OSPAR Guidelines for Completing the Harmonised Offshore Chemical Notification Format (HOCNF)

(OSPAR Agreement: 2012-05. Update 2021)[1]

Introduction

1. The Harmonised Offshore Chemical Notification Format (HOCNF)[2] applies to all chemicals used in connection with offshore exploration and production activities in the OSPAR maritime area. Exact chemical composition of preparations will be held in commercial confidence in the relevant governmental bodies, and will not be published or transmitted to third parties.

Data Requirements

2. For the purpose of these OSPAR guidelines, the following data requirements apply:

   a. All mandatory information designated in Annex 1 of OSPAR Recommendation 2010/3 on a Harmonised Offshore Chemical Notification Format (HOCNF) (as amended), must always be provided;

   b. "Conditional data" become mandatory when the defined conditions mentioned in Annex 1 of OSPAR Recommendation 2010/3 (as amended), or in these guidelines are met;

   c. It is essential that all substances included on a HOCNF also fully comply with the relevant requirements of REACH for that substance. Suppliers are therefore advised to follow the REACH

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1 This version includes:

a) Amendments agreed at OIC 2013 to reflect agreement on the assessment of man-made polymers under OSPAR Decision 2000/02 on a Harmonised Mandatory Control System for the Use and Reduction of the Discharge of Offshore Chemicals.

b) Amendments agreed at OIC 2014 to reflect agreement on: (1) the procedure for the assessement of chemicals where there is a chemical reaction between the component substances; (2) the registration requirements for inseparable mixtures or unresolved complex mixtures and, (3) how the raw data from REACH simulation tests can be used in the Pre-screening scheme set in OSPAR Recommendation 2017/1 on a Harmonised Pre-screening Scheme for Offshore Chemicals, as amended.

c) Amendment in 2015 to take account of the test conditions and use of the Marine BODIS test.

d) Amendments agreed at OIC 2018 to reflect on the use of plastic, microplastic or deliberately added nano materials in offshore chemical products. Moreover, it also includes a registration requirement for products with added biocides. Finally, it includes a method of Determination of the Biodegradability of Solute/Solvent mixtures - enclosed in appendix 7.

e) Amendments agreed in 2020 – addition of text to the end of paragraph 56 and Appendix 8

f) Amendments agreed by OIC 2021 - addition of clarification text to paragraph 30; deleting reference to CHARM model in paragraph 62 and Appendix 6.

2 cf. Annex 1 of OSPAR Recommendation 2010/3 on a Harmonised Offshore Chemical Notification Format (HOCNF) (as amended).

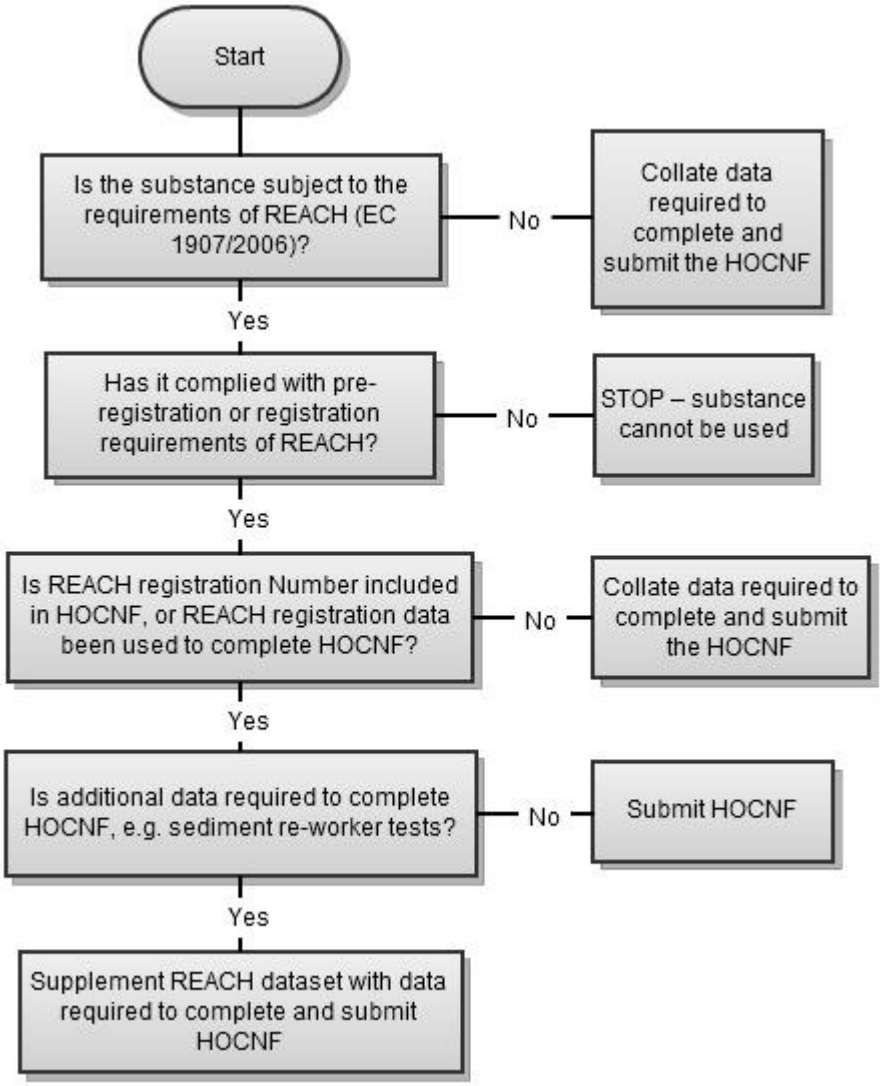
compliance flowchart shown in Figure 1 (overleaf) before they commission relevant tests or complete the HOCNF with existing data.

3. The amended OSPAR guidelines relate to the provisions and requirements stipulated in Annex 1 of OSPAR Recommendation 2010/3 on a Harmonised Offshore Chemical Notification Format (HOCNF) (as amended), and the reference numbers mentioned in the headings and subheadings refer to that Annex.

4. When compiling data for the HOCNF, the 'REACH Guidance on information requirements and chemical safety assessment'[3] provide a good source of information on how to characterise the hazard and risk profile of a substance. In particular the REACH Guidance on information requirements and chemical safety assessment documents under Part B: hazard assessment (R2 – 7), Part B: hazard assessment (R.7.1 - 10), Part C: PBT and vPvB assessment (R11) and Part D: Exposure assessment (R14 – 18) may be particularly useful sources of information.

[3] http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_en.htm#B

Figure 1: REACH Harmonisation Flowchart



Letters of Access

5. Where any information is not fully known by the Company completing the HOCNF, because the information is proprietary to the supplier, the Company may request that the proprietary information is provided directly to the Competent Authority by the supplier. This would involve the supplier sending the Competent Authority a Letter of Access (LoA), permitting the Competent Authority to use the information as part of the required data set.

6. The LoA from the supplier should contain, or reference, all the information that the Company registering the product has indicated will be submitted by the supplier.

7. The LoA and any supplementary supporting information, should be forwarded to the Competent Authority and cross-referenced using a common identifier, so that the Competent Authority can link the LoA to the relevant HOCNF.

8. The Competent Authority will confirm to the Company whether the composite LoA and HOCNF is complete and correct.

Harmonisation of REACH and HMCS data

9. Where HMCS data have not been used to derive the REACH data, and the endpoints derived from the two datasets do not agree, the competent national authority will have to be satisfied of the 'sameness' of the substances, and of the equivalence, adequacy, reliability and relevance of the data for the HMCS, taking account of the ECHA Guidance on Information Requirements and Chemical Safety Assessment, Chapter R4: Evaluation of available information, May 2008 (as amended). The decision on which data takes precedence in pre-screening will be made as follows:

10. Biodegradation: For screening tests a positive result will take precedence over a negative result. Simulation tests will be assessed in line with paragraph 55 of this guidance

11. Bioaccumulation: A BCF value will take precedence over any other data and will be compared to HMCS criteria. Where more than one BCF value occurs the highest value will take precedence. Where only $LogP_{ow}$ data are available the highest value will take precedence. The relevance of WOE approaches conducted under REACH or HMCS will be evaluated on a case by case basis.

12. Toxicity: Where there are less than 3 values for the same endpoint and they are within 1 order of magnitude the lowest value will take precedence. Where there are greater than 3 values for the same endpoint and they are within 1 order of magnitude the geometric mean will be calculated and used in the assessment

13. PNEC values and deterministic risk quotients: These will not be calculated in a directly comparable way by HMCS and REACH. The use of these values for ranking and risk management will be at the discretion of the competent national authority.

PART 1: General information on substances and preparations

§ 1.1 State the trade name or trade names of the substance or preparation

§ 1.2 State the supplier and provide background information as regards the substance or preparation

§ 1.3 The Safety Data Sheet (SDS) must be attached to the HOCNF format. Confirm this by ticking the box "Yes"

§ 1.4 Use and discharge

14. If the chemical is suitable for assessment by CHARM, choose an application from one of the CHARM algorithms (utility and stimulation chemicals are assessed using the "Completion/Workover" algorithm). Complete column 1 of the table in § 1.4 of the HOCNF.

15. If the submitted chemical is not suitable for assessment by CHARM, advice should be sought from the competent national authority on information that may be required in addition to that requested in the HOCNF, to allow assessment by models other than CHARM.

16. Information on the quantities used and discharged and on application categories is required, based on either standardised reference installations or, where appropriate; on site specific use and discharge.

17. The entries "function" and "state the process system to which the substance/preparation will be applied" should confirm the function of the chemical and its mode of use. The function is defined as the process for which the substance/preparation is normally or primarily used, e.g. drilling fluid, biocide, scale-inhibitor, demulsifier, and should be one of those listed Appendix 2. If none of the listed functions are appropriate, enter the function as 'other' and provide a description confirming the mode of use, fate and effect. The application process stream should define the process stream into which production chemicals will be dosed; or list the sections of a well in which the drilling chemicals will be used; or should reflect one of the four CHARM sub-algorithms for cementing or completion chemicals. Complete columns 2 and 3 of the table in § 1.4 of the HOCNF.

18. For water-based drilling chemicals, all the well sections in which the product is likely to be used should be stated. The normal dose rate should be based on optimal technical performance and the hazard assessment based on the appropriate sections of a standard well included in the CHARM model (17½'', 12½'' and 8½'' sections) or, where appropriate, on site specific information. Units should be clearly stated. Complete columns 3 and 4 of the table in § 1.4 of the HOCNF.

19. For production chemicals, state either "water" or "total fluids" as the process system into which the chemical will be applied. "Total fluids" should be selected for chemicals dosed into single-phase hydrocarbon streams (either oil or gas) and the dosage should be based on the sum of the produced water and hydrocarbon production for the appropriate CHARM model platform. "Water" should be selected for injection chemicals and surfactants. State also whether the production chemical is to be used on a gas or oil platform. Complete columns 3 and 4 of the table in § 1.4 of the HOCNF.

20. For all cementing chemicals except spacers, "mixwater" should be stated as the process system. For chemicals used in spacer fluids, enter "spacer" as the function of the chemical. Complete column 2 of the table in § 1.4 of the HOCNF.

21. For completion chemicals, enter the function "surface and well cleaning" for products used as rig washes or "other completion/workover" for any other products used in completion and workover operations. Complete this information in column 2 of the table in § 1.4 of the HOCNF.

22. If a product is used in several different applications, data on function, process stream dosage, discharges etc. must be provided for each application in additional tables in accordance with § 1.4 of the HOCNF.

23. A substance/preparation should be assigned to the category "closed system" only if it remains within a reactor or is transferred from vessel to vessel through closed pipework and therefore accidental spillage is the only likely cause for human exposure or environmental contamination. Substances/preparations that are used in closed systems, but as a consequence of normal use might be released into the environment after use or where significant discharges into the environment cannot be excluded during use, should be assigned to the "open system". If it is assigned "open system" then specify the estimated discharge as a percentage of the use or fraction released. Complete the information in columns 7 and 8 of the table in § 1.4 of the HOCNF.

24. If the above sections have been filled in as indicated, then no more information is required to run the CHARM model. However, an estimation of the "frequency of treatment", "probable scale of use per installation (specify units)", "Probable amount of substance/preparation discharged (specify units)" and "Total estimated amount of discharge (tonnes)" must be reported in columns 6, 9, 10, 11 and 12 of the table in §1.4 of the HOCNF to inform site-specific risk assessment.

25. Data in §1.4 must be provided for all offshore chemicals (as appropriate)

§ 1.5 Fate

26. A description of the likely fate of the substance/preparation must be explained in general terms. This must include whether the substance/preparation is likely to change its form on use and/or will end up in the sediment, the water column, the air or biota.

§ 1.6a) Composition

27. All applications must include the names and data for all deliberately added substances within a preparation. Sufficient information must also be available to operators to allow them to properly assess the chemicals they intend to use.

28. When providing data to non-governmental organisations, information regarding composition should be the same as that normally provided in a Safety Data Sheet (SDS).

29. The full chemical composition must be provided when submitting the form to government bodies. The complete and precise composition of the substance or preparation must be reported, including each "active" substance, "inert" substance, solvent and additive substance and their proportions, using CAS numbers (if available) and recognised chemical formulae or recognised chemical names for all substances.

EINECS, or ELINCS or REACH numbers must also be provided if they are available. Please note that trade names will not be accepted by the government bodies instead of this information. Trade names are, however, useful additional information and should also be provided where possible.

