Applicability of Analytical Tools, Test Methods and Models for Polymer Risk Assessment

Technical Report No. 133-2

EUROPEAN CENTRE FOR ECOTOXICOLOGY AND TOXICOLOGY OF CHEMICALS

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DISCLAIMER: This Technical Report reflects current experience and knowledge and shall be adapted, amended and refined as new evidence on polymer risk assessment becomes available.

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European Centre for Ecotoxicology and Toxicology of Chemicals

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SUMMARY

The first part of the ECETOC TR No. 133 series presented the *Conceptual Framework for Polymer Risk Assessment* (CF4Polymers; ECETOC TR No. 133-1; ECETOC, 2019) providing basic guiding principles to be considered in assessing potential ecological and human health hazards and risks posed by polymer products to facilitate consistency.

This second part, i.e. ECETOC TR No. 133-2, further advances the topic of polymer hazard and risk assessment by providing a detailed review of the applicability of standard analytical tools, *in vitro* and *in vivo* test methods and *in silico* models to assess the physico-chemical, fate, exposure-related, ecotoxicological, and toxicological properties of polymers; complemented by an extensive Glossary providing definitions for all key terms.

The test methods and parameters that are further assessed for their relevance in hazard and/or risk assessment, in line with the recommendations of the CF4Polymers, include:

- Analytical tools to assess physico-chemical properties with a focus on weight-average and number-average molecular weight, acid dissociation constant, n-octanol/water and organic carbon/water partition coefficients, solubility in water or fat, surface tension; further discussing approaches for within-study exposure verification, i.e. analytical measurements of polymers in environmental matrices;
- Test methods addressing the environmental fate properties of biodegradation, abiotic degradation and bioaccumulation/bioconcentration;
- Environmental exposure modelling addressing the compartments of soil, wastewater treatment plants, freshwater and freshwater sediment, and marine surface water and sediment;
- Human health exposure modelling addressing both occupational (industrial and professional) and consumer exposure;
- Ecotoxicological assessments using aquatic organisms, sediment-dwelling organisms, and terrestrial organisms (in-soil organisms, and organisms living above the ground; and plants);
- Human health hazard assessments.

Each section of this report presents a comprehensive overview of available tools, test methods and/or models, preferably referring to formally agreed test guidelines (TGs), such as the ones adopted by the Organisation for Economic Co-operation and Development or by the US Environmental Protection Agency Office for Chemical Safety and Pollution Prevention. Since these TGs were mostly developed for mono-constituent, water-soluble substances, they may have technical limitations when assessing e.g. poorly soluble or solid polymers. Therefore, the report also considers standards and technical specifications published by the International Standardisation Organisation or national/regional standardisation bodies since these were developed specifically for the testing of plastics and as such are generally also applicable to solid, particulate polymers.

For each parameter, scientific evidence is presented and discussed on the applicability of the corresponding tools, test methods and/or models, while highlighting their technical limitations for the assessment of specific types of polymers. Such technical limitations may depend e.g. on polymer size, molecular weight / molecular weight distribution, solubility, or charge density, and they may also relate to the complexity of the polymer products. Polymer products may consist of (mostly high molecular weight) polymeric macromolecules as main

components, and (lower molecular weight) non-intentionally added substances and sometimes intentionally added substances as other components. The different components of the polymer product may exhibit different properties, so that it may be more appropriate to express measurements as ranges instead of unique values.

Suggestions are made as to how specific TGs might be adapted to facilitate the testing of polymers. Further, evidence from the scientific literature for how specific types of polymers have been submitted to the respective tools, test methods and models is presented. However, for many tools, test methods and models, the published evidence on their use for the assessment of polymers is scarce, or even absent. Due to the paucity of information specifically on polymers *per se*, publications on the testing of microplastics, as polymer-based materials, are also referred to in this report since they may provide insight into how specific tools, methods and models are applied for the assessment of polymers in general. In this regard, however, the influence of the polymer matrix upon bioavailability of chemicals present within the polymer product should not be underestimated. Since this is a rapidly evolving area of science, studies addressing the testing of microplastics may provide further insight into the development or adaptation of test methods also for the assessment of polymers.

The detailed review of the applicability of standard analytical tools, *in vitro* and *in vivo* test methods and *in silico* models underlines that fit-for-purpose identification of the polymer product ('as produced' or during different life-cycle stages, as relevant) and/or of specific fractions or constituents is a prerequisite for all assessments of fate, exposure, ecotoxicity and toxicity potential. Such fit-for-purpose polymer identification is pivotal to determine whether a specific parameter will be relevant for hazard, exposure and risk assessment and to establish the applicability of the given test method (or needs for adaptation). Further, the review shows that specific physico-chemical properties that are key parameters for the hazard, exposure and risk assessment of mono-constituent, soluble substances (such as the partition coefficients) may not be relevant for e.g. solid, particulate polymers, for which other properties (such as size and charge density) will be decisive.

Importantly, the ECETOC TR No. 133-2 review of the applicability of tools, test methods and models for polymer hazard and risk assessment should not be misunderstood as a check list for a 'tick-box approach' to hazard and risk assessment. Therefore, this report also includes general guidance on how to identify if a specific test method might be relevant for specific types of polymers and on how relevant test methods might be structured in a tiered approach. Specifically, three conceptual frameworks for polymer (bio)degradation, bioaccumulation and ecotoxicity assessment, respectively, are presented. These three conceptual frameworks complement the ECETOC TR No. 133-1 by 'zooming into' specific steps of the CF4Polymers to show how (bio)degradation, bioaccumulation and ecotoxicity might be addressed within the CF4Polymers. (The CF4Polymers already provided a similar 'zoom in' for human health hazard assessment, so that this approach is referred to in the present report.)

To avoid generating data of questionable scientific relevance thereby resulting in misleading risk characterisation, test methods should preferably not be performed if their technical limitations for the assessment of the given type of polymer cannot be addressed. If a test is performed in spite of recognised technical and/or scientific limitations (e.g. to meet specific legal requirements), these limitations should be clearly described and the relevance and reliability of the test results established (in terms of real-world exposure scenarios). Therefore, the three conceptual frameworks for polymer (bio)degradation, bioaccumulation and ecotoxicity assessment include the following general decision tree to determine if a specific test method is applicable for the given type of polymer, **once the general need to generate data on**

this specific endpoint has been identified (e.g. based upon specific physico-chemical properties indicating likely bioavailability of the polymer):

1. Would the findings from this tool / method add knowledge that would be relevant for risk assessment?
    a. **Yes / Maybe**: Continue to question No. 2
    b. **No**: Test should not be performed
2. Is it physically / technically possible to perform the test following the formal, TG-conforming protocol?
    a. **Yes**: Proceed with testing
    b. **No / Don't know**: Continue to question No. 3
3. Can the testing protocol be adapted to enable testing of the given type of polymer (e.g. by adapting testing conditions and/or duration; or by selection of a specific approach for test item preparation that does not change key properties of the polymer)?
    a. **Yes**: Proceed with testing; clearly describe amendments to standard test protocol and provide justification for why adaptation does not change key parameters of the polymer of interest
    b. **No / Don't know**: Performing the test runs the risk of yielding data that are of questionable scientific relevance thereby resulting in misleading risk characterisation. Therefore, the test should preferably not be performed. If, however, it is performed in spite of these limitations (e.g. in order to meet specific legal requirements), the technical and scientific limitations should be clearly described and the relevance and reliability of the test results established (in terms of real-world exposure scenarios)

The suggested conceptual frameworks for polymer (bio)degradation, bioaccumulation, and ecotoxicity assessment are intended to ensure that only such information is collated (via the proposed TGs) that will be relevant for the hazard and risk assessment of the polymer of interest. They have been drawn up following the state-of-the-art assessment in view of streamlining efforts and expenditure, and (for bioaccumulation and ecotoxicity testing) in view of reducing vertebrate animal testing needs as far as possible and whenever appropriate. Notably, while the three conceptual frameworks provide general outlines for how to address the respective parameters, this does not imply that all of their steps are already in place for all types of polymers. Instead, the conceptual frameworks should be considered 'living' proposals and they are by no means considered prescriptive. Individual test methods (and their order of sequence) should be selected on a case-by-case basis as relevant for the polymer of interest (and depending on the applicable legislative framework).

Generally, while the hazard, exposure and risk assessment and in this regard also the grouping of many polymers is more complex than that of many mono-constituent substances, both the CF4Polymers and the present report show that following a transparent, structured approach will enable more reliable hazard, exposure and risk assessment thereby contributing to the safe use of polymers. Indeed, many polymers can be reasonably assumed not to pose environmental or human health concerns, as also accepted by internationally applied concepts for 'polymer of low concern' (PLC). Nevertheless, knowledge gaps prevail in the hazard and exposure assessment for polymers. These gaps range from the lack of specific methods for the quantification of specific parameters to issues related to test item preparation and opportunities to adapt existing testing protocols. Given the versatility and complexity of polymer products, a variety of different approaches are required to handle different types of polymers and to understand their environmental relevance throughout their life cycle (depending on solubility, charge density, molecular weight / molecular weight distribution, etc.).

Further research is needed to extend the understanding of the applicability of the available tools, methods and models for polymers hazard and exposure assessment. Such future research work might aim at advancing

current tools, methods and models in view of their applicability for specific types of polymers, or to develop new tools, methods, or models for the given purpose, as necessary. Such research work should also aim at streamlining the expenditure for polymer hazard, exposure and risk assessment. The present report refers to ongoing research work conducted within relevant research programmes of the European Chemical Industry Council Long-range Research Initiative (Cefic LRI). The outcomes of these research programmes should be followed to identify opportunities to update the state-of-the-art applicability of tools, methods and/or models for polymers hazard, exposure and risk assessment.

Further, opportunities to address knowledge gaps by future research work have been identified in the present report. ECETOC has mandated an *ad-hoc* committee to follow up and report upon developments and evolution in the knowledge, testing and risk assessment related to polymers. It is intended that ECETOC will proactively and periodically update the Technical Report No. 133 series to keep abreast of the state-of-the-art within this domain.

In the ECETOC TR No. 133-1, five recommendations were spelled out for how to advance the scientific knowledge base underlying polymer hazard, exposure and risk assessment. These five recommendations are revisited below to incorporate the findings from the ECETOC TR No. 133-2:

ECETOC TR No. 133 Series Recommendation 1: *Identify sets of structural and/or morphological descriptors as well as physico-chemical and fate properties that are key parameters for different types of polymer products*.

ECETOC TR No. 133-2 provides further details on research that is merited to identify which specific properties are relevant key parameters for fit-for-purpose identification and grouping of specific types of polymers. Specific key parameters might generally be relevant across different types of polymers, or they might be unique to specific types of polymers. Knowledge on such key parameters will also facilitate the identification of data needs during exposure and hazard assessment. Please refer to the respective sections for details on such further research needs (formatted as 'blue boxes' in the endpoint-specific sections).

ECETOC TR No. 133 Series Recommendation 2: *Consider prevailing technical limitations of available tools, test methods and models for polymer risk assessment*.

ECETOC TR No. 133-2 provides details on the specific technical limitations that currently impair the applicability of specific methods, tools and models for polymer risk assessment. It also indicates further research that is merited to contribute to overcoming such technical limitations when assessing polymers and to permit standardisation of testing. Please refer to the respective sections for details.

ECETOC TR No. 133 Series Recommendation 3: *Maintain the CF4Polymers as a 'living', flexible framework*, *and review and update it in line with emerging knowledge on how it can efficiently and effectively support polymer risk assessment.*

ECETOC TR No. 133-2 complements the ECETOC TR No. 133-1 by providing further evidence to support the general outline of the CF4Polymers and more detailed guidance on how to pass through its successive steps (specifically: CF4Polymers Step 2: Polymer identification; Step 5: Determination of exposure scenarios; Step 6: Exposure characterisation; Step 7: Hazard assessment). Importantly, the information evaluated for the present report did not indicate any current requirement to amend the CF4Polymers.

ECETOC TR No. 133 Series Recommendation 4: *Expand the knowledge base to (1) substantiate the PLC concept and (2) to identify under which conditions the presence of specific structural alerts or physico-chemical properties poses environmental or human health hazard concerns*. Particularly, there is only weak evidence that anionic or amphoteric and water absorbing polymers might generally have a relevant hazard potential.

The information collated in ECETOC TR No. 133-2 supports this goal in an indirect manner by showing how specific parameters related to the PLC concept can be measured. While the PLC concept has been implemented in different non-EU jurisdictions for many years, or even decades, without indications to disprove its validity, the recommended research work shall serve to eventually extend the criteria, if sufficient experimental justification becomes available. For example, there is some evidence indicating that the current thresholds for relative fractions of oligomers in the PLC concept might be too conservative (ECETOC, 2019).

It is expected that the planned case studies, which shall be published in ECETOC TR No. 133-3, will serve to advance the evidence collated in ECETOC TR No. 133-1 and 133-2 that is relevant to advancing Recommendation 4. Further, it is expected that the planned case studies shall serve to enhance an understanding on the opportunities to group polymers by common physical, chemical and/or biological properties.

ECETOC TR No. 133 Series Recommendation 5: *Develop environmentally relevant models, methods and/or criteria to assess (bio)degradation to improve the reliability of exposure and fate assessments important to the risk assessment of polymers*.

ECETOC TR No. 133-2 provides further details on the specific types models and/or criteria to assess (bio)degradation (or other key parameters for polymer risk assessment) taking into account the type of (bio)degradation, its duration (i.e. half-lives), and whether it is intended during the given life cycle stage of the polymer, or not.

While the CF4Polymers provides basic guiding principles to be considered in assessing potential ecological and human health hazards and risks posed by polymer products, the TR No. 133-2 has been developed as a primer to ignite a closer look at existing methods, tools and models and their relevance to the hazard, exposure and risk assessment of polymers. To complement the CF4Polymers (ECETOC TR No. 133-1) and the present review (ECETOC TR No. 133-2), a selection of case studies is planned as ECETOC TR No. 133-3. These case studies shall address different components of polymer hazard and risk assessment (including grouping) to put the CF4Polymers into practice and to further substantiate the need to address specific physico-chemical, ecological and/or human health-related parameters for polymer risk assessment. Further, the case studies shall serve to enhance the understanding on the applicability and/or technical limitations of the corresponding methods, tools and models. It is expected that these case studies will not only serve to identify opportunities and/or the need to refine specific parts of the CF4Polymers, but that they will also provide important insight to advance the understanding on the applicability of standardised methods, tools and models for polymer hazard, exposure and risk assessment. Following such new insight, the *ad-hoc* committee mandated by ECETOC to follow up and report upon developments and evolution in the knowledge, testing and risk assessment related to polymers will proactively initiate an update to the Technical Report No. 133 series to keep abreast of the state-of-the-art within this domain.

