(s))(g/mol): .....

NOTE: If the substance is a polymer, details of its average molecular weight and molecular weight distribution should be provided, along with any solvent content.

<sup>1</sup> This Agreement supersedes OSPAR Agreement 2002-04 on Further Guidance on the Assessment of the Toxicity of Substances under the Harmonized Pre-Screening Scheme of OSPAR Recommendation 2000/4 and OSPAR Agreement 2005-12 on Guidelines for Toxicity Testing of Substances and Preparations Used and Discharged Offshore

<sup>2</sup> OECD Guidance No. 23 (2019) of February 8 2019 (ENV/JM/MONO(2000)6/REV1)

- b. Specific gravity kg/m<sup>3</sup> (state temperature) liquid: ..... solid: .....
- c. Is the substance(s) ionisable? If yes, dissociation constant(s) (pKa): .....
- d. Henry's Law Constant (H) (Pa m<sup>3</sup>/mol).....
- e. Vapour Pressure (Pa) (state temperature) .....
- f. Suspended solids (mg/litre): .....
- g. Dissolved solids (mg/litre): .....
- h. Where liquid state whether: miscible: ..... or immiscible: ..... with sea water.
- i. Solubility in sea water (mg/litre): .....
- j. Does the substance separate in sea water to give:
  - (i) floating (Yes/No): .....
  - (ii) sinking (Yes/No): ..... materials ?
- k. Give the measured or estimated partition coefficient between octanol and water (log P<sub>ow</sub>) for each component present in excess of 10% by weight.
 

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- l. Give the measured or estimated organic carbon adsorption coefficient (K<sub>OC</sub>) for each component for which a log P<sub>OW</sub> value has been provided.
 

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**NOTE:** If the K<sub>OC</sub> is measured, the organic carbon content of the test sediment must be stated; if estimated, an organic carbon content in a muddy sand of 1% by wet weight should be assumed. If this information has not been provided, measure or estimate K<sub>OC</sub> following Karickhoff, S. W., Brown, D. S. and Scott, T. A. (1979) Water Research 13: 241-248 or the alternative methods given in OSPAR Guidelines for Completing the Harmonised Offshore Chemical Notification Format (HOCNF).

**2.3 Examination of Behaviour in Sea Water**

- 2.3.1. Take the sample container and thoroughly homogenise the contents on a shaker-table or roller-mill for 1 hour in a cool environment (15 ± 2°C).
- 2.3.2. Add 1 g of the homogenised sample to 1 litre of filtered (10 µm) sea water in a clean, stoppered, glass flask which holds 1100-1500 ml when full. Shake vigorously, upending for 10 reversals of the contents. Allow to stand for 4 hours and examine. (Swirl the contents gently to remove any material from the walls if visibility is impeded):
- 2.3.3. Determine whether:
  - a. No floating or settled materials, liquid or solid
    - (i) Clear solution/mixture ⇒ Go to 2.4.4
    - (ii) Homogenous emulsion or fine/colloidal suspension ⇒ Go to 2.4.5
    - (iii) Neutrally buoyant droplets, particles, or floc ⇒ Go to 2.4.6
  - b. Floating or settled, liquids or solids ⇒ Go to 2.4.6

**2.4 Appropriate Sample Preparation Procedures for Toxicity Tests**

- 2.4.1. The test substance should be thoroughly homogenised in a sample container. If necessary, this can be achieved by rolling on a roller mill or shaking on a shaker-table in a cool environment (15°C ± 2°C). This process may take e.g. up to 16 hours for some substances. In clean glass aspirators, add the required amounts of re-homogenised sample to the water. If necessary, the preparation should be carried out in the dark as many organic substances, including hydrocarbons may be photo-sensitive.
- 2.4.2. Test substances that are classified as - "difficult to test" substances - according to the OECD Guidance No. 23 (2019) should be tested according to this guidance. Among the typical properties, which can make a substance

"difficult to test", are: poorly water soluble, toxic at low concentration, volatile, photo-degradable, hydrolytically unstable, oxidizable, adsorbing, ionised, subjected to corrosion/transformation, colloids, biodegradable, complexing, coloured, hydrophobic, and multi-component substances and nanomaterials.

2.4.3. The OECD Guidance No. 23 (2019) gives recommendation on how to choose the proper test system and medium preparation method for the above listed "difficult to test" substances. Only the methods for handling of multi-component substances or complex substances are mentioned in the following paragraphs.

2.4.4. In case 2.3.3 a (i) above, the content of the flask from the examination of the behaviour in sea water may be used as the 1000 mg/litre stock concentration and the other test concentrations could be made by serial dilution of this stock solution. Test against the algal, crustacean and fish species, as specified.

2.4.5. According to OECD Guidance No. 23 (2019), testing of aqueous dispersions and emulsions is not generally advocated since the non-dissolved test chemical has e.g. the potential to exert effects on test organisms which are unrelated to chemical toxicity. However, if there is a regulatory requirement or the test substance has an inherent tendency to form an aqueous dispersion or emulsion, the test can be performed using the dispersion/emulsion. The contents of the flask from the examination of the behaviour in sea water may be used as the 1000 mg test chemical/litre stock concentration or the dispersibility limit (i.e., the limit at which phase separation takes place), whichever is lower. Prepare individual stock solutions for the other concentrations to be tested in the same way and test against the algal, crustacean and fish species, as specified.