30. The molecular weights of the named constituent substances must be provided, together with percentage composition of the constituents (in accordance with the allowed variation[4]). Percentage composition is derived from the weight of the substance in the preparation divided by the total weight of the preparation. The resultant figure is then multiplied by 100 to get the percentage composition. It is important that the sum total of the data in column 2 (percentage composition) adds up to 100. Contracting Parties may need to request further information on the composition of the polymer, such as the molecular weight distribution, in order to complete their assessment.

31. All substances known to be deliberately added, including those present at less than 1% by weight of an entire preparation, should be declared. Impurities are not considered to be deliberately added. However, residue substances from the manufacturing process and other impurities present at greater than 1% should be declared as part of the formulation. Substances (except those stated in paragraph §1.6.b of the OSPAR Recommendation 2010/3 on a Harmonised Offshore Chemical Notification Format (HOCNF) (as amended), that are not deliberately added and are present at less than 1% by weight should not be declared.

32. Where two or more substances in the formulation are known to react together upon mixing, and the reaction is not reversed by discharge of the product into the marine environment, the description of the substances listed in the composition table, and the tests conducted, should reflect the substance(s) formed by the reaction.

33. Where a CAS number is not available for a polymer, provide the CAS number (and the EINECS, or ELINCS or REACH numbers if they are available) for the monomer on which it is based. If the molecular weight is not available for a polymer, provide the molecular weight of the monomer. Where there is a molecular weight range for polymers or complex mixtures, the mean value should be provided with an explanation of how this value has been arrived at (e.g. GPC, MS, calculation). Monomers should also be assessed separately if they are present at levels that require them to be declared.

34. Substances which are on the latest REACH Annex IV list or satisfy the criteria detailed in REACH Annex V or which are on the latest OSPAR List of Substances / Preparations Used and Discharged Offshore Which are Considered to Pose Little or no Risk to the Environment (PLONOR) must also be declared. The REACH Annex IV and Annex V lists can be found on the ECHA website at:

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:268:0014:0019:EN:PDF and the OSPAR PLONOR list can be found on the OSPAR website at www.ospar.org

REACH Annex V states 13 sets of circumstances under which ECHA is satisfied that a substance should be exempt from registration, some of which (items 8 & 9) relate to naturally occurring, non-hazardous substances and therefore are equivalent in concept to substances on the PLONOR list. For such substances, the submission of PBT data is not normally required for HMCS registration purposes. In addition, item 7

[4] **Concentration ranges:**

Declared concentration range of the constituent (%w/w absolute)	Permitted variation in initial concentration of the constituent (% relative)
≤2,5%	±15%
>2,5≤10%	±10%
>10≤25%	±6%
>25≤50%	±5%
>50≤100%	±2,5%

may be treated similarly if evidence is provided that the substances are non-hazardous*. Other REACH Annex V exemptions do not provide a basis on which substances can be accepted as PLONOR-like for HMCS registration.

Expert judgement is carried out by authorities based on the Annex V guidance provided by ECHA (https://echa.europa.eu/documents/10162/23036412/annex_v_en.pdf/8db56598-f7b7-41ba-91df-c55f9f626545), in order to establish whether the relevant substance would qualify for a REACH exemption under items 7*,8, or 9. During this process, the supplier may be required to provide additional information such as details of how the substance is extracted and processed. If these qualifying criteria are not met, the substance may not be accepted for registration without PBT data.

*Where item 7 is applicable, authorities would in addition need to be satisfied that the substance was

a) not classified as dangerous according to Regulation (EC) No 1272/2008 in respects that are relevant to the marine environment, and

b) not PBT (persistent, bioaccumulative and toxic) nor vPvB (very persistent and very bioaccumulative) in accordance with the criteria set out in Annex XIII of REACH, nor identified in accordance with Article 59(1) at least two years previously as a substance giving rise to an equivalent level of concern as set out in Article 57(f).

REACH-compliant Safety Data Sheets for the relevant substance should provide such information.

35. Where generic descriptions are provided, such as tall oil soaps, polyoxyalkylated glycols, phenol/formaldehyde resins, reaction-products, copolymer series the source materials must be also be provided, together with the best description of the range of major substances/preparations present. Where solvents or other additives are refinery or petrochemical products consisting mainly or entirely of hydrocarbons, the generic description (e.g. straight-chain alkenes, 3-5 ring aromatics) must be provided together with the concentrations of any aromatics, and the method or methods used for determination of the components should be stated. In such cases, this information must be completed in the “Comment” box.

36. There are a number of offshore chemical products that are composed of, or include, complex mixtures, for example the grease component in pipe dopes or jacking greases. Although the grease component is composed of a number of substances, it is treated as a single substance for the purpose of HOCNF testing, but the additives to the grease must still be tested separately. This approach can be used by Contracting Parties for other inseparable mixtures or unresolved complex mixtures.

§ 1.6b) Contents

37. For substances/preparations where knowledge of the raw materials and the manufacturing processes involved indicate that one or more of the named substances will be present, please tick the appropriate box. Supportive evidence must be provided, where available, in the next table of § 1.6b). If the chemical product contains plastic or microplastic or if nanomaterials are deliberately added, the relevant boxes should be ticked off. If the offshore chemical contains biocides, compliance with the Biocide Product Regulation (EU) 528/2012 should be observed and ticked off in the relevant box.

38. The latest version of the OSPAR List of Chemicals for Priority Action (OSPAR LCPA) and the latest version of the OSPAR List of substances of possible concern (OSPAR LSPC) can be downloaded from the OSPAR website at www.ospar.org.

§ 1.7 General physical properties

39. Enter details of the physical state of the substance or preparation. Most of the general physical properties are described in the Safety Data Sheet (SDS).

Part 2 Ecotoxicological information

40. Ecotoxicological information can be mandatory or conditional. If the offshore chemical is on the PLONOR List or all the relevant ecotoxicological information has already been submitted to the authority, Part 2 of Annex 1 to OSPAR Recommendation 2010/3 on a Harmonised Offshore Chemical Notification

Format (HOCNF) (as amended) need not be completed. Reference should be provided to the document in which this information is given. If the offshore chemical is a man-made polymer, it can be assessed as non-biodegradable, non-toxic and non-bio-accumulative without the provision of test data, but the supplier may prefer to provide such data where they consider it to be relevant to the assessment, or the relevant national competent authority, on the basis of expert judgement, may conclude that the provision of test data is necessary to undertake the assessment. A guidance framework for national competent authorities' assessments and the application of expert judgement is provided in Appendix 4. Where valid test data is provided, it shall take precedence over the default assessment.

41. Whenever possible, ecotoxicological data should be derived from tests performed according to recognised international standard protocols or guidelines (e.g. OSPAR guidelines, ISO test guidelines and OECD test guidelines) and conducted by laboratories working in compliance with the current OECD principles of Good Laboratory Practice (GLP) at the time of testing. Test laboratories must also follow the OSPAR Guidelines for Toxicity Testing of Substances and Preparations Used and Discharged Offshore (Reference number: 2005-12) whenever samples are received for testing. The testing laboratories must also confirm to the supplier, in every case, that the validity criteria for the reference test have been met.

42. Other types of information may be sufficient for completing the HOCNF especially when used in a *Weight of Evidence* approach. Such information could include:

a. Data from *in vitro* or *in vivo* studies that have not been generated in accordance with the latest adopted/accepted version of the corresponding (validated) test method or to GLP (or equivalent)

b. QSAR model outputs

c. SAR model outputs, read across and category approaches.

43. Where data of type 42a are presented they should be evaluated for completeness and quality to assess whether they fulfil the requirements of the HOCNF Guidelines for the substance in question, and whether they are therefore appropriate for use in HMCS pre-screening and CHARM calculations as required. The evaluation of these data should be conducted according to ECHA Guidance on Information Requirements and Chemical Safety Assessment, Chapter R4: Evaluation of Available Information, December 2011[5].

44. Data of type 42b may be used instead of testing when the following conditions are met:

i. Results are derived from a QSAR model whose scientific validity has been established in line with the OECD principles of QSAR validation (see reference*),

ii. The substance falls within the applicability domain of the QSAR model,

iii. Results are adequate of the purpose of pre-screening and CHARM assessment as required,

iv. Adequate and reliable documentation of the applied method is provided.

*The ECHA Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.6: QSAR and Grouping of Chemicals, May 2008[6] should be followed when using QSAR for HMCS purposes.

45. Similarly, when using data of type 42c for HMCS purposes, the ECHA Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.6: QSAR and grouping of Chemicals, May 2008 should be followed.

46. If REACH data for the substance is available and these data have been used in the HOCNF submission, the competent national authority may conduct a Weight of Evidence evaluation (Reference to ECHA REACH

[5] https://www.echa.europa.eu/documents/10162/13643/information_requirements_r4_en.pdf/d6395ad2-1596-4708-ba86-0136686d205e

[6] https://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf/77f49f81-b76d-40ab-8513-4f3a533b6ac9

document R.7b and R.7c) for OSPAR Registration purposes. If REACH Registration data for the substance are not available, then the Weight of Evidence evaluation will only be possible if the competent national authority is satisfied of the sameness (Reference: ECHA Guidance for identification and naming of substances under REACH, June 2007) of the substances.

§ 2.1 Partitioning and bioaccumulation potential

47. N-octanol water partitioning data must be provided for all organic substances with the exception of surfactants. For preparations, information for all the deliberately added substances is required. The data can be derived by measuring, estimating or calculating the partitioning of the substances between water and n-octanol (P_{ow}).

48. Log P_{ow} is used for two purposes. In the pre-screening (OSPAR Recommendation 2017/1 as amended) it is used to advise on bioaccumulation potential, and in the CHARM model it is used to estimate how a substance partitions between oil and water with the aim of predicting the environmental concentration (PEC). As a consequence of this dual use of log P_{ow} data, whenever a range of log P_{ow} values is quoted in a test report, the maximum and minimum value must be stated in the HOCNF. The maximum values will be used for estimating bioaccumulation potential in the pre-screening scheme and the minimum value will be used as an indication of the potential of the substance to partition into the water phase.

49. A standard shake flask method for the determination of P_{ow} is OECD Guidelines for Testing of Chemicals, 1995-107. The shake flask method is applicable for substances that are water soluble and which do not dissociate or associate. The method is not applicable for lipophilic organic substances (of low water solubility), preparations, complex substances, organo-metallic compounds and surface-active agents. Log P_{ow} values in the range –2 to 4 can be measured by this method.

50. A standard HPLC method for the estimation of the log P_{ow} is OECD Guidelines for Testing of Chemicals, 2004 - 117. This method is applicable for preparations and complex substances (for complex substances the log P_{ow} range needs to be stated). Ionisable substances should be measured in their non-ionised form under appropriate pH conditions (pH range of natural seawater). The HPLC method is not applicable to strong acids and bases, metal complexes, substances reacting with the eluent or surface-active agents. Log P_{ow} values in the range 0 to 6 can be estimated using the HPLC method.

51. Reliable methods are now available for calculation of log Pow for many types of organic substances and often laboratory testing can be avoided. Examples of reliable methods are CLOGP, LOGKOW and AUTOLOGP (cf. European Commission, Technical guidance documents on risk assessment in support of the Commission Directive 93/67/EEC on risk assessment for new notified substances; Commission Regulation (EC) No. 1488/94 on risk assessment for existing substances; and Directive 98/8/EC of the European Parliament and of the council concerning the placing of biocidal products on the market (Part II. Published April 2003)). Care should be taken when using models to assess substances for which the protocols have not been verified. Calculations should be fully validated and justified. The latest version of relevant Technical Guidance Documents under the REACH Regulation should also be consulted where appropriate, including the Guidance on information requirements and chemical safety assessment, Chapter R.11: PBT Assessment, May2012.

52. If the calculated or experimentally determined logPow $\geq$3, bioaccumulation will be assumed unless experimental bioconcentration factor (BCF) tests indicate the opposite. While high molecular weight compounds are less likely to bioaccumulate a precise threshold is not recognised; OSPAR has agreed to recognise MW 700 as a limit for bioaccumulation as given in the EU TGD Part II https://echa.europa.eu/documents/10162/16960216/tgdpart2_2ed_en.pdf BCF data are relevant for all deliberately added substances with a log Pow >3. The BCF is determined on the basis of the ratio of animal tissue concentration to water concentration of the test substance at equilibrium, or on the basis of the ratio of the uptake and depuration rate constants. The competent national authority should be consulted beforehand to ensure that the proposed test method is suitable. In general, for fully water miscible substances, either a fish or a bivalve mollusc bioaccumulation test would normally be appropriate (e.g. OECD 305 or ASTM E1022), whereas for substances that give rise to suspended particles. A filter feeding organism such as a bivalve mollusc would be more appropriate.

53. A variety of approaches may be appropriate for assessing the potential for substances to bioaccumulate. A more detailed assessment scheme for the HMCS, which applies substitution warnings to those substances that are likely to exhibit a potential to bioaccumulate, is detailed in Appendix 3 of these guidelines.

§ 2.2 Biodegradability

54. Data on biodegradability in the marine environment are relevant for all organic substances. For preparations or complex mixtures, individual information for all the deliberately added substances is required. Data on the rate of hydrolysis of a substance, with pH conducted to OECD guideline 111, may be useful in assessing the abiotic degradation of some substances. These data may be submitted to the competent national authority and the applicability of the data will be assessed by expert judgement on a case-by-case basis.

55. Where a chemical listed in Section 1.6a is comprised of a solute supplied in an organic solvent, suppliers should first consider whether the chemical complies with the substance definition (See Appendix 1) and where possible, ensure that the solvent is separated prior to testing. If this is not possible, biodegradability data must still be provided for both solute and solvent. Assessment is based on the lower biodegradability figure of the two. Biodegradability of the solute may be determined by difference, using the modifications to the OECD 306 Closed Bottle method that are described in Appendix 7. Alternative methodologies may be submitted to the regulatory authorities for approval at their discretion.