1. INTRODUCTION

This review of the applicability of standard analytical tools, *in vitro* and *in vivo* test methods and *in silico* models for polymer risk assessment is the second part of the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) Technical Report (TR) No. 133 series prepared by the ECETOC Polymers Task Force (TF).

The first part, ECETOC TR No. 133-1, presents the *Conceptual Framework for Polymer Risk Assessment* (CF4Polymers; ECETOC, 2019) providing basic guiding principles to be considered in assessing potential ecological and human health hazards and risks posed by polymer products to facilitate consistency. The CF4Polymers is based upon a review of the state-of-the-art polymer grouping and risk assessment. Within the CF4Polymers, deviations from the internationally agreed paradigm for chemical risk assessment, as published by the World Health Organisation – International Programme for Chemical Safety (WHO IPCS, 2004, 2010), are necessitated by the chemical and physical attributes of polymeric substances and polymer products and their complex markets and uses. Generally, polymers are not present as mono-constituent substances, but as complex polymer products consisting of the polymeric substance (polymeric macromolecules), intentionally added substances (IAS; e.g. additives, stabilisers) and non-intentionally added substances (NIAS; e.g. impurities) (Box 1; see also Glossary for definitions of all key terms). Further, polymer products can change their form during different life cycle stages.

Box 1: Polymer product and its components

Polymer product: A chemical product with a polymeric substance as main component, and NIAS and sometimes IAS as other components (ECETOC Polymers TF working definition*). Polymer products are only in some cases finished articles.

Polymeric substance (polymeric macromolecules): The chemical (co)polymer and possibly present oligomers (both are composed of the same monomeric units) (ECETOC Polymers TF working definition*).

Oligomer: Part of the polymeric substance (at the low end of its molecular weight range). In some contexts, also referred to as NIAS.

Intentionally added substance (IAS): *"A substance added to something in small quantities to improve or preserve it."* (https://en.oxforddictionaries.com/definition/additive); *"A substance which is intentionally added to plastics to achieve a physical or chemical effect during processing of the plastic or in the final material or article; it is intended to be present in the final material or article"* (European Commission, 2011).

Low molecular weight (LMW) compounds: Small oligomers, IAS, and NIAS, including unreacted monomers.

Non-intentionally added substance (NIAS): *"An impurity in the substances used or a reaction intermediate formed during the production process or a decomposition or reaction product"* (European Commission, 2011).

Monomer, unreacted: Depending on the manufacturing process and intended use of the polymer product, unreacted monomers can either be IAS or NIAS.

* See Section 2 in ECETOC TR No. 133-1 (CF4Polymers; ECETOC, 2019) for further discussion, e.g., on how the ECETOC Polymers TF working definitions were selected to comply with current chemical and products legislation.

The CF4Polymers consists of eight steps, i.e. (1) problem formulation - risk assessment scope and protection goal definition; (2) polymer identification; (3) polymer component strategy; (4) grouping approach evaluation; (5) determination of exposure scenarios – the first part of exposure assessment; (6) exposure characterisation – the second part of exposure assessment; (7) hazard assessment – hazard identification and characterisation; and (8) risk characterisation. The CF4Polymers has been designed flexibly and it is not prescriptive. The order of the eight steps can be adapted as necessary depending on the risk assessment needs and/or on data

availability. For each step of the CF4Polymers, the ECETOC TR No. 133-1 provides a detailed outline for how it can be completed, accompanied by explanatory notes and illustrative examples (ECETOC, 2019).

Further, for each step of the CF4Polymers, knowledge gaps are identified in the ECETOC TR No. 133-1. Many of the identified knowledge gaps relate to the applicability of standard analytical tools, test methods and *in silico* models for polymer hazard and risk assessment. A broad spectrum of such tools, methods and models are available to assess the physico-chemical, fate, ecotoxicological, toxicological and exposure-related properties of (non-polymeric) substances. However, these tools, methods, and models may have technical limitations restricting their applicability domain for assessing (specific types of) polymers. In the CF4Polymers, these technical limitations are only generally addressed, indicating that, for the time being, expert knowledge is required to select or adapt the most appropriate tool, method or model to assess a given parameter and to address potential technical limitations of the selected approach (ECETOC, 2019).

The present ECETOC TR No. 133-2 aims at advancing this topic. It encompasses a detailed review of the applicability of standard analytical tools, *in vitro* and *in vivo* test methods and *in silico* models to assess the physico-chemical, fate, ecotoxicological, toxicological and exposure-related properties of polymers. By default, it is assumed that the polymer product is submitted to the assessment, i.e. the chemical product with the polymeric macromolecules as main components, and NIAS and sometimes IAS as other components.

Generally, the ECETOC Polymers TF excluded considerations on nano-sized polymers and environmental pollution by plastics or microplastics from the scope of its work (see ECETOC TR No. 133-1 for further details). Nevertheless, due to the paucity of information specifically on polymers *per se* (and since macro- and microplastics are polymer-based materials), publications on the testing of microplastics (and in a few instances also on the testing of nano-sized polymers) are also referred to in this report since they may provide insight into how specific test methods are applied for the assessment of polymers in general. In this regard, however, the influence of the polymer matrix upon bioavailability of chemicals present within the polymer product should not be underestimated. Since this is a rapidly evolving area of science with initiatives such as the ECETOC Microplastics Scientific Platform, studies addressing the testing of microplastics (or nano-sized polymers) may provide further insight into the development or adaptation of test methods (also for the assessment of polymers) in the future.

In preparing this review, the ECETOC Polymers TF considered all relevant test guidelines (TGs) and Guidance Documents adopted by the Organisation for Economic Co-operation and Development (OECD). Further, the review includes relevant guidance published by the US Environmental Protection Agency (US EPA) Office of Chemical Safety and Pollution Prevention (OCSPP)/Office of Prevention, Pesticides and Toxic Substances (OPPTS) as well as standards and technical specifications from the International Standardisation Organisation (ISO) or regional/national standards institutes, such as the European Committee for Standardisation (CEN) or the American Society for Testing and Materials (ASTM), as applicable.

The review has a focus on identifying those tools, methods and models that are suitable for the assessment of specific types of polymer products and on identifying potential restrictions in the applicability domain or other technical limitations impairing the applicability of specific tools, methods and models for the assessment of specific types of polymer products. In addition, outlines, or conceptual frameworks, are provided for how the available methods might be integrated in tiered approaches for the assessment of polymer (bio)degradation, bioaccumulation, and ecotoxicity potential. These three conceptual frameworks complement the ECETOC TR No. 133-1 CF4Polymers by 'zooming into' specific steps of the CF4Polymers to show how parameters related to (bio)degradation, bioaccumulation and ecotoxicity might be addressed

within the CF4Polymers. (By contrast for human toxicity assessment, a tiered approach has already been described in the CF4Polymers so that it is referred to in the present report.) The conceptual frameworks for polymer bioaccumulation and ecotoxicity assessment aim at identifying those polymers for which the respective endpoint is relevant and then determining which specific test methods are both necessary for hazard and risk assessment and applicable for the given type of polymer. This serves to streamline the testing so that only such information is gathered that is necessary to ensure the safe use of polymers. While testing for (bio)degradation does not include *in vivo* studies, the tiered approaches described in the conceptual frameworks for polymer bioaccumulation and ecotoxicity assessment also serve the mandate to replace, reduce, and refine animal testing (Russell and Burch, 1959) that has been implemented in *Directive 63/2010/EU on the protection of animals used for scientific purposes* (EP and Council, 2010), in the *US Federal Collaboration Toxicity Testing in the 21st Century* (Choudhuri et al., 2018), and in the OECD Testing of Chemicals Programme (http://www.oecd.org/chemicalsafety/testing/animal-welfare.htm).

Both the discussion of applicability of test methods and the conceptual frameworks for polymer (bio)degradation, bioaccumulation and ecotoxicity assessment should be considered together with the overarching outline of the CF4Polymers (ECETOC TR No. 133-1; ECETOC, 2019), and the respective sections from this first part of the TR No. 133 series are cross-referenced in the present report, i.e. the ECETOC TR No. 133-2, as relevant.

To complement the CF4Polymers (ECETOC TR No. 133-1; ECETOC, 2019) and the present review (ECETOC TR No. 133-2), a selection of case studies addressing different components of polymer grouping and risk assessment to put the CF4Polymers into practice is planned as ECETOC TR No. 133-3. It is expected that these case studies will not only serve to identify opportunities and/or the need to refine specific parts of the CF4Polymers, but that they will also provide important insight to advance the understanding on the applicability of standardised tools, methods, and models for polymer hazard and risk assessment.

2. OVERVIEW OF TEST GUIDELINES, STANDARDS AND SPECIFICATIONS INCLUDED IN THIS REPORT

This report on the applicability of standard analytical tools, *in vitro* and *in vivo* test methods and *in silico* models for polymer exposure, hazard and risk assessment encompasses relevant OECD TGs supplemented by TGs issued by the US EPA OCSPP/OPPTS. For some endpoints, potentially relevant non-standard test methods were also considered, even if these have not yet completed validation and/or have not yet gained regulatory acceptance. Since most of the corresponding tools and methods were developed for substances in general, they may have limitations for the assessment of polymers.

By contrast, a number of standards and technical specifications published by the ISO, CEN or ASTM were developed specifically for certain types and/or specific uses of polymers. Therefore, such standards and technical specifications were also included in the present report, and the evaluation of these standards/technical specifications addressed whether the tools and methods described therein might also be applicable to other types of polymers not explicitly included in the scope of the respective document.

Since formal guidance on the performance of analytical tools and test methods is periodically subject to revision as necessitated by emerging knowledge, **the reader is referred to the respective websites listed below as reference to all TGs, standards and technical specifications included in this report to ensure the consideration of the most recent version of the respective document.**

OECD TGs are available at
http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm.

OECD TGs from the following series are included in this report:

- OECD TG 100 series: Physical chemical properties – addressed in Section 3 of this report.
- OECD TG 200 series: Effects on biotic systems – addressed in Section 6.
- OECD TG 300 series: Environmental fate and behaviour – addressed in Section 4.
- OECD TG 400 series: Health effects – addressed in Section 7.

US EPA OCSPP/OPPTS TGs are available at https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances. TGs issued before 22 April 2010 refer to OPPTS, the office name until that date, rather than OCSPP which is the current office name (US EPA, 2019a).

OCSPP/OPPTS TGs from the following series are included in this report:

- 835 series: Fate, transport and transformation TGs – addressed in Section 4 of this report.
- 850 series: Ecological effects TGs – addressed in Section 6.

ISO documents are available at https://www.iso.org/standards.html; CEN documents are available at https://www.cen.eu/Pages/default.aspx; ASTM documents are available at https://www.astm.org.

For ISO and CEN documents, it is further indicated whether they present a standard or a technical specification; https://www.iso.org/deliverables-all.html:

- *"An International Standard provides rules, guidelines or characteristics for activities or for their results, aimed at achieving the optimum degree of order in a given context."*

- *"A Technical Specification addresses work still under technical development, or where it is believed that there will be a future, but not immediate, possibility of agreement on an International Standard."*

3. ANALYTICAL TOOLS TO DETERMINE STRUCTURAL AND MORPHOLOGICAL DESCRIPTORS AND PHYSICO-CHEMICAL PROPERTIES OF POLYMERS

The determination of structural and morphological descriptors and physico-chemical properties of polymers is of utmost importance for fit-for-purpose polymer identification. It also provides information that is relevant for grouping and to identify the need for hazard assessment. Further, knowledge on structural and morphological descriptors and physico-chemical properties of polymers is indispensable for the selection of appropriate ecological or toxicological test methods as well as for their design (e.g. with respect to test substance preparation) and to verify effective concentrations in the studies.

As explained in further detail in the CF4Polymers (ECETOC, 2019), for different types of polymer products, different morphological and structural descriptors and/or physico-chemical properties are expected to be relevant. Depending on the type and life cycle stage of the polymer product under investigation, relevant key parameters may be (no order of properties is inferred):

- Structural descriptors: For example, chemical formula, degree of substitution, tacticity, weight-average molecular weight (M_w), polydispersity, number-average molecular weight (M_n), and reactive functional groups;
- Morphological descriptors: For example, physical state at ambient temperature and pressure (solid, liquid), shape (e.g. spherical, fibre, tubular), physical form (e.g. amorphous, crystalline), particle size if applicable;
- Physico-chemical properties: For example, water solubility, n-octanol/water partition coefficient ((log) K_{ow}), acid dissociation constant (pK_a), net charge (under conditions that are relevant for ecological and human health hazard assessment), zeta potential, vapour pressure, viscosity / melt-flow index / glass transition temperature, density.

A range of internationally (or nationally) agreed TGs is available for measuring the physico-chemical properties of chemicals, and hence potentially also of polymers, e.g., the TGs from the OECD TG 100 series or ISO standards (Table 1). (While this section focuses on OECD TGs, it equally applies to the equivalent TGs in the OCSPP/OPPTS TG series, as available.)

Generally, the nature of a polymer product having multiple components (or in some cases even being *substances of unknown or variable composition, complex reaction products and biological materials* (UVCBs)) drives the determination of all structural and morphological descriptors and physico-chemical properties of polymers. For example, it may be inappropriate or unfeasible to describe the polymer chain length by a specific length, and thus it could be described by chain length distribution with minimum, maximum and mean values. Similarly, measurements for specific physico-chemical parameters will describe the properties of the polymer product as a multi-component substance or mixture while the properties of the single components are subsumed in this final value. If appropriate and needed, the given physico-chemical property of the polymer product (e.g. K_{ow}, pK_a, pH) may be expressed as an approximate range using the minimum and maximum values of the respective property.