2.4.6. Water Accommodated Fractions (WAFs) should be prepared of the test substance in case 2.3.3 b above. The WAFs are prepared individually and not by serial dilution of a single stock WAF. Measured amounts of multi-component substances are added directly to water and mixed for a period of time sufficient to achieve an equilibrated concentration of dissolved and dispersed or emulsified components in the aqueous phase. Following cessation of mixing and a period of settling (to allow phase separation), the aqueous phase, i.e. the WAF, is drawn off for testing. The WAFs are taken by siphoning from the middle of the mixture avoiding contamination by any floating or settled material and used to testing against the algal, crustacean and fish species, as specified. If neutrally buoyant liquids or solids are present (case 2.3.3 (iii) above), centrifuge at 1000 rpm for 10 minutes and use only the supernatant for testing against the algal, crustacean and fish species, as specified. The duration of the mixing and settling phases when preparing a WAF should normally be determined by carrying out a preliminary study. The presence of water-miscible solvents can modify the composition of a WAF and should not be used during the preparation of the medium (OECD Guidance No. 23 (2019)). NOTE: Good guidance on the preparation of WAFs is provided in *ISO 14442: Water quality — Guidelines for algal growth inhibition tests with poorly soluble materials, volatile compounds, metals and waste water* and its use is recommended for relevant toxicity testing at all trophic levels.

**NOTE:**

If the substance

- a. is a sinker; or
- b. has an organic carbon adsorption coefficient ( $K_{OC}$ ) greater than 1000; or
- c. has a  $\log P_{ow} \geq 4$ ; or
- d. is in any other way known to adsorb to particles or end up in the sediment or
- e. contains surfactants

then follow the examination of the behaviour in seawater of the preparation above, and the sample preparation procedure appropriate to 2.4.1-2.4.6 above, but always carry out a sediment-reworker test (*Corophium spp.*) using direct addition with the whole substance homogenised with the substrate for the test in addition to the algal, crustacean and fish tests.

### 3. Reference Standard Test

3.1. The algal, crustacean and fish tests must be carried out at regular intervals using a single concentration of 3,5-dichlorophenol as a standard reference toxicant, and the results reported. The standard concentrations of 3,5-dichlorophenol to be tested are as follows:

- a. **Algae**
  - Skeletonema costatum* 1.6 mg/l (ISO 10253-2006)
  - Phaeodactylum tricornutum* 2.7 mg/l (ISO 10253-2006)
- b. **Crustacea\***

<b><i>Acartia tonsa</i></b>	1.0 mg/l (ISO 14669)
<b><i>Tisbe battagliai</i></b>	2.3 mg/l (ISO 14669)

\*Note For crustacea, the sensitivity of the copepods and conformity to the procedure can alternatively be established by checking that the 48 h LC50 falls within the following concentration ranges:

<b><i>Acartia tonsa</i></b>	0.5-1.5 mg/l (ISO 14669)
<b><i>Tisbe battagliai</i></b>	1.1-3.5 mg/l (ISO 14669)

c. **Fish**

<b><i>Scophthalmus maximus</i></b> * (juveniles)	0.66 mg/l (OSPAR Agreement 2005-11 (as amended) <sup>3</sup> )
<b><i>Cyprinodon variegatus</i></b> (juveniles)	0,66 mg/l (OSPAR Agreement 2005-11 (as amended) <sup>4</sup> )

\* Note If another sensitive fish species is used, it should be tested at its 96h LC50 concentration, the value of which should be confirmed with the competent authority.

3.2. It is recommended that the fish reference standard test should be undertaken at regular, but less frequent, intervals than the algae and crustacea reference standard test (e.g. every 5 years), to ensure there is no genetic drift in the test fish population that could affect their sensitivity. These tests could be co-ordinated by regulators as a ring test or similar procedure.

3.3. It is further recommended that additional fish reference standard tests should be undertaken if there are significant procedural changes, for example when the fish supplier is changed; when the existing supplier confirms that the stock may be different for any reason; when the source of seawater is changed; or when there is a change in the investigator.

3.4. In general, reference results for algae should be between 20 and 80% growth rate inhibition by comparison with controls, and those for crustacea and fish should show 20-80% mortality. If reference results lie outside these limits, a repeat test should be conducted.

3.5 A reference test should be conducted using an appropriate reference item to assess the quality of the culture employed for sediment reworker (*Corophium spp.*). Guidance is provided in *ASTM E1367-03 (2014) Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Estuarine and Marine Invertebrates* and *USEPA EPA/600/R-94/025 Methods for assessing the toxicity of sediment associated contaminants with estuarine and marine amphipods*.

## 4. Test Reports

4.1. Testing and reporting should be carried out according to OECD Good Laboratory Practice or other quality assurance systems approved by national authorities. The testing laboratory should confirm to the supplier, in every case, that the validity criteria for the test have been met. If they were not, a repeat test should be conducted. The results of the most recent reference standard tests must always be reported.

## 5. Review

A review of this agreement should be carried out every five years.

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<sup>3</sup> OSPAR Agreement 2005-11 on Protocols on Methods for the Testing of Chemicals Used in the Offshore Oil Industry (as amended by OSPAR 2006)

<sup>4</sup> OSPAR Agreement 2005-11 on Protocols on Methods for the Testing of Chemicals Used in the Offshore Oil Industry (as amended by OSPAR 2006)