56. If a ready aerobic biodegradation test has not been performed, it will be assumed that the substance is persistent in aerobic conditions unless a simulation test (e.g. OECD 308, OECD 309) is performed which indicates the opposite. Substances for which no biodegradation data are available shall be tested according to the standard test methods for biodegradability, according to either OECD Guidelines for Testing of Chemicals, 1992, 306, Marine BODIS (BOD-test for insoluble substances), Marine CO2 evaluation test and Marine CO2 head space protocols as published in the report "Biodegradability of chemical substances in seawater. Results of the four OSPARCOM ring tests, final report, Elf Akvamiljö, November 1996." Details of the OSPAR ring test are available from OSPAR. A guidance for test conditions and use of the Marine BODIS is provided in Appendix 6. In the absence of valid results for such tests, authorities may accept data from freshwater tests according to OECD Guidelines for Testing of Chemicals, 1992, 301 A-F and freshwater BODIS tests, if these data are already available. In addition, the OSPAR Offshore Industry Committee at the March 2020 meeting accepted the use of the MaP test protocol for the purpose of persistence assessment. Therefore, It is now an accepted marine test protocol for the purpose of establishing greater than 20% biodegradability under section 3.2 g) of the pre-screening scheme (OSPAR Recommendation 2017/1). However, the test protocol cannot be used for the purpose of establishing greater than 60% biodegradability under Section 3.2 i) of OSPAR Recommendation 2017/1. Details are provided in Appendix 8.

57. In a screening test, the highest value for the percentage biodegradation during the period of testing shall be used as a measure of the biodegradation potential provided that the value is not an outlier. The substance will be considered persistent if:

i biodegradation is <20% in OECD 306, Marine BODIS or any other accepted marine protocols or <20 % in 28 days freshwater (ready test).

ii Half-life values derived from aquatic simulation tests (e.g. OECD 308, 309) indicate persistence to REACH (EC 1907/2006) Annex XIII criteria

58. Tests on substances known to be toxic to microbes (e.g. biocides) should follow the recommendations in Annex II of OECD 301 1992.

59. Biodegradability tests on poorly soluble materials should follow the recommendations set out in ECETOC Technical Report No. 20 (1986), Annex III of OECD 301 1992 and ISO Guidance Document ISO 10634.

60. For the purposes of pre-screening, a biodegradability of 20% is considered equivalent to half-life values derived from simulation tests submitted under REACH (EC 1907/2006) of 60 and 180 days in marine

water and sediment respectively (e.g. OECD 308, 309), conducted with marine water and sediment as appropriate. Conversely, no half life values have been defined that are indicative of ready biodegradability, nor can percentage biodegradability figures be derived from half lives reported from simulation tests. However, OSPAR (OIC 2012) has allowed that it is acceptable to derive percentage biodegradability figures from raw data obtained from simulation tests. The method is described in Appendix 5.

§ 2.3 Aquatic toxicity

61. Toxicity data must be provided for all substances. Marine data should be provided where possible but competent national authorities can also accept freshwater toxicity data in lieu of marine data, provided the freshwater tests are carried out using test species mentioned in the OECD 201, 202 and 203 guidelines or any other suitable internationally-accepted protocol.

62. The relevance of toxicity test data other than that specified in the notification format should be agreed in consultation with the competent national authority. For certain substances, additional marine toxicity data may be required at any time, if evidence casts doubt on the relevance of the existing test data.

63. Substance-based testing on representative algal, crustacean and fish species is mandatory[7]. The full OSPAR marine toxicity data set comprises:

a.	Algae	
	Skeletonema costatum; or *Phaeodactylum tricornutum;*	to ISO/DIS protocol 10253
b.	Crustacea	
	Acartia tonsa; or *Tisbe battaglia;*	to ISO protocol TC 147/SC5/WG2
c.	Fish	
	*Scophthalmus maximus (juveniles);*or *Cyprinodon variegatus (juveniles)*	to Part B of the OSPAR Protocols on Methods for the Testing of Chemicals Used in the Offshore Industry (published by OSPAR in 1995, available from the OSPAR web site at www.ospar.org)

64. Where there are no existing fish toxicity test data, it is recommended that a limit test is conducted using the LC50 or EC50 of the most sensitive species of the other taxonomic groups that have been tested. If no significant mortality occurs in this limit test (when compared with the control), it is unnecessary to undertake a full toxicity test and the end point of the fish test should be reported as greater than the concentration tested (> limit concentration).

65. Most substances exhibit a fairly similar degree of toxicity to both algae and crustacea but there are occasions where a substance appears to be very much more toxic to one class of organism than the other. Should the apparent toxicity be found to be due to a physical effect such as chelation and not toxicity, OSPAR recommends that the limit test should be conducted at the LC50 or EC50 concentration of the other species that was tested.

66. A comparative suite of alternative marine or freshwater species are also acceptable if tests are conducted according to recognised protocols such as the OECD 201, 202 and 203 guidelines or any other suitable internationally-accepted protocol.

67. An additional sediment reworker test must be carried out, using *Corophium spp*, as described in Part A of the OSPAR Protocols on Methods for the Testing of Chemicals Used in the Offshore Industry, for

substances which:

[7] If testing another species has already identified a substance for substitution, the fish test is not mandatory although it may still be required at the discretion of the competent authority

a. are "sinkers"; or

b. have a K_{OC} >1000; or

c. have a log Pow>4; or

d. are in any other way known to adsorb to particles or end up in the sediment; or

e. contain surfactants.

Part 3 Confirmation statement

68. The confirmation statement is a written declaration renewable every three years to confirm that:

a. The information in the form still applies precisely to the substance/preparation being manufactured or supplied under that specific trade name. Any change in formulation, by the addition of any new substance, or the removal of any existing substance, other than by trace quantities (<100 ppm or <0,01%), necessitates the immediate action to consider whether new data are required. Any change in composition, i.e., in the concentrations of a substance, unless within the previously accepted range of variability, similarly necessitates the immediate evaluation of the new composition. Any change of name, coding or number requires an immediate declaration that the existing format now applies to this renamed preparation;

b. All laboratory tests results and data referred to in Annex 1 to OSPAR Recommendation 2010/3 on a Harmonised Offshore Chemical Notification Format (HOCNF) (as amended) were either in compliance with the requirements of the relevant REACH registration, or are in compliance with the European Chemicals Agency (ECHA) “Guidance on Information Requirements and Chemical Safety Assessment, Chapter R4: Evaluation of available information, May 2008 (as amended)

69. Suppliers may be asked at any time to submit samples of a substance/preparation to government bodies for analysis or testing. Offshore users may also be asked at any time to submit to government bodies a sample of the preparation currently in use.

70. Before the renewal date, the notifier should consult the competent national authorities. More relevant data, or data not previously supplied, for a substance/preparation, may be required, irrespective of any previous approval for use.

Glossary and Definitions of Terms

1. For the purpose of these guidelines and for the purpose of the Harmonised Offshore Chemical Notification Format (HOCNF) as at Annex 1 of OSPAR Recommendation 2010/3 on a Harmonised Offshore Chemical Notification Format (HOCNF) (as amended):

a. "authority" means the competent national authority of a Contracting Party to the OSPAR Convention;

b. “discharge” means the operational release of offshore chemicals or their degradation and transformation products into the maritime area;

c. "EINECS" means European Inventory of Existing Commercial Chemical Substances;

d. “ELINCS” means European List of Notified Chemical Substances;

e. "HOCNF" means the Harmonised Offshore Chemical Notification Format;

f “Limit test” means a fish toxicity test conducted at a single concentration rather than a range of concentrations (as described in the OSPAR agreement 2005-11)

g. “Microplastics”, as proposed by ECHA[8] : means a material consisting of solid polymer-containing particles, to which additives or other substances may have been added, and where ≥ 1% w/w of particles have:

   i. all dimensions 1nm ≤ x ≤ 5mm, or

   ii.for fibres, a length of 3nm ≤ x ≤ 15 mm and length to diameter ratio of >3.

h. “Nanomaterials” as defined by the EU[9] , being: A natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %. By derogation from the above, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials.

i “offshore chemicals” means all chemicals intentionally used in connection with offshore exploration and production activities in the maritime area. Offshore chemicals comprise both substances and preparations;

j. “OSPAR Decision 2000/2” means OSPAR Decision 2000/2 on a Harmonised Mandatory Control System for the Use and Reduction of the Discharge of Offshore Chemicals;

k. “OSPAR LCPA” means the OSPAR List of Chemicals for Priority Action;

l. “OSPAR Recommendation 2010/3” means OSPAR Recommendation 2010/3 on a Harmonised Offshore Chemical Notification Format (HOCNF) as amended by OSPAR Recommendations 2014/17 and 2019/3;

m. “OSPAR Recommendation 2017/1” means OSPAR Recommendation 2017/1 on a Harmonised Pre-screening Scheme for Offshore Chemicals (as amended);

n. “Plastic” as defined in the marine environment, are - substances that are solid synthetic polymers insoluble in water (ref. OIC Summary Record 2018).

o. "PLONOR" means the OSPAR List of Substances/Preparations Used and Discharged Offshore Which are Considered to Pose Little or No Risk to the Environment

p. “Pow” is equivalent to Kow and means the partition coefficient of a substance between octanol and water, measured or calculated according to the HOCNF;

[8] https://echa.europa.eu/documents/10162/82cc5875-93ae-d7a9-5747-44c698dc19b6

[9] COMMISSION RECOMMENDATION of 18 October 2011 on the definition of nanomaterial (2011/696/EU)

q. “preparation” means a mixture or solution composed of two or more substances;

r. “SDS” means Safety Data Sheet compiled in accordance with Annex II as laid down in Article 31 of Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

s. "sinkers" means those chemicals with a density greater than that of sea water and with a low water solubility;

t. “substance” means a chemical element and its compounds, in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition;

u. “Surfactant” means any substance, which has surface-active properties according to test method A.5 in Regulation EC 440/2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) of 30 May 2008, and which consists of one or more hydrophilic and one or more hydrophobic groups of such a nature and size that is capable of reducing the surface tension of water, and of forming spreading or adsorption monolayers at the water-air interface, and of forming emulsions and/or microemulsions and/or micelles, and of adsorption at water-solid interfaces.

v. "trace" means a substance is represented with less than 0,01 % (<100 ppm) in a preparation;

w. “use”, in relation to an offshore chemical, means any intentional application of the chemical in connection with offshore exploration and production activities in the maritime area under normal operating conditions.

2. Further definitions and information can be found in:

- OSPAR Decision 2000/2 on a Harmonised Mandatory Control System for the Use and Reduction of the Discharge of Offshore Chemicals (as amended);
- OSPAR Recommendation 2017/1 on a Harmonised Pre-screening Scheme for Offshore Chemicals (as amended);
- OSPAR Recommendation 2010/3 on a Harmonised Offshore Chemical Notification Format (HOCNF) (as amended);

which are available for downloading from the OSPAR web site at www.ospar.org.

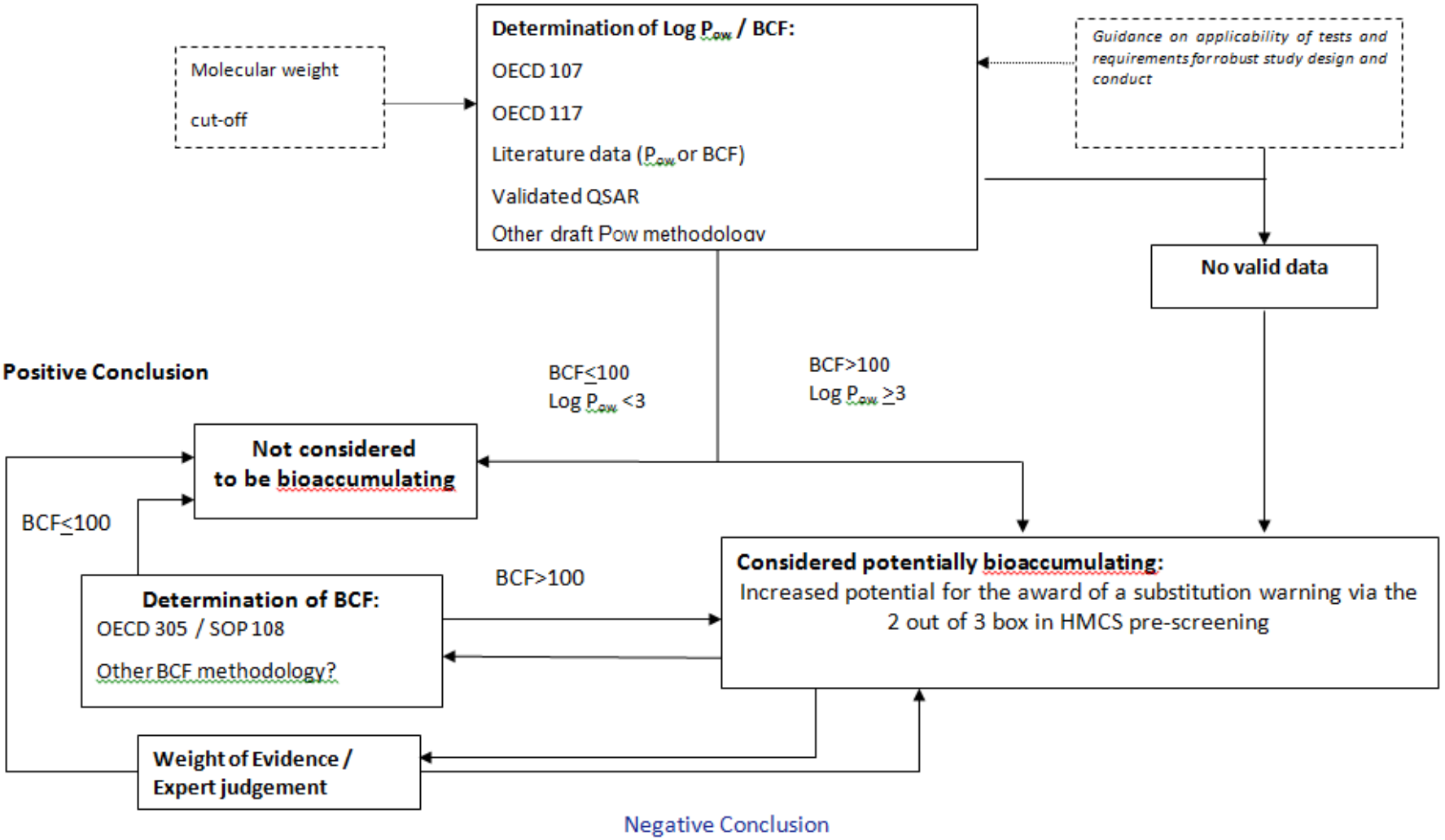
Appendix 2

Functions of Chemicals referred to in paragraph 17 of the OSPAR Guidelines for completing the HOCNF