Table 1: Examples of test guidelines and standards potentially suitable for the measurement of physico-chemical properties of polymers

Characteristic	**Technique**	**Test guideline / standard**
Morphology and structure (polymer composite)	SEM	ASTM E562, ASTM E766, ASTM E896, ASTM E1245, ASTM, E1268, ASTM F1877—16
Morphology and structure	TEM	ASTM F1877—16
Molecular structure (homo-, co-polymers and nature of repetitive units)	NMR; ^{1}H, ^{13}C, other atoms depending on type of polymer	ASTM E2977—15
Molecular structure of functional group	FTIR	ASTM E168, ASTM E1252
Elemental detection	XRF	ASTM D6247 – 18, ISO/TR 18336
Elemental analysis (metals, impurities, minerals)	ICP-AEC, ICP-OES, furnace AAS	Methods generally ion-specific
Tacticity	Proton or carbon NMR	ASTM E2977—15
M_w, M_n, MWD, polydispersity	GPC / SEC Dilute solution viscosity testing Melt flow index testing	OECD TG 118: GPC: M_n and MWD OECD TG 119: GPC: LMW fraction DIN 55672 - GPC - Part 1: THF as elution solvent [a] ISO 13885 - Binders for paints & varnishes, GPC: Part 1: THF as eluent
Absolute M_w	GPC – MALS (MALS detection)	Presently lacks internationally agreed method
Acid dissociation constant	Titration methods; conductometric methods (see Table 2 for details)	OECD TG 112: Titration method, spectrophotometric method, conductometric method
Octanol-water partitioning	HPLC	OECD TG 107: Shake flask method OECD TG 117: HPLC OECD TG 123: Slow-stirring method
Adsorption/desorption and organic carbon/water partition coefficient	Soil, sediment, sludge HPLC	OECD TG 106: Batch equilibrium method OECD TG 121: HPLC

Table 1 continued

Characteristic	**Technique**	**Test guideline / standard**
Solubility in water	Column elution method Flask method	OECD TG 105 OECD TG 120
Solubility in fat	Stirring flask method	OECD TG 116
Surface tension	See Table 3 for details	OECD TG 115
Glass transition temperature (Tg)	DSC	ASTM D3418 - 15 Standard test method for transition temperatures and enthalpies of fusion and crystallisation of polymers by DSC
Viscoelastic behaviour (Tg, miscibility or composition influence and quality control)	DMA / DMTA	ASTM D4065, D4440, D5279
% weight loss [b]	TGA	ASTM E1131, ISO 11358

Footnote to Table 1:

Abbreviations: AAS: Atomic absorption spectroscopy; DIN: Deutsches Institut für Normung (*German Standards Institute*), DM(T)A: Dynamic mechanical (thermal) analysis; DSC: Differential scanning calorimetry; FTIR: Fourier-transform infrared spectroscopy; GPC: Gel permeation chromatography; ICP-AES: Inductively coupled plasma atomic emission spectroscopy; ICP-OES: Inductively coupled plasma optical emission spectrometry; LMW: Low molecular weight; MALS: Multi-angle light scattering; M_n: Number-average molecular weight; M_w: Weight-average molecular weight; MWD: Molecular weight distribution; NMR: Nuclear magnetic resonance; SEC: Size Exclusion Chromatography; SEM: Scanning electron microscope; TEM: Transmission electron microscopy; TGA: Thermogravimetry thermal analysis; THF: Tetrahydrofuran; XRF: X-ray fluorescence.

[a] https://www.beuth.de/en/standard/din-55672-1/249013706.

Further, in contrast to defined small molecules, the determination of the content of a polymeric substance in a polymer product is challenging due to the dispersity of the polymer chains. Depending on the polymer characteristics, i.e. polymer composition, solubility, molecular weight, and molecular weight distribution (MWD), different approaches can be pursued in order to determine a polymer content. Such methods may include e.g. high-performance liquid chromatography (HPLC) or gas chromatography analysis (potentially coupled to mass spectrometry), gel permeation chromatography (GPC), nuclear magnetic resonance, elemental analysis, or total organic carbon (TOC) measurements. For accurate determination, it is vital to have a suitable comparison of known content which can be used for calibration. In most cases, however, such a standard does not exist. Thus, assumptions have to be made or extensive analytical analyses have to be performed to quantify all present IAS and NIAS to calculate the polymer content as the residual percentage. In the latter cases, the accuracy of the content determination is dependent on the quality of the assumptions or the completeness of impurity quantification, respectively. An estimation on the accuracy will be needed to establish if the employed methods meet the requirements for subsequent toxicological studies (if applicable).

For example, the determination of the polymer content via GPC using the refractive index detector would be an ideal procedure to determine the content of a soluble polymeric substance in a polymer product as it can be conducted in a standard GPC measurement with known initial concentration. However, in the absence of a suitable standard, the refractive index increment of the polymer in the used solvent has to be estimated. The typical error in accuracy will be around 5-10%.

To minimise costly analytical efforts, the requirement of accuracy should be determined by the (eco)toxicological facility upfront (if such testing is relevant e.g. depending on the outcome of the analytical assessment). Once the content is determined to a satisfactory accuracy, further concentration controls can be performed more easily using the original polymer product as a reference with known content.

Below, the applicability of specific OECD TGs for the assessment of key parameters of polymers is discussed in further detail, i.e. (Section 3.1) molecular weight; (Section 3.2) acid dissociation constant; (Section 3.3) solubility in water; (Section 3.4) n-octanol/water partition coefficient; (Section 3.5) organic solvent/water partition coefficient; and (Section 3.6) surface tension.

An overview of the corresponding techniques and TGs is provided in Table 1 which also presents structural and morphological descriptors, tacticity, glass transition temperature, viscoelastic behaviour, relative weight loss and charge density (see also Tiwari and Uzun, 2015). Further physico-chemical properties are not included in this section because they are considered to be of less importance for most polymers, e.g. granulometry, zeta potential, vapour pressure, explosivity (of powders, for example), boiling point, flash point. Nevertheless, such parameters might be relevant for fit-for-purpose identification of specific solid or powder polymers, and expert advice should be sought for selecting and applying tools to determine such additional parameters.

These discussions of the applicability of specific analytical tools and TGs to determine specific structural, morphological and physico-chemical parameters are supplemented by (Section 3.7) analytical measurements of polymers in environmental matrices. Therein, options are described for understanding exposure in environmental tests where in-life exposure verifications are generally required. Some of these options for exposure verification (i.e. dose confirmations in the diet or aerosol) are also applicable for mammalian testing.

3.1 Weight-average and number-average molecular weight

The following OECD TGs that specifically relate to polymers describe the determination of M_w, M_n and MWD:

- OECD TG 118: Determination of the M_n and MWD of polymers using GPC; a form of size exclusion chromatography (SEC)
- OECD TG 119: Determination of the low molecular weight (LMW) content of a polymer using GPC

It is impossible to accurately determine the MWD of insoluble polymers using GPC. Therefore, the determination is often restricted to the MWD of the soluble fraction of the polymer. However, the soluble fraction and MWD of a polymer are highly dependent on the chosen analytical tools, the solvent and standards used, and the preparation of the sample. Due to these technical challenges, it may be advisable to use a combination of analytical methods when characterising the MWD of a polymer to describe different aspects of the MWD.

Tools to determine the MWD of soluble polymers include SEC (GPC) where the separation is based on the polymer's hydrodynamic volume. For standard measurements, the detection via differential refractive index or ultra-visible light has been established. GPC is by definition a relative method; therefore, measurements have to be calibrated against standards of known molecular weights. The result of a GPC measurement is complete description of the MWD including the average M_n, M_w and polydispersity (Đ) in equivalents of the standard used for the calibration. The result of the measurement will be as close to the real MWD as suitable calibration standards can be found. However, only a very limited number of standards is available commercially. Therefore, it is generally acknowledged to define standard calibrants for each solvent, e.g. poly(styrene) for tetrahydrofurane, or poly(methyl methacrylate) for N,N-dimethyl acetamide and 1,1,1,3,3,3-hexafluoro-2-propanol. Within limits, the absolute molecular weight can be determined online using light scattering detectors. However, light scattering can only be used for polymers with sufficiently high molecular weight (HMW) or, more precisely, sufficiently large size in solution (> 10 nm). Additionally, the refractive index increment (dn/dc) needs to be known for the polymer in the used solvent. This can be challenging for new polymers where literature data are unavailable.

Kilz and Ehmcke (2004) provide a comprehensive overview of the measurement principles and advantages and limitations of using different light scattering detection techniques, in combination with SEC, to determine the MWD of polymers. In accordance with Kilz and Ehmcke (2004), multi-angle light scattering is the preferred light scattering detector since it provides accurate measurements of the M_w, reliable radius of gyration, long-chain branching and structural information on the polymer of interest, provided that the requirements for a successful light scattering can be met. SEC-light scattering will only work well on samples that are not (partially) crosslinked. If samples are (partially) crosslinked, a very small fraction of particles, even after sample filtration, might be sufficient to significantly bias the results. Since the SEC-light scattering no longer depends on relative standards, it will yield the absolute molar mass distribution. Nevertheless, it does need a fitting for extrapolating the molar mass distribution at the end of its distributions. In consequence, the relative fraction < 1000 Dalton (Da; g/mol), is very hard to reproduce in comparison to conventional calibration.

For polymeric substances with very short chains and LMW, HPLC is an appropriate separation method (that needs to be combined with an appropriate detection method, such as mass spectrometry, to determine the MWD; see Section 3.4 for further details on the application of HPLC for the assessment of polymers).

Finally, for some polymers, a relationship exists between the intrinsic viscosity and the M_w of the crystalline and amorphous phases (Colby et al., 1987). Therefore, viscosity (or the melt flow index) is frequently used as a proxy for M_w to distinguish between different M_w ranges of the same polymer type. For example, a linear polydimethylsiloxane (PDMS) with a viscosity of 100,000 centistokes has a M_n of approximately 74,000 Da (ECETOC, 2011a). Due to this very high M_n, full MWD data for this polymer (or other polymers having similar viscosity ranges) are not relevant for most uses. Notably, such calculations should consider that the measurement of viscosity is never truly straightforward when working across a range of different materials (as may be included in one single polymer product) since viscosity also depends upon the size of the turning spindle used for the measurements.

It is expected that the case studies putting the CF4Polymers into practice, planned as ECETOC TR 133-3, will provide further insight on the applicability and/or technical limitations of analytical tools and TGs to assess the molecular weight of different types of polymers.

3.2 Acid dissociation constant

The following OECD TG describes the determination of dissociation constants:

- OECD TG 112: Dissociation constants in water (distinguishing between titration, spectrophotometric and conductometric procedures)

As described in the OECD TG 112, dissociation is the reversible splitting into two or more chemical species which may be ionic. The dissociation of a substance in water informs on its fate in the environment since it affects the substance's adsorption to soils and sediments as well as its potential for cellular uptake. The acid dissociation constant (K_a or pK_a i.e. the negative base-10 logarithm of the K_a), reflects the tendency of an acid to dissociate (i.e. release a proton) in an aqueous solution (Tay et al., 2016). The dissociation constant may be an important factor in deciding which method or testing conditions should be used to determine the octanol/water partition coefficient (Section 3.4) or the organic carbon/water adsorption coefficient (Section 3.5) (ECHA, 2017a).

Table 2 presents analytical tools to determine the pK_a indicating their suitability to assess (specific types of) polymers. Similar to the determination of molecular weight (Section 3.1), a combination of different techniques (e.g. spectrophotometric, potentiometric and electrophoretic measurements) is recommendable to determine the pK_a for polymers. The selection of techniques should also consider that one single polymeric substance may also have multiple pK_a values depending on the types of functional groups and their position on the polymer chain.

For water-soluble polymers, the pK_a of a given polymer chain in the water phase can be affected by the potential ionisation site that may interact with the surrounding media. Although the potentiometric titration is useful as a quantitative analysis of acid dissociation, acidity cannot be measured directly by aqueous potentiometric titration if the polymer does not dissolve in water. For water-insoluble polymers, spectrophotometric methods (UV–Vis absorption, ^{1}H-nuclear magnetic resonance, Fourier-transform infrared spectroscopy (FTIR), and Raman techniques) could be considered (Tay et al., 2016).

Table 2: Analytical methods potentially suitable to determine the acid dissociation constant of polymers

Analytical method	**Applicability**
Titration methods, spectrophotometric method (e.g. UV spectroscopy)	Generally suitable for the assessment of polymers
Conductometric methods (electrophoresis, capillary electrophoresis)	Generally suitable for the assessment of polymers
Mass spectroscopy	Generally suitable for the assessment of polymers, including complex polymer mixtures
HPLC	Valuable for the separation of weak acids and bases
Calorimetry	Applicable for peptides or similar compounds
Hyper-Rayleigh scattering technique	Only suitable for very specific types of polymers (weak organic acids in protic solvents)
Fluorometric method	Only suitable for very specific types of polymers (soluble arylamines and hydroxy aromatic polymeric substances)
Fluorescence polarisation method	Suitable for peptides and similar compounds, and therefore, possibly also applicable for oligomers or short-chain polymers
Surface plasma resonance	Only applicable for bio-polymers
Nuclear magnetic resonance spectroscopy (protein ligand)	Not suitable for polymers
Solvation model dissociation constant measurement by calculating Gibbs energy change for deprotonation in gas phase	Very complex calculation that is not applicable for polymers

3.3 Solubility in water

One of the most important properties influencing environmental fate of a chemical substance, and exposure in the aquatic environment (or e.g. in aqueous media), is water solubility. The water solubility of a discrete chemical is a well-defined property that represents the maximum dissolved concentration in equilibrium with the pure compound under given conditions. As with other partitioning properties, the solubility of polymer products is more challenging to define because of their multi-component nature. Consequently, the dissolution behaviour of a particular polymeric substance / polymer product will depend on its chemistry, physical state, and MWD. Consequently, some polymeric substances may be highly water soluble (e.g. polyvinyl alcohol), whereas others may be essentially insoluble even if they are of LMW (e.g. PDMS). Further, given the broad range of M_n it can be expected that any given polymer is characterised by a range of water solubilities.

Another possibility is that only certain components or molecular weight fractions of the polymer product may be soluble at concentrations that are relevant from a fate or exposure perspective. In this case, extractability is a term describing the extent to which individual components (e.g. oligomers/homologues or NIAS) or fractions of the non-soluble portion of the polymer product will partition into water. The resulting aqueous concentrations of the components depend on the physical state of the mixture (liquid versus solid) and the nature of their molecular interactions in the mixture and in water. For ideal liquid mixtures (i.e. individual components interact similarly with each other as they do with themselves), the aqueous concentration of a given component will be the Raoult's law product of its water solubility and mole fraction in the mixture, provided that the mixture composition is not altered substantially during equilibration (Banerjee, 1984). For ideal solid mixtures, the expected equilibrium concentration of a given component is identical to its pure

component water solubility. The situation is more complicated for non-ideal mixtures (e.g. those containing both hydrophobic and hydrophilic components and/or those with different ionic strengths, e.g. cationic charges, etc.), especially when the concentration of water in the liquid polymer product (mixture) is substantial or when the aqueous concentration of the hydrophilic components is high. In these cases, the aqueous concentrations of the hydrophobic components of the liquid mixture will be enhanced. For more theoretical details regarding the solubility of organic mixtures in water, the reader is referred to Birch et al. (2019), a comprehensive literature review on water solubility of difficult-to-test substances (and references therein).

Fundamentally, experimental determination of water solubility or extractability involves equilibration of the substance with water and analytical measurement of aqueous component concentrations. There are two OECD TGs describing techniques for the measurement of water solubility, one of which specifically refers to investigation of polymers:

- OECD TG 105: Water solubility
    - Column elution method
    - Flask method
- OECD TG 120: Solution / extraction behaviour of polymers in water

(Further, OECD TG 116 is available for the determination of fat solubility of solid and liquid substances.)