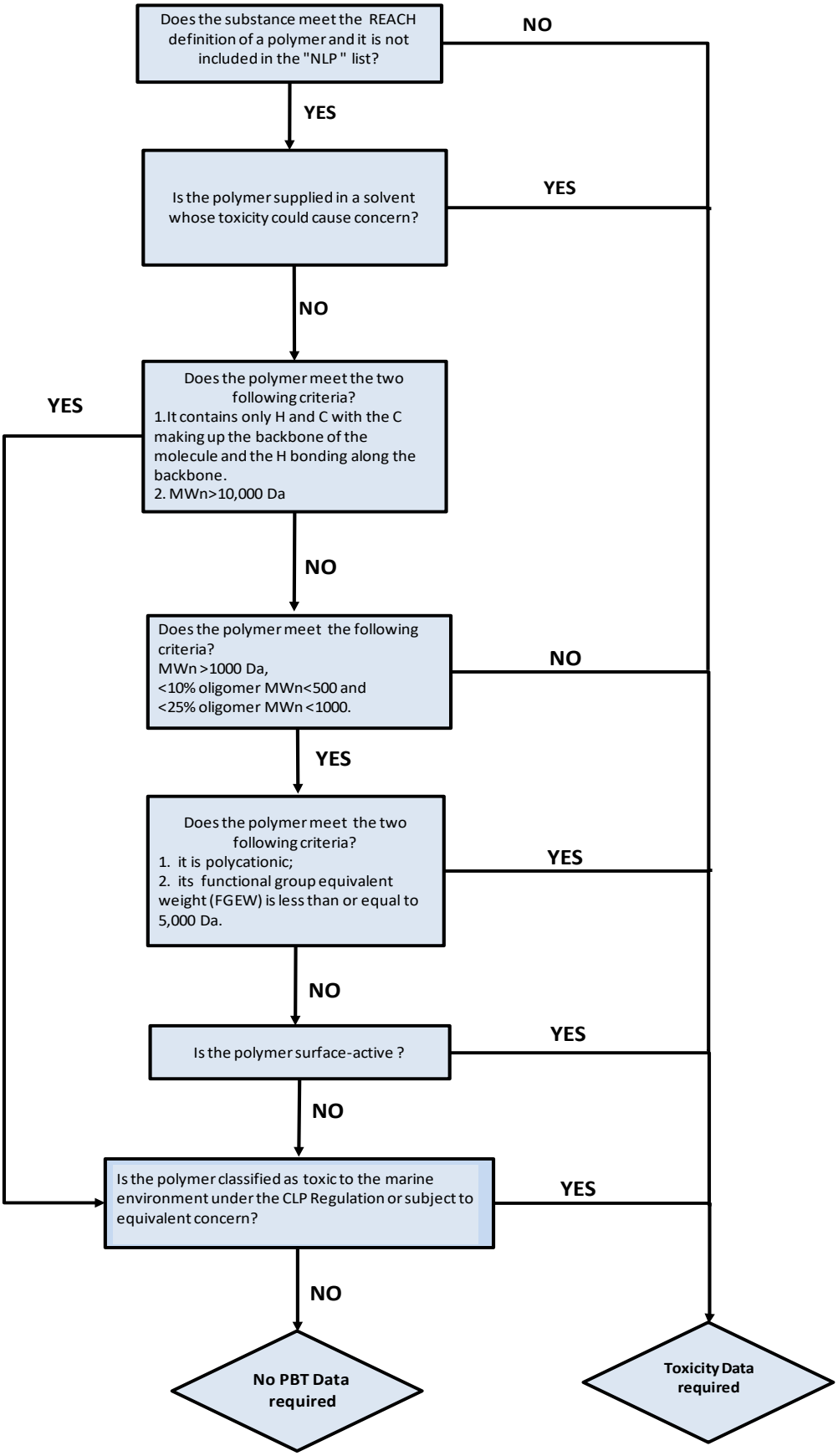
Acidity Control Chemical	Lost Circulation Material
Antifoam (Hydrocarbons)	
Antifoam (Water Injection)	OPF Additive
Asphaltene Dissolver	OPF Base Oil
Asphaltene Inhibitor	OPF Base Synthetic
	OPF Oil based Drilling fluid
Biocide	OPF Synthetic-based Drilling Fluid
Brine (Completion)	Oxygen Scavenger
Carrier Solvent	Pipe Dope
Cement or Cement Additive	Pipe Release Chemical
Coagulant	Pipeline Hydrotest Chemical
Coolant or Coolant Additive	Pipeline Pigging Chemical
Corrosion Inhibitor	Proppant
Crosslinking Chemical	Scale Dissolver
Cuttings Wash Fluid	Scale Inhibitor
	Shale Inhibitor / Encapsulator
Defoamer (Drilling)	
Demulsifier	Thinner
Deoiler	Tracer chemical
Detergent / Cleaning Fluid	
Dispersant	Viscosifier
Drilling Lubricant	
Dye	Water Based Drilling Fluid Additive
	Water Based Drilling Fluid
Emulsifier	Water Clarifier
	Wax Inhibitor
Filter Cake Removal Chemical	Wax Dissolver
Filter Media or Filter Media Additive	Weighting Chemical
Filtrate Reducer	Well Stimulation Chemical
Flocculant	Well Bore Clean-up Chemical
Fluid Loss Control Chemical	
	Other
Gas Hydrate Inhibitor	
Gelling Chemical	

Hydraulic Fluid Hydrogen Sulphide Scavenger Jacking grease	

Bioaccumulation Assessment Methodology for substances for HMCS



Appendix 4 –Flowchart for the assessment of polymers – Glossary of relevant terms and acronyms



a) Polymer definition under REACH, in accordance with REACH (Article 3(5)), is that a polymer is defined as a **substance meeting the following criteria:**

(i) Over 50 percent of the weight of the substance consists of polymer molecules (see definition below); and,

(ii) The amount of polymer molecules presenting the same molecular weight must be less than 50 weight percent of the substance.

In the context of this definition:

Polymer molecule is a molecule that contains a sequence of at least 3 monomer units, which are covalently bound to at least one other monomer unit or other reactant. (See example in the ECHA Guidance for monomers and polymers, Version 2.0, April 2012).

NLP. No longer Polymer. With the implementation of the 7th amendment of Directive 67/548/EEC, some substances which were considered to be polymers under the reporting rules for EINECS are no longer considered to be polymers under the 7th amendment. Therefore, these substances are called "No-Longer Polymers" (NLP). Those no-longer polymers shall be registered as a normal phase-in substance.

The NLP list mainly consists of the following groups:

- alkoxylated substances
- oligomeric reaction products
- oligomers from one monomer only
- dimers and trimers
- polymer-like substances containing 50% or more by weight of species with the same molecular weight

More information on NLP substances can be obtained on the website of the European Commission Joint Research Centre: http://esis.jrc.ec.europa.eu/index.php?PGM=nlp

b) **H**. Hydrogen atom

c) **C.** Carbon atom

d) **MWn.** This is the number-average molecular weight. See OECD Guidelines for the Testing of Chemicals, for the Determination of the Number-Average Molecular Weight and the Molecular Weight Distribution of Polymers using Gel Permeation Chromatography, Test No. 118, adopted on the 14.06.1996.

e) **Da**. **Dalton.** 1.0000 atomic mass unit or 1/12 the mass of a carbon atom of mass 12. Hence, a polymer with a molecular weight of 10,000 atomic mass units has a mass of 10,000 Daltons.

f) **Oligomer.** In the context of this guidance is a low molecular weight species derived from the polymerization reaction, i.e. the low molecular weight polymer content. See OECD Guidelines for the Testing of Chemicals, Determination of the Low Molecular Weight Polymer Content, *Test No. 119*, adopted on the 14.06.1996.

g) **Polycationic polymer.** A polymer that contains cationic groups including phosphonium, sulfonium, or ammonium or can potentially become cationic in water containing amines (primary, secondary, tertiary and aromatic) and isocyanates which hydrolise to form carbamic acid, then decarboxylate to form amines. For a polymer to be exempted (low cationic density), the concentration of cationic functional groups is limited to a functional group equivalent weight of greater than or equal to 5,000 Daltons (1 cationic charge every 5,000 MWn of polymer).

h) **Functional group equivalent weight (FGEW).** The weight of polymer that contains one equivalent of the functional group; or the ratio of number-average molecular weight (NAVG MW) to the number of functional groups in the polymer. The following equation can be used for any reactive functional group in a polymer:

$$FGEW = \frac{FWG \times 100}{W\%G}$$

where FWG is the formula weight of the group and W%' is the weight percent of the group.

When functional groups are introduced into polymers from the precursor monomers, the following equation can be used to estimate the FGEW.

$$FGEW = \frac{FWM \times 100}{W\%G \times NGM}$$

where FWM is the formula weight of the monomer, W%M is the weight percent of the monomer, and NGM is the number of groups in the monomer.

i) **Surface-active**. Any substance, which has surface-active properties according to test method A.5 in Regulation EC 440/2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) of 30 May 2008, and which consists of one or more hydrophilic and one or more hydrophobic groups of such a nature and size that is capable of reducing the surface tension of water, and of forming spreading or adsorption monolayers at the water-air interface, and of forming emulsions and/or microemulsions and/or micelles, and of adsorption at water-solid interfaces.

j) **CLP Regulation**. European Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging of Substances and Mixtures (CLP Regulation).

k) **Toxicity data Required**. Standard HOCNF form with toxicity data.

l) **No PBT data required**. No data for persistence, biodegradation and toxicity are required. The polymer will be assumed to be non-biodegradable, non-toxic and non-bioaccumulative and it will be allocated a substitution warning for non-biodegradability.

The following information will still be required if the polymer meets the non-biodegradable, non-toxic and non-bioaccumulative criteria:

a) List of monomers /reactants (CAS and name) and whether the monomer and reactants are registered under the ECHA Guidance for monomers and polymers, Version 2.0, April 2012.

b) Existing physico-chemical properties on the polymer, as indicated in the safety data sheet (SDS)

c) Molecular weight distribution estimated according to the OECD guidelines for the Testing of Chemicals, Test No. 118 and/or Test No. 119.

Use of the Screening Flow Chart.

a) Does the substance meet the REACH definition of a polymer and is not included in the "NLP" list*?*

A chemical substance must meet the REACH polymer definition to be assessed as a polymer and shall not be classified as "no-longer polymers" under the 7th amendment of Directive 67/548/EEC. The "no-longer polymers"were considered to be polymers under the reporting rules when the European Inventory of Existing Commercial Chemical Substances (EINECS) was being established, but they are no longer considered to be polymers under the 7th amendment. The "no-longer polymers" shall be registered as normal phase-in substances.

b) Is the polymer supplied in a solvent whose toxicity could cause concern?

Polymers meeting the OSPAR substance definition may contain residual quantities of solvent. Consideration of the toxicity of the solvent should be applied before any decision is made concerning the polymer itself.

c) Does the polymer meet the two following criteria?

(i) It contains only H and C with the C making up the backbone of the molecule and the H bonding along the backbone.

(ii) MWn>10 000 Da

This criterion will screen out commodity plastics which are solid, have relatively high molecular weight (>10000 Da) and are not soluble in water. These polymers are deemed to be non-toxic on the assumption of low reactivity and low bioavailability without the need for specific test data.

d) Does the polymer meet the following criteria?

MWn>1000 Da, <10% oligomer MWn<500 and <25% oligomer MWn <1000

The number-average molecular weight (Mn) and the low molecular weight oligomeric species content are generally used to predict the ability of a polymer (or its components) to cross biological membranes. The distinction between MWn and oligomeric content are used to determine if the polymer is assessed only as a polymer, or if oligomers may also need to be addressed. Oligomers may need to be assessed if there is high content of residual monomer and/or the monomer poses known aquatic or human health hazards. The assessment of oligomer toxicity is in addition to, or in lieu of, any polymer specific assessment. The criteria refer to polymers that can be of concern to the aquatic environment, as defined by the guidance adopted by the USA, Canada and Australia.

e) Does the polymer meet the two following criteria?

(i) it is polycationic;

(ii) its functional group equivalent weight (FGEW) is less than or equal to 5,000 Da.

Cationic polymers or polymers that may become cationic in the environment are known to pose a concern for aquatic hazard. Limits on cationic content can be defined in terms of the Functional Group Equivalent Weight (FGEW), a measure of the "dilution" of the cationic charge amongst the polymer's other components. The criterion adopted here has been adopted by the US and Canada.

f) Is the polymer surface-active?

Records of offshore chemicals held on the Cefas data base indicate that there are significant numbers of polymers which are likely to be toxic to the marine environment. More than 90% of these polymers are surfactants. On this basis and in accordance with the precautionary principle, all polymers that are surfactants will require test data.

g) Is the polymer classified as toxic to the marine environment under the CLP Regulation or subject to equivalent concern?

In the event that a polymer passes all the screening criteria, but the regulatory authorities have other reasons to believe that the polymer can be of concern to the aquatic environment (e.g. it is classified as toxic to the marine environment under the CLP Regulation) test data can be requested.

The use of raw data from REACH simulation tests to derive percentage biodegradability figures

1. The practical principle of the OECD 308/309 tests is similar to that of the closed bottle variant of the OECD 306 method, with both utilising the biodegradability of the test substance in marine water (and sediment re. OECD 308), using no added innocula, followed by the analysis of samples to follow the biodegradability process. In typical OECD 306 studies, measurement is made of the dissolved oxygen content, which declines as that oxygen is consumed by bacteria as they consume the test substance.

2. For certain test substances (i.e. those of an inhibitory nature), it is necessary to work at significantly lower concentrations, at which the dissolved oxygen measurement cannot be carried out with sufficient sensitivity. Under these circumstances, the OECD 306 (1992) protocol allows for the use of a radio-labelled test substance enabling the evolved CO_2 to be determined at very low levels.

3. This is possible by trapping the $^{14}CO_2$ in caustic solution, which enables the biodegradability to be expressed thus:

$$\% \text{ biodegradability} = \frac{\text{radioactivity from trapped } CO_2}{\text{Initial radioactivity of test substance}}$$

4. The OECD 308/309 methods utilise the same ^{14}C-labelled chemicals and trapping techniques detailed above, but the radioactivity measurements taken are employed to establish the half life for the degradation. However, OSPAR has allowed (OIC, 2012) that the raw data collected in an OECD 308/309 study can be used as the basis of a valid Biodegradability in Seawater test, if adequate account is taken of other critical parameters, namely:

Test substance: In order to ensure that mineralisation is measured, the radio label must be carried on the most stable part of the molecule.

Temperature: It is noted that the test requirements of the OECD 306 and OECD308/309 protocols are not identical, with OECD 306 (1992) stating that the temperature should be "*controlled to ± 1˚C within a range of 15-20°C*" whilst OECD 309 (2004) requires a "*Controlled temperature (± 2°C) which may be the field temperature or a standard temperature of 20-25˚C*". It is therefore possible that the temperature used in an OECD 308/309 study may exceed the range defined for an OECD 306 test. If this occurs, the authorities should consider the application of a temperature correction based on the Arrhenius equation, in line with the REACH Guidance R.7b.

Marine Water/Sediment: The use of Marine Water is a requirement of the OSPAR Pre-screening scheme for acceptance of OECD 309 data. Likewise a valid OECD 308 study must employ marine sediment.

Trapping of $^{14}CO_2$: the $^{14}CO_2$ should be trapped in KOH traps which should be sampled (volume measured samples taken for radioassay) and changed for fresh KOH throughout the test. Therefore $^{14}CO_2$ must be confirmed by making a composite sample of all KOH samples from the study and treating these with a solution of barium ions to precipitate the $^{14}CO_2$ as barium carbonate. Radioassay should then show that there is no radioactivity in the liquid phase thus confirming that $^{14}CO_2$ is formed. This approach is advocated in order to prevent interference from volatile organic substances from the other trapping media.

Radiochemical purity of the test substance: OECD 309 (2004) and OECD 308 (2002) stipulate that the radiochemical purity should exceed 95%, and it is recommended that purities of over 97% should be obtained where possible.

5. Other method requirements should follow those of the OECD 308/309 protocols as appropriate, including the recovery of radioactivity from the test samples, which should be between 90 and 110%.

GUIDANCE FOR THE TESTING OF CHEMICALS using the Marine BODIS (BOD-test for Insoluble Substances) test

Introduction

This Appendix provides guidance based on the ISO 10708 "Water Quality- Evaluation in an aqueous medium of the ultimate aerobic biodegradability of organic compounds. – Determination of biochemical oxygen demand in a two phase closed bottle test~~" fresh water environments fresh water environments~~. The test is an adaptation of the ISO 10708 to render it applicable to the marine environment (i.e. Marine BODIS) and represents the protocol followed in the OSPAR ring-test draft procedure in 1995 (pages 1-5).

Headings and paragraphs numbers given in the following text correspond to those given in ISO 10708. Only sections of the ISO 10708 that have been modified and adapted for the Marine BODIS test are reported herein.

4. Principle of the test method

The test principle is stated in section 4 of the ISO 10708, the only difference being that the test medium is seawater fortified with nutrients and mineral elements as described in the OECD 306 guidelines (1992). No inoculum is added in addition to the microorganism already present in seawater. The test is based on repeated measurements of consumed O_2 with repeated oxygenation of the test bottle. If a test chemical gives a measured BOD ≥60% of the ThOD or COD (within 28 days), it can be concluded that the test chemical has the potential for biodegradation in the marine environment.

5. Test environment

Incubation temperature should take place at a constant temperature (±1°C) in the range ~~of~~ between 15-20°C, with 20°C ±1°C being the maximum temperature.

6. Reagents

6.2. Composition of the mineral medium

The following stock solutions are prepared using analytical grade reagents:

a) Dissolve anhydrous potassium dihydrogenphosphate (KH_2PO_4)	8.5g
Anhydrous dipotassium hydrogen phosphate (K_2HPO_4)	21.75g
Disodium hydrogenphosphate dehydrate ($Na_2HPO_4.2H_2O$)	33.4g
Ammonium chloride (NH_4Cl)	0.5g

Dissolve in water to make up 1 litre. The pH of the solution should be 7.4

b) iron (III) chloride hexahydrate ($FeCl_3.6H_2O$)	0.25g

Dissolve in water and make up 1 litre.

NOTE: - In order to avoid having to prepare this solution immediately before use add one drop of conc. HCl or EDTA (disodium salt) to attain a concentration of 0.4 g/L.

Mix 10 ml of solution a) with 800 ml dilution water, add 1ml of solution b) and make up to 1 litre with dilution water containing less than 2mg/l of DOC and/or less than 10% of the initial organic carbon content introduced by the test compound.

7. Apparatus

7.5 Water Bath, or device to guarantee an accurate temperature control (±1 °C) in a range between ~~of~~ 15-20 ~~+1~~°C.

8. Procedure

8.1 Preparation of Test and reference compounds

The test is applicable to organic compounds which are: soluble under the test conditions, insoluble, non-volatile, non-inhibitory.

8.1.1 Water-soluble test compounds

Concentration of the test substance in the **stock solution** should be 40 mg ThOD/ml ~~ThOD~~ (or COD). The final test concentration should be approximately 100 mg ThOD/l.

8.1.2 Water-insoluble test compounds

Insoluble compounds must be introduced into the flask in accordance to the ISO 10634 and section 8.1.2 in ISO 10708. The final test concentration should be approximately 100 mg ThOD/l
~~Concentration of the test substance in the stock solution should be 20mg/l ThOD (or COD)~~

For calculation of ThOD and estimation of the COD refer to Annex A and B of the ISO 10708 respectively.

8.1.3 Solution of the reference standard

Prepare a stock solution of the reference standard, sodium benzoate or aniline as indicated in 8.1.3 of ISO 10708 ~~in order to obtain a final test concentration of 40mg/l ThOD (or COD).~~The final test concentration should be approximately 100 mg ThOD/l.

8.2 Seawater

The following paragraph is cited from the OECD 306 Marine Closed Bottle Method.
Collect a sample of seawater in a thoroughly cleansed container and transport to the laboratory, preferably within one or two days of collection. During transport, do not allow the temperature of the sample to exceed significantly the temperature to be used in the test. Identify the sampling location precisely and describe it in terms of its pollution and nutrient status. Especially for coastal waters, include in this characterization a heterotrophic microbial colony count and the determination of the concentrations of dissolved nitrate, ammonium and phosphate.

Provide the following information for the seawater sample itself: -

- *date of collection;*
- *depth of collection;*
- *appearance of sample - turbid, etc.;*
- *temperature at the time of collection;*
- *salinity;*
- *DOC;*

- *delay between collection and use in the test*

If the DOC content is found to be high or if it is thought that the blank BOD to be more than 30% of the reference substance after 28 days, it is recommended that the seawater is aged for about a week prior to use.

Aging of seawater should be carried out in the dark or under diffuse light conditions aerobically.

Prior to use, pre-treat seawater to remove coarse particles by filtration through a nylon filter or coarse paper filter (not membrane of GF filters), or by sedimentation and decanting. This procedure and filter type must be reported.

The microbial colony count of the original seawater sample should normally fall within the range 10^4-10^6 colonies/litre. ***No inoculum is added in addition to the micro-organisms already present in seawater.***

Where artificial seawater is used the specific parameters should not deviate significantly from that outlined in the ISO 16221:2001 (BS 6068-5.29: 2001). Where artificial seawater is used, 30mg/l wet weight inoculum obtained from the filter of a marine aquarium should be used.

8.3 Test procedure

Each test bottle (with magnetic stirrer inside) is filled to 2/3 of the free volume with mineral medium (pretreated seawater with nutrients and mineral elements). All incubation should be prepared in triplicate. Each test series is accompanied by a triple blank, triple reference and toxicity controls.

The test should be run at a constant temperature (±1°C) in a range between 15-20°C ~~within +1°C~~.

The pH of natural seawater is typically around 8.0 and adjustment for pH is not likely to be necessary. Any adjustment should be carried out prior to start.

NOTE- Magnetic stirrers and pH meter will be required to fulfil the test requirements and ensure the experiment is within test guidelines.
Measurements should be made at time 0, 7, 14 and 28 days and should in any case be reported. The test should be prolonged beyond 28 days to identify a plateau if the curve shows that biodegradation has started and not reached a plateau.

8.3.4 Analysis

The saturation value for dissolved oxygen varies with salinity. The equipment for dissolved oxygen measurement must be able to correct for salinity, and it must be correctly calibrated at the salinity of the seawater before every measurement series. Saturation values for dissolved oxygen at 20 °C and 101.5 kPa atmospheric pressure are as follows for selected salinities:

35‰ sal.	7.4 mg/l
30‰ sal.	7.7 mg/l
25‰ sal.	7.9 mg/l
20‰ sal.	8.2 mg/l

Oxygen readings should be taken once the reading has stabilised (usually within 2 minutes). After the oxygen measurement is taken measure and record the pH while keeping stirring.

9. Calculation and expression of results

9.1 Calculation

Apply the following modification to the ISO 10708.

Cs, saturation value for dissolved oxygen at normal atmospheric pressure at 20 °C, is set according to the salinity of the seawater.

Oc, total oxygen capacity of a flask, is calculated from $Oc= V_{air} \times 0.280 + V_l \times 0.008$ Where:
V_{air}= Volume of air in the flask (in ml)
V_l= Volume of liquid in the flask (in ml)
0.280= Oxygen content in mg/l of normal air
0.008= oxygen content in mg/ml of saturated seawater

10. Validity of the test

The results are valid if the following conditions are met:

- The degradation of the reference compound should reach 60%(of ThOD) within a reasonable time span. For sodium benzoate and aniline the degradation must be compared to that obtained in the EEC ring test, as given below:
    - o Lag phase (t_L) and the time to achieve 50% biodegradation (t_{50}) for :
        - ▪Sodium benzoate: (t_L)= 0 to 2 days and (t_{50})= 1 to 4 days (50%)
        - ▪Aniline: (t_L)= 0 to 7 days and (t_{50})= 2 to 12 days (50%)
- The total oxygen uptake in the blanks should not exceed 30% of that of the reference standard. If it is not possible to meet this criterion using freshly collected seawater, the seawater must be aged before use.
- In a toxicity test containing both the test substance and a reference substance, if less than 25% degradation occurs in 7 days, then the test chemical can be assumed to be inhibitory and the study should be repeated at a lower concentration.

For nitrogen containing substances the possibility that nitrification processes may affect the result should be considered as given in Annex C of ISO 10708.

11. Test report

The test report should include the items specified in the ISO 10708, with the exception of that related to the inoculums. Instead, report should include the following information about the seawater:

- Location, description of the sampling site: pollutional and nutrient status (colony count, nitrate, ammonium, phosphate if appropriate);
- Characteristic of the sample (date of sampling, depth, appearance, temperature, salinity, DOC, delay between collection and use in test);
- Method used (if any) for ageing the seawater;
- Method used for pre-treatment (filtration/sedimentation) of the seawater;
- Method used for determining the number of ~~hererotrophs~~ heterotrophs in the seawater (plate count method or alternative procedure)
- Method used ~~to~~ for determining DOC in seawater.

In addition the test report should include information about:

- Equipment used for the oxygen measurements
- Method used for the COD determination (if performed)

Bibliography

- ISO 15462:2006 Water quality – Selection of tests for biodegradability
- ISO 10634: 1995 Water quality- Guidance for the preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium.
- ISO 10708: 1997 Water quality – Evaluation in an aqueous medium of the ultimate aerobic biodegradability of organic compounds – Determination of biochemical oxygen demand in a two-phase closed bottle test.
- OECD Guidelines for Testing Chemicals (1992) Test No. 306: Biodegradability in Seawater.
- OSPAR Ringtest (1995) Draft Procedure – Biodegradability in seawater: BOD test for insoluble substances (Marine BODIS-test).