The optimal choices of the TG equilibration technique, and accompanying analytical methods (quantitative and qualitative) for determination of dissolved components, depends on the expected dissolution behaviour of the polymer under investigation, as well as the type and specificity of information required. The standard OECD TG 105 flask method is the method of choice for well-soluble hydrophilic liquid polymers, and it is especially useful if chemical instability in water is a concern. For hydrophobic liquid or solid polymers, the flask method can lead to overestimation of solubility due to formation of microemulsions/microcrystals caused by vigorous agitation, which might be difficult to detect and remove. For this type of polymer, a slow-stirring technique, such as that described in OECD TG 123 for determination of K_{ow}, is a preferred alternative to minimise formation of insoluble particulates. The main disadvantage of the slow-stirring technique is slower equilibration kinetics, which could render it impractical for components having limited stability in water. The OECD TG 105 column elution method may be a suitable alternative for scarcely soluble (< 10 mg/L) polymers, especially solids, provided the polymer can be deposited on a solid support or can be packed in the column in bulk. Use of the column elution method with liquid polymers must be approached cautiously, since they might be displaced from the support as microdroplets, again leading to overestimation of the aqueous phase concentration. If the column elution technique is used for the evaluation of a polymer, the recirculating pump option should be used to promote attainment of equilibrium concentrations in the eluate water.

The OECD TG 120 is a modification of the OECD TG 105 flask method, which describes in detail the determination of the solution/extraction behaviour of solid polymers with a solubility of < 10 g/L in water. While the TG states that the method is not applicable to liquid polymers, such use is advocated by the New Substances Program in Canada to satisfy the requirement for water availability information under the New Substances Notification Regulations (Government of Canada, 2005). A separate Canadian guidance document addresses technical issues related to application of the OECD TG 120 to polymers (Environment Canada, 2009). Although the guidance permits use of low-speed centrifugation and filtration to remove suspended material from the aqueous phase, the formation of stable emulsions is not considered to invalidate use of the method, as this is considered to be part of the water-available fraction in the aquatic environment under the New

Substances Program. The main differences between the OECD TG 105 flask method and OECD TG 120 (inclusive of the additional recommendations in the Canadian guidance) lie in the prescriptive nature of the latter, which specifies precise test conditions related to particle size (solid polymers), sample-to-water ratio, temperature, water pH, stirring rate, and mixing time. Notably, the OECD TG 120 stipulates a 24-hour mixing time, while the OECD TG 105 targets attainment of saturation equilibrium. Clearly, the choice of method to evaluate water solubility/extractability depends on the nature of the polymer itself, as well as the specific considerations associated with use of the results for fate or exposure assessment under particular emission scenarios.

An equally important aspect of the experimental measurement of water solubility/extractability of polymers is the analytical determination of aqueous phase concentrations of dissolved components. The OECD TG 105, which was developed for discrete chemical substances, states a preference for substance-specific analytical methods, in part to avoid errors associated with co-existence of soluble impurities. In contrast, the OECD TG 120 provides more detailed guidance on the types of methods that might be used for analysis of components of the polymer product. Applicable analytical methods can range from determination of non-specific parameters (such as the total weight of extracted components or TOC/elemental analysis) to qualitative and quantitative analysis of individual components or component groups by molecular spectroscopy or chromatographic separations and/or mass spectrometry. In some cases, direct analysis of the water sample may be possible, while in other cases the solubilised components may need to be isolated by solvent extraction. Obviously, the specific details of the sample preparation method and the analytical technique used to identify and/or quantify the water-soluble components or fractions will greatly influence the granularity of the information that can be obtained from the experiment. Considering the breadth of polymer chemistries and properties, the most appropriate analytical strategy must be selected on a case-by-case basis, and should take into account the nature of the polymer as well as the specific concerns and goals associated with a given assessment.

To account for the potentially differing solubilities of different components of a given polymer product, the solubility in water (or fat) can be measured with different loading rates in the respective medium.

It is expected that the case studies putting the CF4Polymers into practice, planned as ECETOC TR 133-3, will provide further insight on the applicability and/or technical limitations of analytical tools and TGs to assess the solubility of different types of polymers in water (or fat).

Future efforts are needed to develop guidance on how the range of water solubilities (and other key physico-chemical parameters) that characterise any given polymer should be used in environmental fate distribution models for risk assessment.

3.4 n-Octanol/water partition coefficient

The n-octanol/water partition coefficient ((log) K_{ow}) describes the equilibrium concentration ratio of a discreet un-ionised chemical substance between n-octanol and water. N-octanol serves as a surrogate for lipid-rich phases in different environmental or biological compartments, facilitating exposure estimation. The K_{ow} also serves as a general indicator of chemical hydrophobicity/lipophilicity, and it is frequently used to predict other properties, such as the organic carbon/water partition coefficient (K_{oc}; Section 3.5), or bioaccumulation (Section 4.2), and also during environmental exposure modelling (Section 5.1).

However, usage of K_{ow} (and/or K_{oc}) values is not universally meaningful for all types of polymers. In the context of polymer risk assessment, the relevance of determining the K_{ow} will depend on the nature of the polymeric substance or polymer product of interest and the assessment objectives. For example, the K_{ow} might not be a necessary or useful parameter for polymer products whose molecular weight exceeds the threshold of concern in terms of systemic bioavailability (Section 4.2.1). Notwithstanding, the K_{ow} may be highly relevant for the LMW compounds (NIAS, IAS, oligomers / monomers) associated with the polymer product. If it is possible to attain pure samples of these LMW compounds or oligomer fractions, it may be preferable and simpler to assess their K_{ow} values independently of the other components. If not, the entire polymer product should be assessed. Typically, the LMW fraction is only present at very low levels, which may pose practical challenges for their identification, extraction and quantification. For cationic polymers especially, simple partitioning between bulk phases, such as described by K_{ow} (and/or K_{oc}) values, do not dominate their fate interactions, but charge and viscosity (molecular weight / MWD and polydispersity) will. Section 4.2.1.2 discusses opportunities and limitations of using the K_{ow} as indicator of polymer bioaccumulation.

Furthermore, while it is possible to derive a single K_{ow} value for a particular MWD of the polymer product, for example by calculating a composition-weighted average using values for individual homologues, it is not clear how such a value alone can be used in polymer risk assessment. The same applies to any other single partitioning value representing the polymer product as a whole (e.g. K_{oc} or n-octanol/air partition coefficient (K_{oa}; see Section 4.2.3.2). Whenever K_{ow} values are reported, it should be considered that the different components of the polymer product may exhibit a wide range of behaviours in the water-octanol system, so that a single value may or may not be sufficient for a given assessment objective. If a 'representative' average value is reported, it should be specified how it was determined (along with minimum and maximum values), and it should be stated clearly what is known about the portion of the product represented by the results, for example, its MWD and content relative to the overall composition.

Three OECD TGs are available for the measurement of the (log) K_{ow}, i.e.

- OECD TG 107: Shake flask method
- OECD TG 117: HPLC method
- OECD TG 123: Slow-stirring method

According to the text of the OECD TG 107 and 123, these test methods should generally only be used for pure substances since the components of (polymer) mixtures can affect each other's solubilities (activity coefficients) in the different phases. In this case, the measured concentration in the respective phase (and hence also the log K_{ow}) is dependent on the original sample weight and hence is arbitrary. As described above, it may be preferable to assess only the LMW components of the polymer product of interest. If the entire polymer product needs to be submitted to the assessment, adaptations to the original test protocols may be necessary to account for mixture interactions, e.g. by operating at the lowest practicable solution / loading. Hence, for many polymer products, it will not be possible to determine the K_{ow} in full compliance with the TGs. In such cases, the TGs should be followed as closely as possible and necessary deviations from the test protocol clearly identified and justified.

Against this background, specific issues to be considered when applying the OECD TG 107, 117 or 123 to polymers are addressed below.

The OECD TG 107 shake flask method and OECD TG 123 slow-stirring method enable the direct measurement of the K_{ow}. Both methods involve equilibration of the test substance in the biphasic octanol/water system with subsequent analytical determination of substance concentrations in each phase. The OECD TG 123 is preferred

over the OECD TG 107 method for substances having expected log K_{ow} values > 4-5, as the OECD TG 107 could produce erroneous results due to formation of microemulsions in the aqueous phase. Generally, both methods are suitable for polymers that are at least partially soluble in octanol or water. The most appropriate TG for the polymer under investigation needs to be determined on a case-by-case basis, paying special attention to the applicability domains of the analytical tools and the limits of detection in water and octanol (approx. 1 mg/L). Taking into account the detection limits, concentrations of the polymer of interest can generally be determined in the water phase via sum parameter TOC. By contrast, it is not possible to determine the TOC in octanol. The analysis of polymer components in octanol is generally more challenging due to complications associated with the physico-chemical properties of octanol and incompatibility with common methods of sample preparation and instrumental analysis. Notably, just as may stand true for non-polymeric substances, the experimental K_{ow}, or any other sorption coefficient, of a mixture (if it is based on total concentrations of mixture components), may not be a single number because it could be dependent on the volume ratio of the two phases, such as water-octanol, water-sediment, etc. (Verhaar et al., 1995).

The use of non-specific analytical methods that rely exclusively on a bulk property or sum parameter to express a total polymer concentration in each phase might be the best available option, but must be considered carefully since they give no information about homologue distributions, which could be very different in each phase. A K_{ow} calculated from such data makes it an operationally defined quantity having more limited use since the results depend both on the composition of the polymer product and the properties of the individual components as well as on the chosen measurement parameter. The use of radiolabelled substances and non-specific measurements of total radioactivity exhibits the same limitation and has the additional concern for potentially large errors associated with labelled impurities with K_{ow} values that significantly differ from the main substance. Even a low level (e.g. parts per million) of such an impurity can introduce log unit scale bias in apparent K_{ow}. For these reasons, the suitability of non-specific concentration measurements to determine the K_{ow} for a polymer product should be considered on a case-by-case basis, and the results interpreted appropriately based on the recognised limitations. Use of a well-characterised polymer sample, and limited qualitative analysis of the resulting components in the water and/or octanol phases, could inform this evaluation.

The third OECD TG for determining K_{ow}, i.e. OECD TG 117, describes an indirect measurement based on the empirical correlation between a substance's K_{ow} and chromatographic retention in reversed-phase HPLC, using reference substances with known K_{ow} values. This correlation is a form of single-parameter linear free energy relationship relating a property that can be rather easily measured (capacity factor) to one that is more difficult to measure directly (K_{ow}). The main advantage of this method is that it does not require concentration measurements, thus having the potential to reduce time and cost greatly, especially for multi-constituent substances, including many polymer products. However, implicit in the use of the HPLC method is an assumption that the mechanisms and interactions governing retention in the chosen HPLC system (defined as column and mobile phase) are similar in type and relative contribution to those governing octanol/water partitioning. Since few systems comply with this assumption as such, and use of those systems may not be practical (e.g. because they produce excessive retention times), it is critical to select calibration substances that are chemically similar to the ones for which the K_{ow} is being determined. The OECD TG 117 contains a list of recommended reference substances, but these are small organic molecules including a preponderance of aromatic hydrocarbons and their derivatives. These substances could be well-suited for estimating K_{ow} values of LMW compounds in polymer products, but might lead to erroneous results for the polymeric substance, whose retention might be influenced by mechanisms other than partitioning (e.g. adsorption versus

absorption) or limited by the inability of the polymeric substance to enter completely into the stationary phase due to its size (steric exclusion). Further, if the polymeric substance contains a functional group that may be completely or partially ionised under the chosen chromatographic conditions, ionic interactions will influence its retention, and hence apparent K_{ow} values. Thus, many polymer products cannot be assessed chromatographically following the HPLC conditions mandated in OECD TG 117.

In summary, the selection of the most appropriate test method for determining the K_{ow} should consider the size and chemical nature of the polymer, as well as the availability of a suitable analytical method for making concentration measurements in water and octanol (OECD TG 107 and 123), or a separation system and reference substances whose domain (defined by molecular weight, chemical class and K_{ow} range) includes the substance of interest (OECD TG 117). Generally, while the HPLC method (OECD TG 117) is considered suitable for non-ionic (polymeric) surfactants, the slow-stirring method (OECD TG 123) is the most widely applicable experimental method for generating K_{ow} values for surface-active test compounds: Assessing four surfactant classes (non-ionic, anionic, cationic and amphoteric) including a few LMW polymeric substances in the test set, Hodges et al. (2019) showed that the HPLC method (OECD TG 117) generates consistently higher log K_{ow} values than the slow-stirring method (OECD TG 123) for non-ionics, but this positive bias could be removed using reference surfactants with log K_{ow} values determined using the slow-stirring method. Further, the slow-stirring method was identified as the most widely applicable experimental method for generating log K_{ow} / n-octanol/water distribution coefficient data for all the surface-active test compounds (Hodges et al., 2019).

In addition to the three OECD TGs, for HMW solid polymers or hydrogels, contact angle measurements and/or swelling ratio can be used to estimate the apparent hydrophobicity of the polymer's surface. Magenau et al. (2015) present an approach where they correlate the surface-normalised log K_{ow} values of the respective monomers with a polymer's hydrophobicity.

Further research work is merited to address the suitability of the approach presented by Magenau et al. (2015) to predict the K_{ow} for different types of polymers.

Future efforts are needed to develop guidance on how the range of n-octanol/water partition coefficients (and other key physico-chemical parameters) that characterise any given polymer should be used in environmental fate distribution models for risk assessment.

The K_{ow} can also be estimated based on the ratio of solubilities in water-saturated octanol (no guideline available) and water (OECD TG 105). However, this approach has drawbacks, as described in the European Chemicals Agency (ECHA) *Guidance on information requirements and chemical safety assessment Chapter R.7a* (ECHA, 2017a). In the case of polymeric substances (homopolymers and, even more so, copolymers), the approach could be highly misleading as the octanol-soluble and water-soluble fractions may represent very different portions of the homologue distribution.

Use of quantitative structure-activity relationships (QSARs) to estimate log K_{ow} values for polymers lacks a sound science basis, since no, or only very few, experimental data for polymers have been included in QSAR training sets so far. Therefore, use of QSARs (to predict the K_{ow} based upon the presence or absence of a specific structural alert) should only be used with great caution, and should be limited to the components of the polymer product that lie within the model applicability domain.

A pragmatic, but not yet validated, approach to establish a K_{ow} may be to measure its adsorption/desorption distribution coefficient (K_d) in activated sludge, sediment or soil (see Section 3.5). The K_d value is then used to calculate a log K_{oc}, which can then be used to back-extrapolate a log K_{ow}. However, considering that this

approach has not yet been validated (let alone validated for the assessment of polymers), the relevance of the resulting K_{ow} values should be evaluated with caution.

Overall, very limited log K_{ow} data for polymers are found in the open literature, suggesting this property is not routinely determined. Some company-internal studies report negative log K_{ow} values for anionic and cationic polymers tested with OECD TG 107 (Procter & Gamble, 2000).

Further research work is merited to demonstrate the utility of partitioning parameters for polymer risk assessment, i.e. their predictivity of specific fate properties of specific types of polymers. During environmental exposure modelling, such issues need to be considered (see Section 5.1).

For those types of polymers for which partitioning parameters are identified as relevant for risk assessment, further research work is merited to identify means to adapt test protocols to enable quantification of their concentrations in the different phases.

3.5 Adsorption/desorption and organic carbon/water partition coefficient

A number of TGs and one ISO standard describe the measurement of adsorption/desorption parameters, i.e. the distribution coefficient (K_d) and the organic carbon/water adsorption (partition) coefficient (K_{oc}):

- OECD TG 106: Adsorption/desorption using a batch equilibrium method (K_d and K_{oc})
- OECD TG 121: Estimation of the organic carbon/water partition coefficient (K_{oc}) on soil and sewage sludge using HPLC
- OPPTS 835.1110: Activated sludge sorption isotherm
- OPPTS 835.1220: Sediment and soil adsorption/desorption isotherm
- ISO 18749: Water quality – Adsorption of substances on activated sludge – Batch test using specific analytical methods

As explained in OECD TG 106, **adsorption** represents the process of the binding of a chemical to surfaces of soils, sediments or sludges. This TG does not distinguish between physical and chemical adsorption or further specific processes such as surface catalysed degradation, bulk adsorption or chemical reaction. **Desorption** describes the reversibility of adsorption. Further, the general term **sorption** is used to encompass the processes of absorption, adsorption, ion exchange, and chemisorption (US EPA, 1997a).

The **distribution coefficient (K_d)** is the ratio between the content of the substance in the solid phase and the mass concentration of the substance in the aqueous solution, under the given test conditions, when adsorption equilibrium is reached. The organic carbon-normalised **adsorption (partition) coefficient (K_{oc})** relates the K_d to the content of organic carbon of the soil, sediment or sewage sludge sample (OECD TG 106).

The OECD TG 121 method is in many respects similar to the OECD TG 117 described in Section 3.4 for the determination of the K_{ow} – both are indirect measurements that are based on an empirical correlation between the endpoint and chromatographic retention in reversed-phase HPLC, using reference substances. Therefore, the same limitations as described for the OECD TG 117 above apply. (Notably, the cyanopropyl stationary phase, specified as column material in OECD TG 121, is likely to be suitable for few polymer products, only.)

The K_{oc} for adsorption to activated sludge, relevant for the removal rate in wastewater treatment plants (WWTPs), can be determined using ISO 18749 or OPPTS 835.1110. The K_{oc} for adsorption to sediment and soil can be determined following OPPTS 835.1220. The protocols for these methods may need to be adapted for the assessment of water-insoluble polymers to enable the distinction between adsorption to solids (as would occur in contact with sewage sludge) and physical removal (as would occur e.g. in skimming or sedimentation tanks in a WWTP). Protocol adaptations may for example include additional quantification of the polymer adsorbed to the solids, rather than quantification of polymer in the water column only.

The applicability of the methods will generally depend on the ability to evenly disperse the polymer in a slurry of water and soil, sewage sludge or sediments, and to measure the reduction in polymer concentration in the liquid phase. It can be challenging and time consuming to measure the K_{oc} for polymers due to technical limitations in assessing HMW substances. A major challenge is the development of suitable analytical methods and careful adaptation of those methods originally developed for soluble (organic) compounds to insoluble and poorly soluble polymers. For example, the separation of sludge cannot be easily performed by centrifugation as described in OPPTS 835.1110.

Theoretically, adsorption/desorption parameters can be recalculated for different solid phases based on normalisation of the organic carbon content. However, this approach might not be suitable for polymers for which ionic interactions play a major role in the adsorption/desorption process. In this case, normalisation for the cationic or anionic exchange capacity may be worthwhile to understand the adsorption/desorption mechanisms involved. Overall, the experimental derivation of a K_{oc} value for every polymer using a series of tests is likely not feasible with the existing test methods, so that read-across and data extrapolation to similar polymers will be a practical necessity.

Jop et al. (1997) successfully determined adsorption and desorption properties of two polycarboxylates in a series of experiments beginning with preliminary and screening tests and progressing to kinetics (equilibrium) and isotherm studies. The study confirmed the overall suitability of OECD TG 106, except that activated sludge was used as a sorbent instead of soil.

The use of ^{14}C-labelled test materials will strongly facilitate adsorption/desorption testing. Procter & Gamble (in HERA, 2014a) studied the sorption behaviour of radiolabelled non-biodegradable anionic and cationic polymers (molecular weight ranges approx. 2,800-70,000 Da) to activated sludge. The influence of water hardness with calcium and magnesium ions acting as a bridge between the polymer and the sludge was also studied. For the cationic polymers, results suggest that these polymers are removed primarily by electrostatic interaction between the positive charges on the polymer backbone and negative charges on the sewage sludge particles. Factors that appear to increase sorption are (1) a higher molecular weight of the polymer backbone; (2) increased charge density; and (3) the presence of a hydrophobic tail. LMW anionic polymers showed very low sorption, while for HMW anionic polymers increased sorption was observed (based on precipitation and calcium bridging mechanisms). Within the Human and Environmental Risk Assessment (HERA) project jointly run by the International Association for Soaps, Detergents and Maintenance Products (AISE) and the European Chemical Industry Council (Cefic), the report *Polycarboxylates in detergents* (HERA, 2014a, b; www.heraproject.com) also informs on the sorption of polycarboxylates to sewage sludge.

As discussed above in this subsection, removal from wastewater by adsorption is of relevance for polymers that are dissolved or at least evenly dispersed in water. Insoluble or poorly soluble polymers can be effectively removed from sewage water by physical removal already in the first tanks of a WWTP, before even getting into contact with activated sludge, and this is of high relevance for the protection of the aquatic environment

(Carr et al., 2016; Murphy, 2016; Talvitie, 2017). Depending on the density of the polymer, the physical removal may occur in the sedimentation tanks, floatation tanks, or skimming tanks. The polymer particles will then reside in the scraps of these tanks. As these scraps have high calorific value, they are incinerated in good practice, resulting in significant reduction of environmental exposure. Alternatively, when sludge is used as fertiliser / organic material on land, the polymer particles will have a final sink in agricultural land.

Further research work should aim at collating available K_{oc} data for different types of polymers. Based thereupon, rules of thumb should be developed to allow read-across to other polymers with similar characteristics and to estimate K_{oc} from K_{ow}. Further research work is also merited to improve the understanding of the partitioning behaviour of semi-solid polymers, e.g. hydrogels, and to determine if they rather behave like water-soluble polymers (so that the K_{oc} is applicable) or more like water insoluble particulates (so that settling under gravity and aggregation to organic solids will drive sedimentation).

3.6 Surface tension

Surface tension is defined as "*the free surface enthalpy per unit of surface area*", and it is expressed in units of N/m (with 1 N/m corresponding to 10^3 dyne/cm in the obsolete centimetre-gram-second system) (Council, 2008). Surface tension is not used as such in risk assessment, but it can be a factor to decide how to perform certain tests (e.g. log K_{ow}) to correctly manage exposure in test systems, to monitor irritation effects, etc. For example, following the standard information requirements laid down in Annex VII of *Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals* (REACH; EP and Council, 2006), information on surface tension can be required for substances manufactured or imported in quantities of 1 tonne or more if surface activity is expected or can be predicted based on structure or if it is a desired property of the material.

The surface tension of an aqueous solution of a substance can be used to determine whether the substance is surface active. Such measurements are also relevant since decreasing the surface tension of the aqueous solution may impact on the properties of the solution and other physico-chemical measurements.

The surface tension of solid polymer surfaces or polymer melts is not discussed here, as this is considered out of the scope of this report. Also, surface tension effects in solvents other than water are not considered. (Of note, surface energy might also be a useful physico-chemical property for fit-for-purpose identification of polymers. However, currently, the correlation between surface energy and surface tension appears unclear.)

Importantly, the observation of some surface tension-lowering effects does not necessarily qualify a substance as a 'surfactant' for technical applications.

OECD TG 115 (Surface tension of aqueous solutions) describes the following methods:

- Plate method (i.e. the Wilhelmy method)
- Stirrup method
- Ring method (i.e. the Du Nouy method)
- OECD harmonised ring method

In the OECD TG 115, it is noted that all of these methods are fully described in ISO 304 (*Surface active agents — determination of surface tension by drawing up liquid films*).

Generally, the methods described in OECD TG 115 will be applicable to polymers or polymer blends (i.e. mixtures of two or more polymers that have been blended together to create a new material with different physical properties; https://application.wiley-vch.de/books/sample/3527331530_c01.pdf) provided that they are soluble in water. It is useful to have preliminary information on the polymer's water solubility, structure, hydrolysis properties and critical concentration for micelle formation before performing the respective test. Surface tension measurements require a test material that is stable against hydrolysis during the test period and soluble in water at concentrations of > 1 mg/L. Measurements should be performed on a solution at either 90 % of the solubility limit or 1 g/L (where viscosity permits), whichever is smaller (ECHA, 2017a). For polymers with high M_n, it should be considered that the remaining monomers and short-chained components are likely to dissolve thereby potentially affecting surface tension.

Table 3 presents methods that are recognised for the determination of surface tension-lowering properties of liquids or liquid mixtures and their expected applicability for the assessment of polymers, including methods that are not described in OECD TG 115, e.g. bubble pressure method, hanging drop method, and capillary rise method (https://www.kruss-scientific.com/services/education-theory/glossary/surface-tension/).

Hu et al. (1991) used the bubble pressure method and the ring method to measure the surface tension of four aqueous solutions of HMW polymers (i.e. polyacrylamide, polyacrylic acid, carboxymethyl cellulose and hydroxyethyl cellulose) over a 20-65 °C temperature range. For a fixed concentration, all of the polymer solutions exhibited a decrease in surface tension with increasing temperature level. Depending on the polymer under investigation and their concentration, surface tension values above, equal to or lower than that of water were recorded (Hu et al., 1991). Miaw (1978) reported that the ring method is not suitable for polymer solutions above 500 ppm.

Table 3: Analytical methods potentially suitable to determine the surface tension-lowering properties of polymers

Method	**Applicability**
OECD TG 115: Plate method, stirrup method, ring method, OECD harmonised ring method	Generally suitable if polymer can be brought into solution
Capillary rise method	
Stalagmometer method (drop weight method)	
Maximum bubble pressure method	
Analysis of shape of hanging or sessile liquid drop or gas bubble	
Dynamic methods	Very specific for oscillating liquid; not commonly used for polymers
Inverse gas chromatography	Useful for the characterisation of polymer films, beads, powders, also used to study surface properties of polymer formulations

3.7 Analytical verification of polymer concentrations in environmental media

The OECD TGs for the assessment of environmental fate and effects on biotic systems generally require analytical verification of exposure concentrations, and such verification is highly recommended whenever the organisms are exposed to the polymer externally (e.g. in aqueous media or sediment). Different approaches are available for the measurement of polymer concentrations in environmental media, including:

- Section 3.7.1: Cold (i.e. non-radiolabelling-based) analytical approaches that rely on the quantification of a specific component of the polymer that is not a specific molecular entity or on the quantification of suitable substitutions or constituents of the polymer product (e.g. specific molecules from the polymer distribution with some assumptions regarding behaviour);
- Section 3.7.2: Radiodetection techniques following radiolabelling of the molecule using e.g. ^{3}H or ^{14}C;
- Section 3.7.3: Use of a stable isotope (nonradioactive) label (typically ^{15}N or ^{13}C or both) as is commonly used in ecological investigations of nutrient pathways in ecological systems.

Cold analytical approaches and radiolabelling will also be applicable in the context of human health hazard assessment while use of stable isotope methodologies is rather specific to ecological investigations.

Relatively few examples exist of the quantification of specific polymers in environmental media, either in the laboratory or the field (for example, monitoring data); discussed in further detail in Sections 3.7.1-3.7.3. Generally, the very nature of polymer products being multi-constituent substances, or even UVCBs, makes quantification extremely challenging, but not unattainable. The most appropriate approaches for exposure quantification will vary greatly depending on the type of study and on the properties of the polymer and the medium. In all cases, it is pivotal to begin with a well-characterised polymer. Knowledge on its physico-chemical properties (Sections 3.1-3.6) is indispensable to properly develop dosing solutions in water, food, soil or sediment and to provide context for performing the fate or ecotoxicological investigations (Sections 4 and 6). A single approach and analytical method are unlikely to be suitable for every form of investigation. The selected method(s) should be well documented and validated, and they should employ typical quality control measures to minimise bias due to contamination or other sources. In all cases, the advantages and disadvantages, as well as underlying presumptions of the selected approach / analytical method(s), should be clearly described.

It is expected that the case studies putting the CF4Polymers into practice, planned as ECETOC TR 133-3, will provide further insight on the applicability and/or technical limitations of specific approaches to verify the concentrations of different types of polymers in different environmental media.

Notably, all approaches presented below (cold analytical approach with specific homologue detection, radioanalytical and stable isotope approaches) each carry a certain set of underlying assumptions to varying degrees given that polymers can contain hundreds to thousands of chemically-related constituents and that it is not possible to measure these individually. This is even more complex when addressing environmental fate (e.g. biodegradation, wastewater simulation studies, bioaccumulation) where metabolite formation may contribute to the complexity of structures to be analysed. Presently, it is not practical to address potential metabolites; however, if they do form, the intrinsic hazard of these can be positioned using non-standard (to OECD) ecotoxicological methods, such as testing of wastewater effluents or assessing aged test solutions. The

fact that many polymers do not degrade, at the present time, suggests that this need is not critical unless it is shown the polymer indeed undergoes a certain level of degradation.

3.7.1 Cold analytical approaches for polymers

A good example for a cold (i.e. non-radiolabelling-based) analytical approach is the detection of silicon (Si) in environmental samples such as has been performed with PDMS. Fendinger et al. (1997a, b) monitored PDMS concentrations in wastewater, activated sludge, soil and sediment by extracting the test item using tetrahydrofuran followed by GPC-inductively coupled plasma (ICP) or HPLC-ICP. The recovery rate of field spikes varied by environmental medium and generally ranged from approx. 95-105% except for dilute effluent and river water samples (average: slightly above 50%). Detection limits were generally around 5 µg/L for aqueous samples and 1 mg/kg for extracted solids samples. Limitations of the approach are the ubiquity of Si in the environment and knowledge regarding the expected environmental distribution of PDMS (which was more limited in the 1990's than it is today).