Determination of the Biodegradability of Solute/Solvent mixtures

NOTE: The terms "solute" and "solvent" are used for convenience only. The approach is potentially applicable to all substances which are supplied mixed with an organic solvent, including emulsions and suspensions.

It is recommended that the procedure for the OECD 306 Closed Bottle Method is followed, except that:

Two additional sets of bottles are prepared to facilitate duplicate testing of the solvent alone, i.e. bottles will feature sea water with mineral nutrients and solvent only. These must be tested in parallel with the sample, standard and blank.

Calculate the biodegradability of the solute using the following formula:

$$\%\text{Biodegradability} = \frac{\{(m_{blank(t)}-m_{sample(t)})-(F \times (m_{blank(t)}-m_{solvent(t)}))\}^{+}}{C \times ThOD} \times 100\%$$

Where:

$m_{blank(t)}$ = Dissolved oxygen concentration of sea water blank (mgO_2/L) at time t

$m_{sample(t)}$ = Dissolved oxygen concentration of sample including solvent (mgO_2/L) at time t

$m_{solvent(t)}$ = Dissolved oxygen concentration of solvent (mgO_2/L) at time t

F = Ratio between the concentration of solvent in the sample bottles and the concentration of solvent in the solvent only bottles

C = Concentration of solute in the test vessel (mg/L)

ThOD = Theoretical Oxygen Demand of the solute (mgO_2/mg)*

*Elemental Analysis may assist in the establishment of this value. Where a ThOD value cannot be obtained, the chemical oxygen demand (COD) of the solute may be used instead. This should be determined by the difference between the COD of the combined solute/solvent sample and the COD of the solvent alone, adjusted for the proportion of solvent present. For water-insoluble samples, attention must be paid to ensuring that the sample is fully exposed to the oxidising medium, and Contracting Parties may reject results where this has not been achieved, or where the COD calculated for the solute cannot be justified.

+ The term $\{(m_{blank(t)}-m_{sample(t)})-(F \times (m_{blank(t)}-m_{solvent(t)}))\}$ in the above equation represents the depletion in the oxygen concentration that can be attributed to the solute. This assumes that $m_{blank(0)} = m_{sample(0)} = m_{solvent(0)}$, where

$m_{blank(0)}$ = initial blank dissolved oxygen concentration,

$m_{sample(0)}$ = initial sample dissolved oxygen concentration,

$m_{solvent(0)}$ = initial solvent dissolved oxygen concentration.

If $m_{blank(0)}$ does not equal $m_{sample(0)}$, or $m_{solvent(0)}$, use

$((m_{sample(0)} - m_{sample(t)}) - (m_{blank(0)} - m_{blank(t)}))$ in place of $(m_{blank(t)}-m_{sample(t)})$, and $((m_{solvent(0)} - m_{solvent(t)}) - (m_{blank(0)} - m_{blank(t)}))$ in place of $(m_{blank(t)}-m_{solvent\ (t)})$.

Marine biodegradation screening test for chemical persistence assessment (MaP test)

Introduction

1. Biodegradation screening tests (BSTs) are stringent tests, which aim to provide a conservative assessment of chemical fate, and in doing so screen out chemicals, which are easily degraded in all environments. BSTs have historically formed the foundation on which regulatory frameworks were developed to protect the environment. However, in recent years, BSTs, have not evolved at the same rate as regulatory concerns, which now place an increased emphasis on environmental persistence.

2. Currently available BSTs (e.g. OECD 301, 306 and 310) represent the first tier in persistence assessment, despite not being particularly suited for screening out all non-persistent chemicals. These laboratory based short-term tests are variable and stringent, therefore prone to false negatives (Figure 1).[1–7] This can result in additional costly biodegradation tests and potentially unnecessary bioaccumulation and toxicity tests.[5]

3. BSTs' reliability can be increased by improving the representation of the environmental microbial community in the test vessel through increasing biomass and diversity in the BSTs to levels that a chemical is more likely to encounter in the environment (Figure 1).[5,8–12] This consequently increases the likelihood of including potential degraders of chemicals that show variable degradation results in BSTs but are non-persistent.

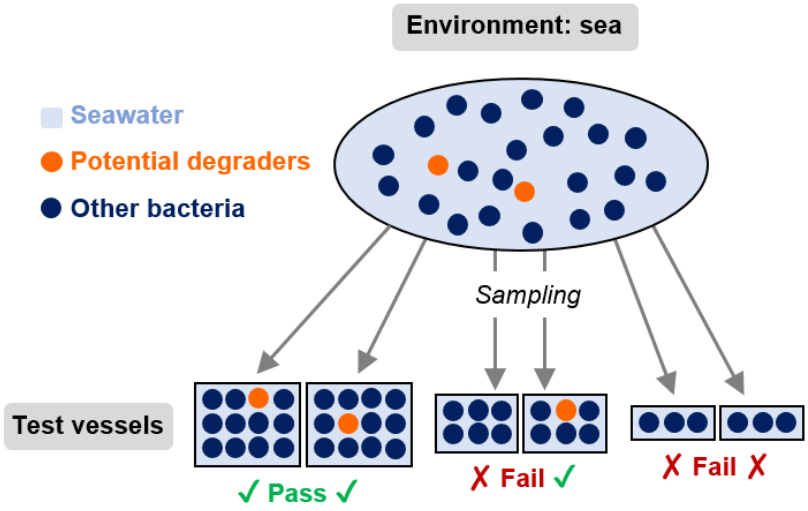


Figure 1: Schematic overview explaining the effect of increased sampling size on the inclusion of potential degraders and improved representation of the sampled environment in the test system.[11]

4. The marine biodegradation screening test for chemical persistence assessment (MaP test) incorporates 100-fold nominally increased biomass concentrations to better represent the microbial diversity inherent in the sampled environment. The MaP test runs for 60 days. The test is a seawater variant of the OECD 301F Manometric Respirometry test [13], incorporating aspects of the OECD 306 Biodegradability in Seawater test.[14]

5. The method was finalised as a result of the Cefic LRi ECO11 ring test in 2017 organised by Newcastle University [11,12,15,16], which closely followed the OECD Guidance Document 34 on the correct validation of new or

updated test methods.[17] The ring test compared the MaP test (there so-called $imBST_{MR}$) with a standard OECD 306 Closed Bottle Method and found the MaP test to be more reliable and less variable in screening for non-persistence than the OECD 306 method.[16]

Principle of the test

6. A measured volume of test medium, containing a known concentration of test substance (usually 100 mg test substance/ L giving at least 50-100 mg ThOD/ L) as the nominal sole source of organic carbon, is stirred in a closed flask in the dark at a constant temperature controlled to ± 1°C within a range of 15-20^{o}C for up to 60 days. The consumption of oxygen is determined either by measuring the quantity of oxygen (produced electrolytically) required to maintain constant gas volume in the respirometer bottle, or from the change in volume or pressure (or a combination of the two) in the apparatus. Evolved carbon dioxide is absorbed in a solution of potassium hydroxide or another suitable absorbent. The amount of oxygen taken up by the microbial population during biodegradation of the test substance (corrected for uptake by blank, run in triplicate) is expressed as a percentage of ThOD or, less satisfactorily, COD. As described for the OECD 301F method, optionally, primary biodegradation may also be calculated from supplemental specific chemical analysis made at the beginning and end of incubation, and ultimate biodegradation by DOC analysis.

7. For the test medium, mineral nutrients are added to pre-treated natural seawater. Seawater pre-treatment incorporates 10 µm pre-filtration to remove coarse particles and tangential flow filtration (TFF) to increase biomass concentration 100-fold nominally. In a comparison of different methods for increasing biomass concentrations in marine BSTs, TFF ranked highest by accurately representing the microbial community of the initial sampled environment while allowing for a high sample throughput.[18] Interestingly, the original OECD 306 protocol already considered biomass concentration for marine BSTs, but at the time, the investigated technologies employed were not successful.[14]

8. Due to reported oxygen limitations in the OECD 306 Closed Bottle Method [14], the MaP test measures biodegradation with manometric respirometers. The headspace provides the microorganisms with more oxygen for a prolonged test duration (60 days instead of 28 days) and thus renders seawater ageing to reduce background dissolved organic carbon concentrations unnecessary. Other advantages of manometric respirometers are that they require less seawater than sacrificial bottles, continuous biodegradation curves can be monitored which are already accepted by regulators (see OECD 301F). Since manometric respirometers have a lower sensitivity compared to the dissolved oxygen measurement in the OECD 306 Closed Bottle Method, higher chemical test concentrations are required.

9. In the ECO11 ring test, standard OECD 306 seawater pre-treatment (filtration or sedimentation followed by ageing) had a variable effect on bacteria concentrations in the OECD 306 Closed Bottle Method. For some testing facilities, the OECD 306 pre-treatment decreased cell numbers from the raw seawater while for others, it increased bacteria concentrations more than 100-fold. Incubation conditions during ageing have previously been reported to exceed selection pressure and alter the microbial community composition from the original seawater sample.[19] When the test chemical is added, the bacterial community may have become atypical of the environment. This may lead to a higher or lower biodegradation potential to be observed and consequently increases the uncertainty and inaccuracy of extrapolating laboratory biodegradation data to the environment.[19] Due to this, ageing is not recommended for the MaP test.

10. While the MaP test aims to better represent the microbiome of the sampled environment by capturing 100-fold more biomass in the test vessel [18], it is still a conservative screening test with unrealistically high test chemical concentrations to overcome analytical constraints. The ratio of bacterial cells to test chemical in the MaP test is comparable to the standard OECD 306 method given the higher test chemical concentrations employed in the former and the variable bacterial cell concentration effects of ageing in the latter. Seawater pre-treatment in the MaP test with TFF results in better representation of the environmental microbial community in the test system, decreases variability between replicates and improves the ability to screen for non-persistence. Previous studies have shown that biodegradation kinetics in BSTs with increased biomass concentrations can be indistinguishable from those in current BSTs.[5]

11. The ECO11 ring test validated the MaP test for test durations up to 120 days.[16]

Applicability and limitation of the test

12. The MaP test is intended for chemicals that are likely to be used or likely to end up in the marine environment. Chemicals with properties suitable for the OECD 301F test (see Table 1, OECD 301) are also suitable for the MaP test. Modifications for poorly soluble chemicals as described in OECD 301 Annex III and by other methods [20,21] can be applicable.

13. In the ECO11 ring test, manometric respirometers (closed system and oxygen replenishing systems) proved suitable for monitoring biodegradation in seawater, but reliability beyond 28 days varied depending on the system used. Laboratories are advised to confirm the suitability of their respirometer systems with manufacturers for prolonged experiments and, if necessary, amend their setups. If reliability cannot be proved beyond 28 days only values up to 28 days should be considered.

14. The results of the MaP test give a first impression of biodegradability in seawater. If the result is positive, it may be concluded that there is a potential for biodegradation in the marine environment and the chemical is non-persistent. The test recommends a number of end-point criteria since there are differing interpretations of what constitutes a non-persistent chemical; OSPAR states that ≥ 20% ThOD biodegradation in 28 days in the existing OECD 306 is evidence for a non-persistent chemical [23] whereas under REACH a pass of ≥ 60% ThOD biodegradation in 60 days is required to demonstrate non-persistence.[24] In the ECO11 ring test, extending the test duration beyond 28 days to 60 days improved non-persistence screening. The 60 day-60% biodegradation threshold was more accurate and reliable than the 28 day-20% biodegradation threshold in characterising the biodegradation behaviour of the reference chemicals (non-persistent or potentially persistent).[16]

15. The MaP test complements the existing OECD 306 method, which is still pertinent for identifying rapidly degrading chemicals, but the MaP test offers a cost-effective screening test for non-persistence when the OECD 306 test gives the result “not rapidly biodegradable”. A negative result in the MaP test does not preclude biodegradation potential but indicates that further study is necessary. In either case, if a more definitive value for the rate or degree of biodegradation in seawater at a particular site is required, other more complex and sophisticated, and hence more costly, methods would have to be applied. Within REACH, the MaP test could form part of the first tier of persistence screening before the more complex, costly and time-consuming simulation tests.[24]

Information on the test substance

16. In order to know whether the test may be applied to a particular substance, some of its properties must be known. The empirical formula is required so that the theoretical oxygen demand (ThOD) may be calculated (see OECD 306, Annex 3); otherwise the chemical oxygen demand (COD) of the compound must be determined to serve as the reference value. The use of COD is less satisfactory since some chemicals are not fully oxidised in the COD test.

17. The solubility of the substance should be at least 100 mg/ L, though in principle less soluble compounds could be tested (paragraph 12). Information on the purity or the relative proportions of major components of the test material is required in order that the results obtained can be interpreted, especially when the result lies close to the "pass" level.

18. Information on the toxicity of the substance to bacteria e.g. as measured in short-term respiration tests may be very useful when selecting appropriate test concentrations and may be essential for the correct interpretation of low biodegradation values. However, such information is not always sufficient for interpreting results obtained in the biodegradation test and the procedure described in paragraph 48 is more suitable.

Reference compounds

19. Suitable reference compounds must be used to check the microbial activity of the seawater sample. Aniline, sodium acetate or sodium benzoate (for example) may be used for this purpose. A degradation of these compounds of at least 60% (of their ThOD) must occur within a reasonably short time span, otherwise it is recommended that the test be repeated using another seawater sample.