Another approach that has become available due to ongoing advances in analytical instrumentation is quantitative mass spectrometry. Specifically, Flow-Injection High-Resolution Quantitative Time-of-Flight Mass Spectrometry can be used to generate polymer-specific fragments (daughter ions) and provide relatively fast and robust measurement of low levels of cationic polymers (Lam et al., 2019; findings from the Cefic Long-range Research Initiative (LRI) ECO46 project *Improved aquatic testing and assessment of cationic polymers* (iTAP[1]) in cooperation with Aarhus University, Denmark). The general strategy of the approach is to choose and follow a minimum number of specific daughter fragments representative of the full polymer distribution whose aggregate concentration is then used to extrapolate to the entire distribution. Detection of selected polyquaterniums has been shown to be quantitative in some instances to 1 mg/L with performance improvements possible by injecting larger volumes or by the application of concentration techniques (Lam et al., 2019). The approach is analogous to earlier mass spectrometry techniques that were used to quantify alcohol ethoxysulfate and alcohol ethoxylates in environmental samples (Popenoe et al., 1994).

Finally, Hüffer et al. (2017) offer an overview of analytical techniques to assess concentrations of microplastics.

3.7.2 Radioanalytical approaches for polymers

Radiolabelling of polymers can be a useful technique to follow the movement of polymers in environmental studies or to quantify exposures in some circumstances. The technique is especially useful for homopolymers and those that have predictable distributions or reactivities. ^{14}C-labelled PDMS and ^{14}C-labelled olestra (a synthetic sucrose polyester, approx. 1,200 Da) have been used in fate and ecotoxicological investigations of the respective polymers (Overcash et al., 1994; McAvoy et al., 1996; Fendinger et al., 1997a). However, it is a significant challenge to produce polymers uniformly labelled with ^{14}C (see also Section 4.1.2). Studies using ^{14}C-labelling of polycarboxylates to determine different fate properties showed varying degrees of success and inference of positioning the radiolabel in the carboxyl group or in the chain, underscoring the difficulties

[1] http://cefic-lri.org/projects/eco-46-improved-aquatic-testing-and-assessment-of-cationic-polymers-itap/

associated with radiolabelling techniques (HERA, 2014a). Alternatively, trituration using ^{3}H-exchange that allows saturation of exchangeable sites has been found useful for certain cationic polymers (Procter & Gamble, 2014), but has the drawback of potential long-term instability (re-exchange between ^{3}H and water).

3.7.3 Stable isotope approaches for polymers

Stable isotopic signatures are widely used in ecological investigations. The general principle is that naturally occurring stable isotopes fractionate through biotic systems in accordance with their relative mass. Heavier, but rarer, stable isotopes (e.g. ^{15}N versus ^{14}N) are found at predictable levels through the food chain reflective of their trophic position (with predators being ^{15}N-enriched). Morrall et al. (2006) used taxon-specific ^{13}C and ^{15}N to sort out trophic positions in an experimental model ecosystem (stream mesocosm) dosed with a proprietary cationic surfactant (Procter & Gamble, 2001; Morrall et al., 2006). The success of this study led to the use of a uniformly labelled ^{15}N polymer dosed into stream mesocosms. Detection of the polymer was quantitative to 100 µg/L in river water and was used to assess potential movement of the polymer into sediment and biota. While stable isotope labelling of polymers is cost-intensive, it has considerable potential for use, also in research into general principles of environmental fate.

4. TEST METHODS TO ASSESS POLYMER ENVIRONMENTAL FATE

This section presents and discusses the applicability of standard test methods to assess the environmental fate, a key parameter in ensuring the safety of polymer products (or any other product). The focus lies on test methods for assessing (bio)degradation (Section 4.1) since this is by far the most relevant elimination process for organic material in the environment. Thereafter, Section 4.2 discusses the applicability of bioaccumulation test methods for the assessment of polymers (see Box 2 for definitions related to environmental fate and (bio)degradation and Box 3 in Section 4.2 for definitions of terms related to bioaccumulation).

Box 2: Definitions of terms related to environmental fate and (bio)degradation

Biodegradability: "*The ability of a material to decompose after interactions with biological elements*" (Goswami and O'Haire, 2016).

Biodegradation: "*The process by which organic substances are decomposed by micro-organisms (mainly aerobic bacteria) into simpler substances such as carbon dioxide, water and ammonia*" (OECD Glossary of Statistical Terms; https://stats.oecd.org/glossary/detail.asp?ID=203).

Composting (industrial): A controlled process (i.e. with respect to humidity, temperature) that can be divided into two distinct phases i.e. active composting (rotting) followed by curing (post-rotting). The duration of the active composting phase depends on the type of composting, and it may include both aerobic thermophilic processes, but also anaerobic processes (adapted from European Bioplastics, 2015).

Degradation, decomposition, or depolymerisation: *"A type of chemical change in which a polymeric substance breaks down into simpler, smaller weight substances as the result of (for example) oxidation, hydrolysis, heat, sunlight, attack by solvents or microbial action"* (US EPA, 1997b).

Disintegration: The physical breakdown of a material into fragments (ISO 24513).

Environmental fate: The destiny of a substance after release into the environment (adapted from https://www.informea.org/en/terms/environmental-fate).

Persistence: *"Length of time a substance stays in the environment (or body organs) after its introduction."* (http://www.businessdictionary.com/definition/persistence.html)

From a regulatory perspective, biodegradation and bioaccumulation assessments are required to identify **persistent, bioaccumulative and toxic (PBT)** and/or **very persistent and very bioaccumulative (vPvB)** substances (e.g. in accordance with Annex XIII of the EU REACH Regulation (EP and Council, 2006)) or **persistent organic pollutants (POPs**; i.e. PBT/vPvB substances with long-range environmental transport potential (UNEP, 2017)). A further regulatory use of information on biodegradation and bioaccumulation is for the generic classification of the environmental hazard potential of the given substance, e.g. in accordance with the *Globally Harmonised System of Classification and Labelling of Chemicals* (United Nations, 2017). When identifying PBT and/or vPvB substances, biodegradation testing informs "P" and "vP", and bioaccumulation assessments inform "B" and "vB". Further, ecotoxicological and toxicological testing (addressed in Sections 6 and 7, respectively) inform on toxicity ("T").

A range of TGs is available for determining the environmental fate and behaviour of chemicals, e.g., the OECD TG 300 series, the US EPA OCSPP/OPPTS 835 series and a set of ISO standards/specifications. The OECD TG 300 series include TGs to determine biodegradation in different environmental compartments, bioaccumulation in different organisms, phototransformation, and emission estimation, as well as further

parameters, such as *in vitro* intrinsic clearance and the dispersion stability of nanomaterials, that are not included in this report.

Generally, the OECD TG 300 series may be used to support the assessment of polymers. However, most of the OECD TG 300 test methods were originally developed for well-defined LMW substances that are typically mono-constituent, water-soluble and uniformly dispersed within the aqueous solution, and the corresponding ring trials did not include polymers. Therefore, care has to be taken to account for specific technical limitations that arise due to the specific physico-chemical properties of polymers. Such specific technical limitations are addressed in detail below. Notably, specific physico-chemical properties of polymers can also affect their fate in the environment. For example, water soluble, poorly soluble, and water insoluble/particulate polymers will show quite different fate behaviour.

4.1 (Bio)degradation

(Bio)degradation is one of the predominant mechanisms for loss or removal of materials from environmental compartments. The OECD TG 300 series distinguish between **primary biodegradation**, i.e. biotransformation resulting in the loss of a specific property of the original substance, and **ultimate biodegradation**, i.e. mineralisation by microorganisms to CO_2, water, new microbial cellular constituents (biomass), and other inorganic substances (e.g. NH_3) (OECD, 2006; ECETOC, 2018a).

As regards types of biodegradation tests, the OECD TG 300 series distinguishes between (Section 4.1.1) **biodegradation screening tests** and (Section 4.1.2) **simulation biodegradation tests**. Further, Section 4.1.3 deals with **abiotic degradation** (photodegradation and hydrolysis), and Section 4.1.4 presents other biodegradation methods that may provide useful information under certain engineered disposal conditions, with a focus on **industrial composting**. In completing the discussion of individual (bio)degradation test methods, Section 4.1.5 suggests a conceptual framework for polymer (bio)degradation assessment.

When selecting the most appropriate method for polymer (bio)degradation testing, it is critical to address its methodological limitations for the assessment of the given polymer. Many polymers are present as polymer products that include multiple constituents, such as polymeric macromolecules of different molecular weights, residual monomers, residual starters, IAS and NIAS, and some polymer products are of HMW and exhibit limited solubility. All such issues might impair the applicability of a specific (bio)degradation test method. However, some technical limitations may be addressed by appropriate adjustments of the method. For example, if poorly soluble and particulate polymers exhibit reduced bioavailability in specific test systems, either due to transfer limitations in dilute test systems and/or high sorptivity, this might be addressed by changing the test configuration and/or the dosing procedure, and/or by extending the duration of the test. Another example is inclusion of pre-exposure techniques to allow for adaptation to occur in polymer biodegradation laboratory studies. In a comprehensive literature review, Baptiste et al. (2019) highlighted the importance of adaptation in the evaluation of persistence of chemicals and, specifically, that adaptation is a naturally occurring phenomenon that has been observed in all environmental compartments and can be induced in the laboratory.

Generally, appropriate preparation of samples of the polymer product of interest is also important to enhance the relevance and reliability of test results. For example, ISO 10210 (*Plastics - Methods for the preparation of*

samples for biodegradation testing of plastic materials) provides a reference for the particle size distribution for the testing of solid polymers.

Further, it is important to consider that different constituents of the polymer product might biodegrade at individual kinetics. Where feasible, the duration of a biodegradation test should generally be extended until complete mineralisation of both the polymeric substances and all of the further constituents can be observed. Notably (and just as may also stand true for non-polymeric substances), in closed test systems the pre-requisites of biodegradation, i.e. active microbiota, may not always be maintained throughout longer test periods. Also, lag-periods may be needed to adjust for competent microbiota. Adaptations of test durations should consider that extensions might result in a decrease in microbial biodiversity or an overall decrease in the numbers of microbes present in the test system over time.

Some polymeric substances biodegrade in a different manner than traditional soluble chemicals. For example, the biodegradation of non-soluble polymeric substances may be surface-mediated (Zumstein et al., 2018). For solid polymers, surface-mediated biodegradation may be driven by the ratio between surface and volume. Further, surface-mediated biodegradation (of polymeric and non-polymeric substances) may be affected by the circumstance that the number of hydrolytic enzymes available in the extracellular fractions of activated sludge are generally much lower than those present within the microbiota (Boczar et al., 1992, 2001). Such issues may increase the time needed for biodegradation.

A topic that is gaining increasing importance with respect to the biodegradation of e.g. petroleum-based polymers upon disposal is that fungi have the ability to invade substrates by using substrate-unspecific, detoxifying enzymes, and that they can further produce hydrophobins for surface coating to attach hyphae to hydrophobic polymers that then penetrate the polymers thereby enhancing their biodegradation (Kang et al., 2019; Sánchez, 2019). However, to the best of the ECETOC Polymers TF's knowledge there are currently no standardised test methods that specifically address fungi-mediated biodegradation. Therefore, this issue is not further addressed below.

Tables 4A-4C provide an overview of the available (bio)degradation test methods. In addition to the OECD TG 300 series which address the environmental fate of substances in general, a number of standards and technical specifications were specifically designed to assess the biodegradability of (specific types and uses of) plastics. Many of these standards and technical specifications are also applicable to other types of polymers, as they account for e.g. the need for prolonged test durations (the exact duration of which would need to be determined on a case-by-case basis considering observation of (bio)degradation, steady-state or absence of (bio)degradation).

It is expected that the case studies putting the CF4Polymers into practice, planned as ECETOC TR 133-3, will provide further insight on the applicability and/or technical limitations of the different available biodegradation screening and simulation test methods for the assessment of different types of polymers.

4.1.1 Biodegradation screening tests

Screening biodegradation studies are conservative test methods that all utilise indirect methods to quantify mineralisation. The commonly accepted indirect methods are O_2 consumption and CO_2 evolution. The use of these indirect methods results in the need to dose test systems at high concentrations to overcome background levels of O_2 consumption or CO_2 evolution resulting from normal microbial processes. The high

concentrations of test materials applied in biodegradation screening tests are not environmentally relevant, but a technical requirement. However, because these methods follow O_2 consumption or CO_2 evolution as the analytical endpoint, they are generally applicable to the evaluation of polymers.

It is imperative in using biodegradation screening tests to have an accurate theoretical O_2 demand (ThOD) or theoretical CO_2 evolution ($ThCO_2$) depending on the analytical endpoint. The ThOD demand is the stoichiometric amount of O_2 required to oxidise a compound to end products (Baker et al., 1999). It may be known from the synthesis route or quantified using elemental analysis in some cases. The $ThCO_2$ is the calculated amount of CO_2 that can evolve during ultimate biodegradation. It can be measured using a total organic carbon analyser and coupling that system with solid sample combustion unit for poorly soluble or insoluble polymers. Since polymers can be complex mixtures, it can be difficult to adequately quantify the ThOD or $ThCO_2$.

In addition to O_2 consumption and CO_2 evolution, dissolved organic carbon (DOC) can be followed as an indirect analytical measurement for soluble polymers.

4.1.1.1 Aerobic biodegradation screening tests

This section on aerobic biodegradation screening tests is organised to present (A) screening tests using aqueous media; (B) inherent biodegradability tests; (C) screening tests using marine water/sediment as the test matrix; and (D) screening tests using soil as the test matrix. Generally, all screening methods support the assessment of the ultimate mineralisation of the substance (Table 4A).

In the screening tests using aqueous media (A) and the inherent biodegradability tests (B), pH buffered aqueous mineral media is prepared in the laboratory, and high concentrations of the test substance and small amounts of the inocula (e.g. from activated sludge, sewage effluents, surface water or soils) are added to the media. Hence, all of these methods include dilute inocula.

In the screening tests using marine water/sediment (C) or soil (D) as test matrices, the respective test matrices are typically supplemented with mineral nutrients as needed. Test substances are dosed at high concentrations (not environmentally relevant) in order to utilise non-specific analytical methods (as discussed above).

Generally, the physical form and size of the polymer under investigation should be similar to the reference materials as the level of bioavailability and biodegradation may be related to the size of the (solid) polymer (further discussed under (D) screening tests utilising soil as the test matrix).