20. In the ECO11 ring test where seawater samples were taken at different locations and at different times of the year, the lag phase (t_L) and the time to achieve 50% degradation (t_{50}), including the lag phase, were 2 ± 1 days and 4 ± 2 days respectively for sodium benzoate.[16]

Description of the method

Apparatus

21. Normal laboratory apparatus and:

a) Suitable respirometer;
b) Several 2-, 3- and 4- litre volumetric flasks for the preparation of the experiment and for the filling of the respirometer bottles;
c) Waterbath or constant temperature room for keeping the bottles at constant temperature (± 1°C or closer) with the exclusion of light;
d) 10 µm filter;
e) Tangential flow filtration unit with 0.22 µm filter and pumps;

Seawater collection

22. Collect the seawater sample in a thoroughly cleansed container and transport to the laboratory, preferably within one or two days of collection. During transport do not allow the temperature of the sample to exceed significantly the temperature to be used in the test. At the laboratory, storage at 4ºC in the dark is recommended.

23. Identify the sampling location precisely and describe it in terms of its pollution and nutritional status. Include in this characterisation a cell count and the determination of concentrations of dissolved nitrate, ammonium and phosphate. For cell counts, culture-independent (e.g. flow cytometry with optional live/dead staining of cells or haemocytometer) or culture-dependent (e.g. heterotopic microbial colony count) methods can be used. Note that culture-dependent methods only measure a small fraction of total cell counts (0.01-1% [25]) and that different media and methods for culture-dependent methods affect the number and types of microorganisms recovered.[26]

24. Provide the following information for the seawater sample itself:

a) date of collection;
b) depth of collection;
c) distance from the coast;
d) appearance of sample - turbid etc.;
e) temperature at the time of collection;
f) dissolved organic carbon (DOC);
g) cell count
h) delay between collection and use in the test.

Seawater pre-treatment

25. Pre-filter the seawater through a 10 µm filter to remove coarse particles. Determine cell counts in the 10 µm pre-filtered seawater. This cell count will later be compared to the cell count in TFF processed seawater to determine the TFF concentration factor and assess the TFF efficiency.

26. 10 µm pre-filtered seawater is processed with TFF to increase biomass concentrations 100-fold nominally. This means that for instance, 1 L of 100-fold nominally concentrated biomass in seawater is thus prepared from 100 L of natural pre-filtered seawater. In TFF, water is pumped tangentially across the filter surface to reduce the chance of filter cake formation. Seawater including salts passes the 0.22 µm filter membrane as a partial flow and is removed as filtrate (also called permeate) while bacteria remain in the retentate and are enriched in the feed tank (Figure 2).

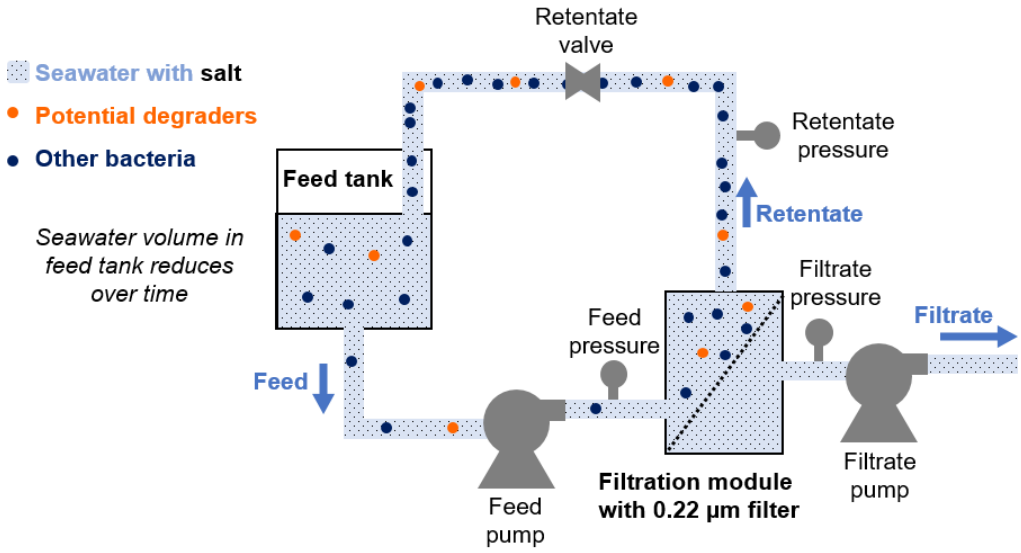


Figure 2: Schematic tangential flow filtration setup to increase biomass in seawater.[16,27]

27. Typically, TFF applications adjust the pressure in the system with a retentate valve where the filtrate flows uncontrolled and unrestricted out of the module. This is the simplest type of operation, but for the MaP test it is helpful to use a filtrate control as shown in Figure 2. Using very open membranes (as here with a pore size of 0.22 µm), the membrane permeability is so high that nearly all of the crossflow is converted to filtrate. Although this results in high fluxes, it is similar to operating a normal flow filtration mode (dead-end filtration) and the benefits of the tangential flow are lost. Often, very high wall concentrations and high membrane fouling occur, especially during the start-up of the process. To reduce the filtrate rate, the filtrate flow must be controlled. In a controlled flow filtrate operation, a pump (as described here and tested for the ECO11 ring test, see Figure 2 and paragraph 31-32) or valve on the filtrate line restricts filtrate flow to a set value to maintain adequate tangential flow.

28. Operation of a TFF system typically consists of the following steps:

a) test pumps to determine revolutions per minute – flow correlation using seawater (*);
b) setup the TFF and install the filters;
c) flush the TFF system with distilled water;
d) sanitize the TFF system with sanitizing solution;
e) flush the TFF system with distilled water;
f) measure the normalized water permeability (NWP) of the membrane to assure cleanness;
g) concentrate biomass in seawater 100-fold nominally;
h) recirculation step (optional);
i) flush the TFF system with distilled water;
j) clean the TFF system with cleaning solution;
k) flush the TFF system with distilled water;
l) measure the NWP to determine cleaning efficacy;
m) flush the TFF system with storage solution;
n) take apart the TFF system and store filters in storage solution;
(*) only conducted at beginning when new test system is established.

29. The choice of sanitizing, cleaning and storage solution including circulation times depend on the filter material. Methods given by the manufacturer of the TFF system should be followed. During these steps, both, feed

and permeate channels are flushed. For the feed channel flushing, the feed pump is switched on, the permeate pump is switched off and the retentate valve is fully opened. For the permeate channel flushing, the feed pump is switched on, the permeate pump is switched on to give a permeate flow of 30% of the feed flow and the retentate valve is partially closed.

30. To calculate the NWP, permeate flow, water temperature, feed pressure, retentate pressure and permeate pressure are measured under a set flow rate with deoionized water. Detailed methods on NWP determination are given by the manufacturer of the TFF system. The initial NWP of a new membrane is used as the basis to determine membrane recovery, i.e. how effectively the membrane was cleaned back to its original state. The first NWP after processing may be up to 20% lower than the initial NWP. This decrease is normal and is a result of the membrane being conditioned. No additional decline in NWP should occur for several process runs. If the NWP decreases significantly from run-to-run, cleaning procedures may be inadequate. Alternative cleaning agents and procedures should be investigated.

31. TFF setup and seawater viscosity determine the flow rates for the biomass concentration. The feed flow rate for TFF depends on the TFF system and membrane material and is provided by the manufacturer of the equipment. The permeate flow should not exceed 50% of the feed flow. If significant decreases in permeate flow are observed during the biomass concentration (at same pump speed), it is recommended to reduce the permeate flow for future concentrations to operate a more robust TFF process. Note that during the biomass concentration, the retentate valve is open.

32. For example, the TFF system used in the ECO11 ring test had a maximum feed flow of 6 L min^{-1} m^{-2}. With a total filter area of 0.5 m^2, the feed pump was set to pump at a feed flow rate of 3 L min^{-1}. To operate the TFF process stably across laboratories with varying seawater characteristics, the permeate flow was set to 2.2 L min^{-1} m^{-2}. Consequently, the permeate pump was set to pump at a permeate flow of 1.1 L min^{-1}, translating to 37% of the feed flow.[16] For testing facilities with less viscous (clearer) seawater, permeate flow could be increased to reduce filtration time while maintaining conditions of minimal fouling and operating a steady process.

33. A recirculation step at the end of the biomass concentration can be beneficial to flush any bacteria sticking to the membrane into the retentate. For this, filtrate is collected during concentration and then flushed through the system at high feed pump speed and clamped filtrate tubing. This filtrate is then added to the concentrated retentate. Assure to take this volume change into consideration. For instance, to obtain 3 L of concentrated seawater, the retentate could be reduced from 300 L to 2 L and then topped up with 1 L of recirculated filtrate to achieve a final volume of 3 L. In the ECO11 ring test, two recirculation steps with each 1 L of collected filtrate were conducted. The filtrate was flushed through the system at maximum feed pump speed (here 6.7 L min^{-1} m^{-2} feed flow) and clamped filtrate tubing (0 L min^{-1} m^{-2} permeate flow) for a cycle of 2 min run, 1 min break and 2 min run and then added to the retentate.[16]

34. Backflushing at the end of the concentration can be employed to wash any biomass sticking on the membrane into the retentate but note that not all filters are designed to withstand this reversed pressure. For clarification, contact the manufacturer of the filters.

35. Determine cell counts in TFF processed seawater and calculate the concentration factor by dividing the cell concentration in TFF processed seawater with the cell concentration in 10 µm pre-filtered seawater. If the

concentration factor is significantly lower than 100, the TFF process could be improved by decreasing permeate flow to reduce filter cake formation and/or performing recirculation steps to wash biomass off the filters in the retentate.

36. The TFF time to increase biomass 100-fold nominally in seawater depends on the seawater volume to filter (defined by test setup e.g. number of test chemicals, replicates and test volume), the filter surface and seawater characteristics.

37. While the ECO11 ring test employed TFF to increase biomass concentrations in seawater [16,18], other concentration methods could also be applicable, but should first be validated to ensure adequate representation of the sampled environment in the concentrate.

38. The TFF processed seawater should be used as soon as possible for the test setup, preferable within one or two days of concentration. During storage, do not allow the temperature of the sample to exceed significantly the temperature to be used in the test. Storage at 4ºC in the dark is recommended. The TFF processed seawater should be well mixed before use.

Stock solutions for mineral nutrients

39. Prepare the following stock solutions using analytical grade reagents:

a) Potassium dihydrogen orthophosphate, KH_2PO_4 — 8.50 g
Dipotassium hydrogen orthophosphate, K_2HPO_4 — 21.75 g
Disodium hydrogen orthophosphate dihydrate, $Na_2HPO_4.2H_2O$ — 33.30 g
Ammonium chloride, NH_4Cl — 0.50 g
Dissolve and make up to 1 litre with distilled water.

b) Calcium chloride, $CaCl_2$ — 27.50 g
Dissolve and make up to 1 litre with distilled water.

c) Magnesium sulphate heptahydrate, $MgSO_4.7H_2O$ — 22.50 g
Dissolve and make up to 1 litre with distilled water.

d) Iron (III) chloride hexahydrate, $FeCl_3.6H_2O$ — 0.25 g
Dissolve and make up to 1 litre with distilled water.

Note: Precipitation in solution (d) may be prevented by adding one drop of concentrated HCl or 0.4 g ethylenediaminetetra-acetic acid (EDTA, disodium salt) per litre.

40. If a precipitate forms in a stock solution, replace it with freshly made solution.

Preparation of test medium

41. Mix 10 mL of solution (a) with 800 mL TFF processed seawater, then add 1 mL of solutions (b), (c) and (d) and make up to 1 L with TFF processed seawater.

42. Saturate the test medium with air at the test temperature by aerating with clean compressed air for about 20 minutes. Determine the concentration of dissolved oxygen for control purposes. The saturated concentration of

dissolved oxygen as a function of salinity and temperature may be read from the nomogram enclosed in OECD 306 test guideline (Annex 4).

Inoculum

43. Do not add a specific inoculum in addition to the microorganisms already present in the TFF processed seawater.

Preparation of test bottles

44. Prepare respirometer bottles for the determination of the biological oxygen demand (BOD) of the test and reference substances in simultaneous experimental series. Perform all analyses in triplicate bottles (blanks, reference and test substances).

45. Test and reference substances are normally tested at 100 mg chemical/ L (giving 50-100 mg ThOD/ L). Calculate the ThOD on the basis of formation of ammonium salts unless nitrification is anticipated, when the calculation should be based on nitrate formation (see OECD 306, Annex 3). Nitrification inhibitors should not be used as these are usually not validated by the manufacturers for test periods beyond 28 days, and could possible degrade and interfere with the test chemical degradation.

46. Prepare separate solutions of test and reference substances in large bottles of sufficient volume by first adding test and reference substances either directly or by using a concentrated stock solution to the partly filled large bottles. Add test medium at desired test temperature to give the final desired concentrations.

47. An oxygen blank must be determined in respirometer bottles containing neither test or reference substance.

48. If the toxicity of the test substance is to be determined, prepare a further solution in test medium containing both test and reference substances at the same concentrations as in the individual solutions.

49. In the ECO11 ring test, respirometer bottles (500-510 mL) were filled with 250 mL of test medium including test chemicals [16], but higher or lower fill volumes can be employed for the MaP test. Note that higher test volumes increase biomass in the test while lower test volumes increase headspace and consequently available oxygen.

Physical-chemical control test (optional)

50. If the option of using specific analyses is used, a physical-chemical experiment may be performed in order to check whether the test material is removed by abiotic mechanisms, such as hydrolysis or adsorption. A physical-chemical control test may be performed by adding a toxic substance at an appropriate concentration to duplicate flasks with test material in order to stop microbial activity. A significant decrease in specific compound concentration in the course of the test indicates abiotic removal mechanisms.

Number of bottles and volume of seawater in a typical run

51. In a typical run the following bottles are used:

a) Bottle 1,2,3 – containing test substance and test medium (test suspension)
b) Bottle 4,5,6 – containing test medium only (blank)
c) Bottle 7,8,9 – containing reference compound and test medium (procedure control)
d) Bottle 10,11,12 – containing test substance, sterilising agent and test medium (abiotic sterile control, optional)

e) Bottle 13,14,15– containing test substance, reference compound and test medium (toxicity control, optional)

52. Assuming 9 bottles in a standard run, each filled 250 mL, 2.25 L of test medium is required, equivalent to approximately 2.25 L of TFF processed seawater, which means 225 L of raw seawater. To account for spillage and solution preparations, at least 250 L should be collected and processed with TFF.

PROCedure

53. Assemble the equipment, assure the batteries are full, start the stirrer, check that the equipment is air-tight, and start the measurement of oxygen uptake. Usually no further attention is required other than taking the necessary readings and making daily checks to see that the correct temperature and adequate stirring are maintained.

54. When an automatic respirometer is used, a continuous record of oxygen uptake is obtained. For non-automatic respirometers daily readings will be adequate.

55. Calculate the oxygen uptake from the readings taken at regular and frequent intervals, using the methods given by the manufacturer of the equipment.

56. If required, withdraw samples from the respirometer flasks, initially and at the end of the experiment, for analysis of DOC and/or specific chemical (see OECD 301F, annex IV.4). At the initial withdrawal, ensure that the volume of test suspension remaining in the flask is known.

57. At test termination, record test media temperatures in all respirometer bottles. With the incubator temperature within ± 1°C of the set temperature, the media temperature after 60 days should not alter more than ± 2°C. In the ECO11 ring test, temperature increases >+ 2°C were observed after 120 days, probably caused by residual heat from the stirring motion in the respirometer bottles or from the stirring platform on which they sit.[16] To mitigate such variation, the use of water baths instead of incubators, reducing stirrer speed, or incubation temperature may help.

Data and reporting

Treatment of results

58. Data should be entered onto the attached data sheet (Annex 1).

59. First, calculate the BOD exerted after each time period by subtracting the oxygen depletion of the blank (mg O_2 L^{-1}) from that exhibited by the test substance (mg O_2 L^{-1}). Divide this corrected depletion by the concentration of the test substance (mg L^{-1}), to obtain the specific BOD as mg oxygen per mg test substance. Calculate the percentage biodegradation by dividing the specific BOD by the specific ThOD (calculated according to OECD 306, Annex 3) or COD (determined by analysis, see OECD 301F, Annex IV.3, OECD 301D). Thus:

$$BOD\ (mg\ O_2/mg\ test\ substance) = \frac{mg\ O_2/L\ uptake\ by\ test\ substance - mg\ O_2/L\ uptake\ by\ blank}{mg\ test\ substance/L\ in\ vessel}$$

$$\%\ biodegradation = \frac{BOD\ (mg\ O_2/mg\ test\ substance)}{ThOD\ (mg\ O_2/mg\ test\ substance)} \times 100$$

60. It should be noted that these two methods do not necessarily give the same value; it is preferable to use the ThOD, since some chemicals are not fully oxidised in the COD test.

61. For test substances containing nitrogen, use the appropriate ThOD (NH_4 or NO_3) according to what is known or expected about the occurrence of nitrification (OECD 306, Annex 3). If nitrification occurs but is not complete, calculate a correction for the oxygen consumed by nitrification from the changes in concentration of nitrite and nitrate during the 60 days of the test (OECD 301F, Annex V). Test laboratories should also provide a rationale for their correction (i.e. measured nitrate levels) and present all data and calculations used, assumptions and calculations for full nitrification in addition to partial nitrification.

62. Illustrate the course of the degradation test graphically in a diagram (Figure 3).

63. Calculate the time to reach 50% degradation, t_{50} (this descriptor is different to the t_{50} descriptor mentioned in the OECD 306 – see below), time to reach 10% degradation, i.e. lag time, t_L, and $dt_{50} = t_{50} - t_L$ (this descriptor is equivalent to t_{50} as mentioned in OECD 306) based on visual assessment of the biodegradation curve (Figure 3). The distinction between t_{50} and dt_{50} is made to assess the effect of lag phases.

64. Refer to relevant regulations for pass threshold (% biodegradation in x days).

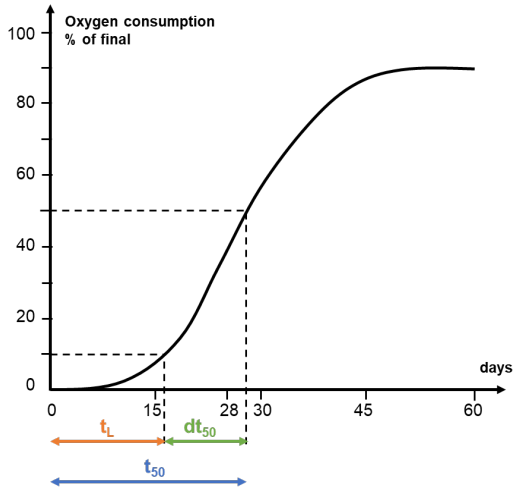


Figure 3: Example to determine values of t_L, dt_{50} and t_{50}.

Test report

65. The test report must contain the information as described in Annex 1 including all raw data and individual replicate data.

Validity of results

66. The oxygen uptake of the blank is normally 20-30 mg O_2/ L and should not be greater than 60 mg O_2/ L in 28 days. Values higher than 60 mg/ L require critical examination of the data and experimental technique.

67. The possibility that nitrogen-containing compounds may affect the results should be considered.

68. Results obtained with the reference compound should be comparable to the results obtained in the ring test (see paragraph 20). If results obtained with reference compounds are atypical, the test should be repeated using another seawater sample.

69. The test substances can be considered to be inhibitory to bacteria (at the concentration used) if the BOD of the mixture of reference and test substances is less than the sum of the BOD of the separate solutions of the two substances.

Abbreviations

BOD:	biological oxygen demand
BST:	biodegradation screening test
COD:	chemical oxygen demand
DOC:	dissolved organic carbon
dt_{50}:	t_{50} - t_L
MaP test:	marine biodegradation screening test for chemical persistence assessment
NWP:	normalized water permeability
t_{50}:	time to reach 50% degradation
t_L:	time to reach 10% degradation, lag phase
TFF:	tangential flow filtration
ThOD:	theoretical oxygen demand

Literature

1. ECETOC. *Workshop on Biodegradation and Persistence No. 10. Workshop Report* (2007).
2. Gartiser, S., Schneider, K., Schwarz, M. A. & Junker, T. *Assessment of environmental persistence: regulatory requirements and practical possibilities - available test systems, indentification of technical constraints and indication of possible solutions.* (2017).
3. ECHA. *Guidance on information requirements and chemical safety assessment, chapter R . 7b : endpoint specific guidance (version 4.0)*. (2017). doi:10.2823/84188
4. Gerike, P. & Fischer, W. K. A Correlation Study of Biodegradability Determinations with Various Chemicals in Various Tests. *Ecotoxicol. Environ. Saf.* **3**, 159–173 (1979).
5. Martin, T. J. *et al.* Environmentally Relevant Inoculum Concentrations Improve the Reliability of Persistent Assessments in Biodegradation Screening Tests. *Environ. Sci. Technol.* **51**, 3065–3073 (2017).
6. Nyholm, N., Lindgaard-Jørgensen, P. & Hansen, N. Biodegradation of 4-nitrophenol in standardized aquatic degradation tests. *Ecotoxicol. Environ. Saf.* **8**, 451–470 (1984).
7. Painter, H. A. Detailed review paper on biodegradability testing. *OECD Environ. Monogr.* **98**, 1–162 (1995).
8. Thouand, G. *et al.* Bacterial inoculum densitiy and probability of para-nitrophenol biodegradability test response. *Ecotoxicol. Environ. Saf.* **30**, 274–282 (1995).
9. Ingerslev, F., Torang, L. & Nyholm, N. Importance of the Test Volume on the Lag Phase in Biodegradation Studies. *Environ. Toxicol. Chem.* **19**, 2443–2447 (2000).
10. Goodhead, A. K., Head, I. M., Snape, J. R. & Davenport, R. J. Standard inocula preparations reduce the bacterial diversity and reliability of regulatory biodegradation tests. *Environ. Sci. Pollut. Res.* **21**, 9511–9521 (2014).
11. Ott, A. *et al.* Improving the biodegradability in seawater test (OECD 306). *Sci. Total Environ.* **666**, 399–404 (2019).
12. Ott, A., Martin, T. J., Snape, J. R. & Davenport, R. J. Increased cell numbers improve marine biodegradation tests for persistence assessment. *Sci. Total Environ.* **706**, 135621 (2019).
13. OECD. *OECD guideline for testing of chemicals (301): ready biodegradability*. *OECD Guideline for testing of chemicals* (1992).
14. OECD. *OECD guideline for testing of chemicals (306): biodegradability in seawater*. *OECD Guideline for testing of chemicals* (1992). doi:http://dx.doi.org/10.1787/9789264070486-en
15. CEFIC-LRi. ECO11-UNEW: towards rationally designed hazard, risk and persistency assessment: putting the 'bio' back into biodegradability tests. (2019). Available at: http://cefic-lri.org/projects/eco11-towards-rationally-designed-hazard-risk-and-persistency-assessment-putting-the-bio-back-into-biodegradability-tests/. (Accessed: 17th July 2019)
16. Ott, A. *et al.* Multi-laboratory validation of a new marine biodegradation test for chemical persistence assessment - manuscript submitted for publication. (2020).
17. OECD. *OECD 34 Guidance document on the validation and international acceptance of new or updated test methods for hazard assessment*. **34**, (2005).
18. Martin, T. J., Goodhead, A. K., Snape, J. R. & Davenport, R. J. Improving the ecological relevance of aquatic bacterial communities in biodegradability screening assessments. *Sci. Total Environ.* **627**, 1552–1559 (2018).
19. ECETOC. Persistence of Chemicals in the Environment. ECETOC technical report. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels. (2003).
20. ISO. *ISO 10634:1995. water quality; guidance for the preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium*. (1995).
21. Birch, H., Hammershøj, R., Comber, M. & Mayer, P. Biodegradation of hydrocarbon mixtures in surface waters at environmentally relevant levels - Effect of inoculum origin on kinetics and sequence of degradation. *Chemosphere* **184**, 400–407 (2017).
22. Brown, D. M., Hughes, C. B., Spence, M., Bonte, M. & Whale, G. Assessing the suitability of a manometric test system for determining the biodegradability of volatile hydrocarbons. *Chemosphere* **195**, 381–389 (2018).
23. OSPAR. *Guidelines for completing the harmonised offshore chemical notification format (HOCNF) (OSPAR agreement: 2012/05. Update 2015)*. (2015).
24. ECHA. *Guidance on information requirements and chemical safety assessment, chapter R.11: PBT/vPvB assessment (version 3.0)*. (2017). doi:10.2823/128621
25. WHO. *Heteorotrophic Plate Counts and Drinking-water Safety*. (IWA Publishing, 2003). doi:10.7717/peerj.1259
26. Gensberger, E. T., Gössl, E.-M., Antonielli, L., Sessitsch, A. & Kostić, T. Effect of different heterotrophic plate count methods on the estimation of the composition of the culturable microbial community. *PeerJ* **3**, e862 (2015).
27. Millipore. *Protein Concentration and Diafiltration by Tangential Flow Filtration*. *Technical Brief* (2003).

ANNEX 1: Data sheet

1. Laboratory:
2. Date at start of test:
3. Test substance
   - Name:
   - Stock solution concentration: mg/ L
   - Initial concentration in seawater, C_0: mg/ L
   - Volume in test bottle (V): mL
   - ThOD or COD: mg O_2/ mg test substance (NH_4, NO_3)
4. Seawater
   - Source:
   - Date of collection:
   - Depth of collection: m
   - Appearance at time of collection (e.g. turbid, etc.):
   - Salinity at collection: ‰
   - Temperature at collection: ^oC
5. Seawater pre-treatment
   - 10 µm filter material:
   - Microbial cell count (method)
     - After 10 µm pre-filtration: cells/ mL
     - After TFF pre-treatment: cells/ mL
     - Concentration factor TFF:
   - TFF system with filter material and filter surface:
   - Start date TFF process:
   - TFF process time: h
   - Start and end volume of seawater L
6. Test medium
   - Temperature after aeration: ^oC
   - O_2 concentration after aeration and standing before start of test: mg O_2/ L
7. O_2 uptake, biodegradability
   - Type of respirometer:
   - Temperature test medium in bottles after 60 days: ^oC
   - t_L, dt_{50}, t_{50} values for each test chemical replicate, mean and standard deviation

		Time (days)				
		n_1	n_2	n_3	n_4	n_x
O_2 uptake by test chemical (mg)	a_1 a_2 a_3					
O_2 uptake by blank (mg)	b_1 b_2 b_3 $b_m mean$					
Corrected O_2 uptake (mg)	$a_1 - b_m$ $a_2 - b_m$ $a_3 - b_m$					
BOD (mg O_2/ mg test substance)	$\frac{a_1 - b_m}{C_0 V}$ $\frac{a_2 - b_m}{C_0 V}$ $\frac{a_3 - b_m}{C_0 V}$					
% degradation D $\frac{BOD}{ThOD} \times 100$	$D_{1(a1)}$ $D_{2(a2)}$ $D_{3(a3)}$ $mean^*$					

*D_1, D_2 and D_3 should not be averaged if there is a considerable difference.

Note: Similar formats may be used for the reference compound and toxicity control.
For correction for nitrification, carbon analysis, specific chemical analysis and abiotic degradation see OECD 301F data sheets.