A. Screening tests using aqueous media

Screening tests using aqueous media include:

- OECD TG 301A-F: Ready biodegradability screening tests
- OECD TG 310: Ready biodegradability: headspace test
- ISO 14851: Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium: method by measuring the O_2 demand in a closed respirometer
- ISO 14852: Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium: method by analysis of evolved CO_2

Table 4A: Biodegradation screening test methods potentially suitable for the assessment of polymers

Test guideline / standard (endpoint)	Comments	Examples of tested polymers
Aerobic biodegradation screening tests		
Aerobic screening tests using aqueous media		
OECD TG 301A-F and 310 (ready biodegradability)	Methods following O_2 consumption and CO_2 generation are applicable for all test materials; whereas methods following DOC are not applicable to poorly soluble or sorptive materials Bioavailability limitations of some polymers may require test duration extension (see introduction to Section 4.1 for further details)	Poly(3-hydroxybutyrate)-co-(3-hydroxy valerate), PHBV (McDonough et al., 2017)
ISO 14851 (ultimate aerobic biodegradability of plastic materials in aqueous medium: O_2 demand in closed respirometer)	Method developed for plastics, but can be applied to polymers because O_2 consumption is followed as endpoint	HDPE/PCL and HDPE/PLA blends (Moura et al., 2010) Starch, cellulose, PCL, PLA, PEG, polyethylene (Guo et al., 2012)
ISO 14852 (ultimate aerobic biodegradability of plastic materials in aqueous medium evolved CO_2)	Method developed for plastics, but can be applied to polymers because CO_2 evolution is followed as the endpoint	
Inherent biodegradability tests		
OECD TG 302B-C (inherent biodegradability) [OECD TG 302A: Historical data]	OECD 302B can be modified with a trapping train and CO_2 evolution followed as an endpoint in order to evaluate biodegradation of poorly/non-soluble polymers OECD 302C is directly applicable to polymers	Polyacrylate, 302A (HERA, 2014a) Polyacrylic/maleic acid copolymers, 302A and 302B (HERA, 2014b)
Aerobic screening tests using marine inocula		
OECD TG 306 (biodegradability in seawater)	TG was developed for well-defined LMW and for water-soluble substances, but can be modified to follow CO_2 evolution or O_2 consumption so also applicable to many types of polymers	
ISO 18830 (aerobic biodegradation of non-floating plastic materials in seawater / sandy sediment interface)	Only applicable for solid materials that can be held at the water / sediment interface. Different structural and morphological descriptors of the polymer (including shape and size) may affect test result; therefore, outcomes should preferably be compared to data for (similar) reference materials	
ISO 19679 (aerobic biodegradation of non-floating plastic materials in seawater / sediment interface)		Mater-Bi [a]
ISO/DIS 22403 Plastics – Assessment of the intrinsic ultimate biodegradability of materials exposed to marine inocula under mesophilic aerobic laboratory conditions: Test methods and requirements [draft specification]	Different structural and morphological descriptors of the polymer (including shape and size) may affect test result; therefore, outcomes should preferably be compared to data for (similar) reference materials	

Table 4A continued

Test guideline / standard (endpoint)	Comments	Examples of tested polymers
ISO 22404 Plastics — Determination of the aerobic biodegradation of non-floating materials exposed to marine sediment: Method by analysis of evolved CO_2	Different structural and morphological descriptors of the polymer (including shape and size) may affect test result; therefore, outcomes should preferably be compared to data for (similar) reference materials	
ISO/CD 23977: Plastics – Determination of the aerobic biodegradation of plastic materials exposed to seawater (Part 1 / 2: evolved CO_2 / O_2 demand) [draft standard]		
Aerobic screening tests using soil inocula		
ISO 17556 (Plastics - determination of the ultimate aerobic biodegradability of plastic materials in soil by measuring the O_2 demand in a respirometer or the amount of CO_2 evolved	Method developed for plastics but can be applied to polymers as O_2 consumption or CO_2 evolution are followed as the endpoint Different structural and morphological descriptors of the polymer (including shape and size) may affect test result; therefore, outcomes should preferably be compared to data for (similar) reference materials	see Annex of ISO 17556; Mater-Bi (NICNAS, 2009)
Anaerobic biodegradation screening tests		
OECD TG 311 (anaerobic biodegradability)	Method follows biogas generation so applicable to polymers	PEG 400: Reference substance in standard
ISO 13975 (ultimate anaerobic biodegradation of plastic materials in controlled slurry digestion systems; biogas production)	Different structural and morphological descriptors of the polymer (including shape and size) may affect test result; therefore, outcomes should preferably be compared to data for (similar) reference materials	
ISO 14853 (ultimate anaerobic biodegradation of plastic materials in an aqueous system; biogas production)		PHB, cellulose, PEG 400: Reference substances in standard PHB, PCL (Heerenklage et al., 2001)
ISO 15985 Plastics — Determination of ultimate anaerobic biodegradation under high-solids anaerobic-digestion conditions; analysis of released biogas		

Footnote to Table 4A:

Abbreviations: HDPE: High-density polyethylene; PCL: Polycaprolactone; PEG: Polyethylene glycol; PHB: Polyhydroxybutyrate; PHBV: Poly(3-hydroxybutyrate-co-3-hydroxyvalerate); PLA: Poly(lactic acid).

[a] https://ec.europa.eu/environment/ecoap/etv/aerobic-biodegradation-mater-bi-af03a0-and-mater-bi-af05s0-mater-bi-third-generation-under_en.

The OECD TG 301 and 310 ready biodegradability tests are the most common aerobic aqueous medium screening tests. High concentrations of the test material ranging from 2 to 100 mg/L are applied, and biodegradation is measured by non-specific parameters (OECD, 2006). While developed to evaluate the biodegradation of small discrete soluble compounds, the OECD TG 301 and 310 can be valuable screening tools for the assessment of polymers even though they are very conservative with regard to the test material to microbial biomass ratio. Following the OECD TG 301B test protocol, poly(3-hydroxy butyrate)-co-(3-hydroxy valerate) with a molecular weight of 485,000 Da was readily biodegradable reaching 65.4 ± 4.1% evolved CO_2 within 5 days (McDonough et al., 2017). Historical research has shown that (non-polymeric) substances that biodegrade in an OECD TG 301 will also biodegrade in relevant environmental compartments (Struijs and van den Berg 1995; Federle et al., 1997).

Notably, the typical test duration of up to 28 days (as described in OECD TG 301 and 310) may not be applicable to poorly soluble polymers due to limitations in the mass transfer and bioavailability limitations in terms of exposure to microorganisms in the test system (i.e. medium, test material, inoculum) that will lead to slower biodegradation rates (e.g. Battersby, 1990). Therefore, it is important to ensure necessary extensions of the test duration (see Section 4.1). Further, the applicability of pass/fail criteria for the OECD TG 301 and 310 studies is not demonstrated for polymers.

Generally, methods relying on measurement of O_2 and CO_2 are better suited for polymers, as methods relying on the measurement of DOC may not be relevant for poorly soluble or non-soluble polymers. Hence, OECD TG 301B, 301C, 301D, 301F and 310 that use O_2 consumption or CO_2 evolution as the mineralisation endpoint support the screening assessment for all types of polymers. By contrast, the OECD TG 301A and 301E address DOC and will therefore not be relevant for poorly soluble or non-soluble polymers. The OECD TG 301D closed bottle test is particularly useful in evaluation of biodegradation of volatile substances to reduce the losses of the test item through evaporation.

Given the complex composition of polymers, where appropriate, the 10-day window criterion can be waived in the ready biodegradability studies (OECD (2006), Paragraph 43).

ISO 14851 and ISO 14852 were specifically developed for the assessment of ultimate aerobic biodegradation of plastic materials in the aquatic compartment (see also Gartiser et al., 2017). The ISO methods support the use of well-defined biodegradable polymers as reference materials in addition to or instead of aniline, unlike OECD TG 301 and 310 where reference material is limited to discrete, rapidly degradable substances such as aniline (freshly distilled), sodium acetate and sodium benzoate.

ISO 14851 relates biodegradation to O_2 consumption and ISO 14852 to CO_2 evolution. While both standards were developed for polymers in powdered form, they can also be applied to other types of polymers. Care should be taken on the choice of the reference material for similarity in form and size to account for bioavailability limitations and material type to verify the activity of different inocula.

B. Inherent biodegradability tests

Inherent biodegradability test methods are described in

- OECD TG 302B: Zahn-Wellens/EMPA test; measurement of DOC or chemical O_2 demand
- OECD TG 302C: Inherent biodegradability: modified MITI test (II); measurement of biochemical O_2 demand

The OECD TG 302A (*Inherent biodegradability: modified semi-continuous activated sludge test*), which is rarely used, is not further addressed here; however historical data from such testing may be available, also for polymers (see e.g. Table 4A).

The procedures of these aerobic tests *"allow prolonged exposure of the test substance to microorganisms and a low ratio of test substance to biomass, which offers a better chance to obtain a positive result compared to tests for ready biodegradability"* (OECD, 2006). The OECD TG 302B and 302C utilise activated sludge as inoculum and are used to assess whether a chemical has the potential to biodegrade under aerobic conditions.

For specific types of polymers, inherent biodegradability tests (that allow addressing CO_2 evolution as a parameter) may serve as a link from ultimate biodegradation recorded in the screening tests towards the higher realism of simulation tests (i.e. higher inoculum concentration, etc.)

The inherent biodegradability tests have similar overall limitations as described above for the OECD TG 301 and 310 ready biodegradation screening tests, although specific care has to be given to adsorption aspects due to a higher concentration of organic inoculum. On the other hand, for the very same reason, they are considered to be more potent. The OECD 302B measurement of DOC has been combined with CO_2 measurements to better reflect ultimate biodegradation (Strotmann et. al., 1995).

C. Screening tests utilising marine water/sediment as the test matrix

Polymers may enter the marine environment either through direct release (drilling for oil and gas), direct discharge (drilling fluids) or via surface water. The potential for mineralisation of substances in the marine environment can be screened on the basis of existing TGs. However, since these studies use environmental samples as inoculum, they tend to demonstrate great variability. Depending on the properties of the polymer under investigation (that might e.g. be sorbed to particles or dissolved), different sub-compartments (e.g. seawater column or sediment) are most relevant.

Specific guidance for screening tests addressing the various marine habitats is available or under development including:

- OECD TG 306: Biodegradability in seawater
- ISO 18830: Determination of aerobic biodegradation of non-floating plastic materials in a seawater/sandy sediment interface: method by measuring the O_2 demand in closed respirometer
- ISO 19679: Determination of the aerobic biodegradation of non-floating plastic materials in a seawater/sediment interface: method by analysis of evolved CO_2
- ISO 22404: Plastics — determination of the aerobic biodegradation of non-floating materials exposed to marine sediment: method by analysis of evolved CO_2
- ISO/CD 23977-1: Plastics – determination of the aerobic biodegradation of plastic materials exposed to seawater – Part 1: method by analysis of evolved CO_2
- ISO/CD 23977-2: Plastics – determination of the aerobic biodegradation of plastic materials exposed to seawater – Part 2: method by measuring the O_2 demand in closed respirometer
- ASTM D6691-17: Standard test method for determining aerobic biodegradation of plastic materials in the marine environment by a defined microbial consortium or natural sea water inoculum

Technical specification:

- ISO/DIS 22403: Plastics – assessment of the intrinsic ultimate biodegradability of materials exposed to marine inocula under mesophilic aerobic laboratory conditions – test methods and requirements

The assignment of OECD TG 306 as screening test, but distinct from the OECD TG 301 and 310 ready biodegradability tests, is also supported in the introduction to OECD TG 306 where it is stated that studies following this TG *"are not tests for ready biodegradability since no inoculum is added in addition to the micro-organisms already present in the seawater. Neither do the tests simulate the marine environment since nutrients are added and the concentration of test substance is very much higher than would be present in the sea."*

OECD TG 306 was developed for LMW substances, but can be applied to polymers when taking into account their specificities (Table 4A). The OECD TG 306 follows DOC or O_2 consumption as endpoint. As stated above, for polymers of limited solubility, DOC is not a relevant endpoint so that O_2 consumption should be followed. Alternatively, the general methodology and conditions of the OECD TG 306 could be applied, but following CO_2 evolution as the analytical endpoint.

The source of inocula used in OECD TG 306 are microorganisms present in sampled marine water. While the test reflects biodegradation in the open marine environment, the samples, and hence also test results, can demonstrate great variability. The limitations in the predictivity of biodegradation potential have triggered research to improve the reliability of the OECD TG 306 test method. Recent research conducted within the Cefic LRI ECO11 project *The effect of including environmentally relevant microbial diversity in biodegradation screening tests for persistence assessments* has shown that the amount of inocula, diversity of the microorganism community, and test duration can significantly affect the accuracy of an OECD TG 306 study in predicting biodegradability in the marine compartment (Ott et al., 2019). This research further showed that the use of tangential flow filtration with a 100-fold increase in cell concentration using a 0.22 µm pore filter was optimum for preparing inoculum for the OECD TG 306 test system (Martin et al., 2018). The increase in cell concentration positively impacted the reliability of the results of the marine screening test by decreasing within- and between-test variability as well as the lag period and by increasing the probability of reaching a pass criterion for known biodegradable materials. These findings led to an international ring test with thirteen laboratories to validate the improved marine biodegradation screening test method, which incorporated increased bacterial cell numbers and an increased test duration (≥ 60 days). Results of the ring test showed that the improved test method resulted in a less variable and more reliable characterisation of persistence for five reference compounds with varying degrees of biodegradability (see also http://cefic-lri.org/projects/eco11-towards-rationally-designed-hazard-risk-and-persistency-assessment-putting-the-bio-back-into-biodegradability-tests/). The LRI ECO11 project team, and the ECETOC Polymers TF, advise to integrate the outcome of this research into an improved testing protocol for marine screening assessments.

In addition to the OECD TG 306, a number of ISO standards have been developed specifically for the assessment of plastics. ISO 18830 and ISO 19679 allow determining the degree of biodegradation in a laboratory environment for the interface between seawater and sediment, either by measuring O_2 consumption (ISO 18830) or CO_2 development (ISO 19679). However, ISO 18830 and ISO 19679 are not applicable for polymers that cannot be held at the sediment-water interface. By contrast, the (not yet finalised) Committee Drafts ISO/CD 23977-1 and 23977-2 (and similarly ASTM D6691-17) describe a screening test to assess biodegradation in seawater, with Part 1 addressing CO_2 evolution and Part 2 O_2 consumption. Further, for determining the aerobic degradation (of plastics) in marine sediments by following CO_2 evolution, ISO 22404 screening method can be applied.

The technical specification ISO/DIS 22403 further specifies the methods and criteria for showing intrinsic ultimate biodegradability in marine environments. This specification is under development and is expected to provide further details of relevance for ISO 18830, ISO 19679, ISO 22404, ISO/CD 23977-1 and -2, as well as

ASTM 6691-17. As mentioned in ISO/DIS 22403, the material or organic constituents tested shall display biodegradation ≥ 90% within 2 years, absolute or relative to the reference material, to indicate intrinsic biodegradability. Also, the biodegradation result of the whole material by any of the specified standards should be supported by data on biodegradability for single constituents present in the material at a certain range of concentrations.

D. Screening tests utilising soil as the test matrix

The following ISO standard describes a screening test using soil as the test matrix:

- ISO 17556: Plastics - determination of the ultimate aerobic biodegradability of plastic materials in soil by measuring the O_2 demand in a respirometer or the amount of CO_2 evolved

The ISO 17556 test allows incubation until a constant level of biodegradation has been attained (e.g. 6 months) or for up to 2 years. If testing is extended up to 2 years, consideration should be given to the viability of the soil. Biodegradation is measured preferably from 20-28 °C by determining O_2 consumption or CO_2 evolution during incubation. Non-adapted soil is used as an inoculum. Well-characterised biodegradable polymers (e.g. microcrystalline-cellulose powder, cellulose filter or poly(β-hydroxybutyrate) as well as non-biodegradable polymers (e.g. polyethylene)) are used as reference materials.

The physical form and size of the polymer under investigation should be similar to the reference materials (e.g. powder with a maximum diameter of 250 µm) as the level of bioavailability and biodegradation may be related to the size of the (solid) polymer. For example, Chinaglia et al. (2018) evaluated the impact of particle size on polybutylene sebacate (milled pellets) biodegradability in soil. Biodegradation was directly proportional to the available surface area: Particles with the highest surface area (and hence smallest size) biodegraded most rapidly, and the intrinsic biodegradation rate could be expressed as a function of the total available surface (Chinaglia et al., 2018).

Since ISO 17556 follows O_2 consumption or CO_2 evolution as analytical endpoints to assess biodegradation, it can be readily applied to the evaluation of polymers – be they water-soluble, poorly soluble or insoluble in water. In the appendix of ISO 17556, biodegradation data are presented for various polymers. Soil type as well as nutrient content can significantly affect the overall biodegradation (Briassoulis and Mistriotis, 2018). Following the ISO 17556 protocol, Prapruddivongs et al. (2018) assessed the biodegradation potential of poly(lactic acid) (and chemically crosslinked poly(lactic acid) filled with different types of SiO_2). After 60 days, approx. 57% of the non-crosslinked poly(lactic acid) had biodegraded (Prapruddivongs et al., 2018).

Concerns have been raised about the environmental relevance of the ISO 17556 test method because high test material loading to the soil may be needed in order to distinguish CO_2 that is formed from the test material from CO_2 that develops from background respiration (Ardisson et al., 2014). However, usage of non-environmentally relevant test concentrations is an issue faced with all screening tests. Therefore, this does not put the ISO 17556 into question, but reinforces that it is a conservative screening test and not a simulation test.

4.1.1.2 Anaerobic biodegradation screening tests

Anaerobic biodegradability screening tests include (Table 4A):

- OECD TG 311: Anaerobic biodegradability of organic compounds in digested sludge: method by measurement of gas production
- ISO 13975: Plastics - determination of the ultimate anaerobic biodegradation of plastic materials in controlled slurry digestion systems: method by measurement of biogas production
- ISO 14853: Plastics - determination of the ultimate anaerobic biodegradation of plastic materials in an aqueous system: method by measurement of biogas production
- ISO 15985: Plastics - determination of ultimate anaerobic biodegradation under high-solids anaerobic-digestion conditions: method by analysis of released biogas

The OECD TG 311 screening method is used to evaluate the potential for anaerobic biodegradability of organic substances utilising anaerobic digestor sludge as the inoculum. Anaerobic biodegradation is measured by non-specific parameters such as total inorganic carbon, CO_2 and CH_4 production (OECD, 2006). As with other screening methods, a non-environmentally relevant high concentration of the test material is used to overcome analytical constraints.

The ISO 14853 is another anaerobic biodegradation screening method that utilises anaerobic digestor sludge as the inoculum and non-specific parameters to quantify biodegradation. The method is similar to OECD TG 311. In principle, both methods can be utilised for polymers as they follow biogas production utilising non-specific analytical methods. Similar to ISO 14853, ISO 13975 uses a slurry system as matrix, and ISO 15985 is intended for anaerobic biodegradation under high-solids anaerobic digestion conditions.

For these anaerobic tests, similar issues related to physical and chemical properties of the polymers of interest and biodegradation test durations need to be observed as discussed for the aerobic screening tests (Section 4.1.1.1).

4.1.2 Simulation biodegradation tests

Simulation biodegradation test methods include OECD TG 303A-B, 307, 308, 309, and 314A-E (Table 4B). These tests, that are further discussed in the environmental compartment-specific sections below, simulate *"aerobic and anaerobic biodegradation in a specific environment by use of indigenous biomass, media, relevant solids (i.e. soil, sediment, activated sludge or other surfaces) to allow sorption of the test material, and a typical temperature which represents the particular environment*" (OECD, 2006).

Generally, results of simulation tests may include:

- Degradation half-lives ($T_{1/2}$);
- The disappearance time 50 (DT_{50}), i.e. the time within which the test material concentration is reduced by 50% (which is different from the half-life when transformation does not follow first order kinetics).
- Complete mass balances of test systems including quantification of parent and metabolites over time in each test compartment allowing for accurate quantification of biodegradation rates and extent at test completion for parent and metabolites.

Table 4B: Simulation biodegradation test methods potentially suitable for the assessment of polymers

Test method / guideline (endpoint)	Comments	Examples of tested polymers
Biodegradation in soil		
OECD TG 307 (biodegradation in soil)	TG developed for well-defined LMW, water-soluble substances but can be applied to some types of polymers through the use of radiolabelled materials or specific analytical methods. When assessing particulate polymers, care should be taken e.g. regarding effects of size distribution and shape on biodegradability, analytical methods, alternatives to ^{14}C labelling etc., criteria for pass levels & time frames, influences of blending, copolymerisation, additives, etc.	
Biodegradation in surface water and sediment		
OECD TG 308 (aerobic and anaerobic transformation in aquatic sediment systems)	TG developed for well-defined LMW, water-soluble substances but can be applied to some types of polymers through the use of radiolabelled materials or specific analytical methods. When assessing particulate polymers, care should be taken e.g. regarding effects of size distribution and shape on biodegradability, analytical methods, alternatives to ^{14}C labelling etc., criteria for pass levels & time frames, influences of blending, copolymerisation, additives, etc.	
OECD TG 309 (aerobic mineralisation in surface water)		
ISO/DIS 22766 (disintegration, plastic materials, marine habitat, real-field conditions) [draft]	Different structural and morphological descriptors of the polymer (including shape and size) may affect test result; therefore, outcomes should preferably be compared to data for (similar) reference materials	
Biodegradation during wastewater treatment		
OECD TG 303 simulation test - aerobic sewage treatment	TG developed for well-defined LMW, water-soluble substances but can be applied to some types of polymers through the use of radiolabelled materials or specific analytical methods. When assessing particulate polymers, care should be taken e.g. regarding effects of size distribution and shape on biodegradability, analytical methods, alternatives to ^{14}C labelling etc., criteria for pass levels & time frames, influences of blending, copolymerisation, additives, etc.	Polyacrylate in TG 303A (HERA, 2014a) Polyacrylic/maleic acid copolymers in TG 303A (HERA, 2014b)
OECD TG 314A (sewer compartment)		
OECD TG 314B (activated sludge wastewater treatment)		
OECD TG 314C (anaerobic digester sludge)		
OECD TG 314D (treated effluent surface water mixing zone)		
OECD TG 314E (untreated discharge surface water mixing zone)		

Simulation studies, in the great majority of cases, will require the synthesis of radiolabelled test materials. However, such radiolabelling may be particularly challenging for polymers. The position of the radiolabel needs to be carefully chosen, preferably in the part of the polymer that is expected to be most resistant to degradation, normally the polymer backbone. Depending on the chemical nature of the polymer, multiple labelling positions or even multiple test materials labelled in different positions may be required. Furthermore, care needs to be taken to match other polymer characteristics when synthesising the radiolabelled test material e.g. MWD, M_n and LMW fraction. The technical challenges associated with the radiolabelling of polymers and ensuring test material purity and representative identity can result in significant time and costs associated with the evaluation of biodegradability using simulation studies.

Considering the challenges associated with the preparation of radiolabelled substances (e.g. related to their MWD, M_n and LMW fraction), it could be preferable to start with the identification of enzymatic cleavage sites within the polymer chain and then to proceed with radiolabelled fragments for further biodegradation testing.

Application rates in simulation tests should generally reflect the predicted environmental concentration (PEC) in the respective compartment or, as conservative presumptions, 5-fold or 10-fold higher concentrations. However, in contrast to the biodegradation screening tests, they should always lie within an environmentally relevant concentration range. Clearly, application rates must also consider the limit-of-quantification of the respective analytical method while being low enough to ensure that the biodegradation kinetics in the test reflect the degradation kinetics in the field. This may result in application rates that exceed the maximum PEC for the given compartment yielding conservative dose levels of 5- or 10-fold PECs.

Following the quality criteria described in the corresponding OECD TGs, the level of recovery at the end of the simulation biodegradation tests (expressed as mass balance) should ideally be 90-110% of the applied dose for radiolabelled test items and 70-110% for non-labelled test items. When the polymer product is a highly complex mixture, it can be difficult to quantify the parent compound. Similarly, it can be challenging to demonstrate that the mass balance-based quality criteria have been fulfilled, especially for non-labelled test items, unless they do not biodegrade and no non-extractable residues are formed. Further, and as is also the case for non-polymeric substances, identifying and quantifying the spectrum of metabolites (by-products of biodegradation) is challenging even with a radiolabelled backbone.

Compared to other methods, the applicability of OECD TG 307, 308, 309 and the gain of knowledge on the biodegradability of specific types of polymers can be rather limited. Generally, simulation tests have shown limited applicability to non-polymeric UVCBs, and similar limitations have already been observed for complex polymer products with very dissimilar components (see preceding paragraph). Also, the determination of a DT_{50} may be inaccurate for polymer products that are not highly homogeneous since different constituents may be biodegraded at different rates. Such issues were not considered when the simulation tests were first developed, in view of the assessment of mono-constituent substances.

4.1.2.1 Biodegradation in soil

Polymers may reach the soil e.g. upon direct application of formulated plant protection products, fertilisers and soil improvement products onto agricultural land or through land application of biosolids generated during wastewater treatment.

The standard TG to determine the biodegradation of substances in soil is:

- OECD TG 307: Aerobic and anaerobic transformation in soil

This TG is applicable for both water-soluble and insoluble polymers. While it is generally recommended that OECD TG 307 studies should not exceed a duration of longer than 120 days, it can be extended up to 6 months. For polymers that are slow to biodegrade, the test duration might have to be extended beyond 6 months in order to evaluate the complete biodegradation potential of the test item in the soil compartment, while considering that such extensions might result in a decrease in microbial biodiversity or an overall decrease in the numbers of microbes present in the test system over time.

The choice of a specific type of soil in combination with specific properties of the polymer product (e.g. cationicity, anionicity, presence of specific functional groups) may have an impact on bioavailability and thereby also on biodegradation rate. For example, when clay soils are used for the assessment of cationic and anionic polymers, differing cation-exchange capacities might influence the binding potential thereby reducing bioavailability following significant formation of non-extractable residues, with consequences on the biodegradation half-life. Soil-dependent differences in biodegradation rates are revealed by following the OECD TG 307 test protocol that includes assessments in different soil types.

The application rate for a surface-treated application (e.g. agrochemical) should be at the maximum envisaged treatment rate. For polymers entering the soil via sludge application, the application rate should be based on modelled PECs in sludge (Section 5.1.4). If non-direct application is relevant and the input to the terrestrial compartment is from sludge amendments, the test substance can be applied directly to control sludge at the max PEC in sludge and applied to the soil incubates.

The ECETOC Polymers TF is unaware of any scientific publications presenting OECD 307-type soil studies conducted with polymers. A review paper from Scalenghe (2018) cites a number of soil degradation studies for a variety of different types of polymers. However, many of these consider the isolation of specific microorganisms and apply this to the polymer degradation studies.

4.1.2.2 Biodegradation in surface water and sediment

For polymers where a significant portion will potentially be released into the surface water compartment (e.g. effluent from WWTPs, runoff of plant protection products, fertilisers, soil improvement products into water bodies), biodegradation in surface water and sediment needs to be studied, e.g. using:

- OECD TG 308: Aerobic and anaerobic transformation in aquatic sediment systems
- OECD TG 309: Aerobic mineralisation in surface water – simulation biodegradation test

Both TGs are applicable for both water-soluble and poorly soluble polymers (see also Table 4B).

The OECD TG 309 simulation test allows quantifying aerobic biodegradation in natural water (fresh, brackish, or marine). It includes a shake flask batch test system that typically relies on radiolabelled test substances to allow dosing at environmentally relevant concentrations and to quantify primary and ultimate biodegradation rates, but it can also utilise specific analytical methods to quantify primary and ultimate biodegradation. The test can be conducted using surface water only or surface water supplemented with suspended solids or sediment. Generally, OECD TG 309 is applicable to non-volatile or only slightly volatile test substances. As with the other aqueous test systems discussed above (OECD TG 301 and 310, ISO 14851 and 14852), the OECD TG 309 is a dilute test system, and poorly soluble polymers will have bioavailability limitations in terms of their exposure to the microorganisms in the test system. Therefore, extensions of the test duration should be considered to assess the complete level of mineralisation possible for the polymer.

The OECD TG 308 is a multi-compartment test system with both aqueous and sediment phases which can yield complex test results. The test can be conducted using an aerobic and an anaerobic test system. Further, as discussed above for the OECD TG 309, radiolabelled test materials are often used to allow for dosing at environmentally relevant concentrations, quantification of primary and ultimate biodegradation rates, and to enable complete mass balance of the test systems. Notwithstanding, specific analytical methods can also be utilised, if available. OECD TG 308 is not applicable for volatile substances. Depending on their physical or chemical properties, the assessment of polymers using OECD TG 308 can pose specific challenges that need to be addressed in designing and evaluating the study. For example, highly sorptive polymers may be irreversibly bound to sediment so that their bioavailability is significantly reduced. It will be important to carefully apply the OECD TG 308 test method and to critically analyse the test results to obtain a comprehensive understanding of the fate of the polymer in sediment.

Shrestha et al. (2016) systematically compared the OECD TG 308 and 309 test systems using four radiolabelled (non-polymeric) substances with differing biodegradation and sorptive behaviour to evaluate and address any methodological shortcomings. This research highlighted the importance of test conditions (sediment amount and redox conditions due to stirring versus shaking) and the impact of the presence of non-extractable residues on test results. Given that many polymeric materials are sorptive, it will be important to apply learnings from this research in applying these methods to sorptive polymers. It will also be important to consider bioavailability limitations of non-extractable residues in evaluation of biodegradation results when significant portions of the test material are strongly sorbed to the sediment and not bioavailable.

Finally, a test method to determine biodegradation in the marine environment under real field conditions is being developed as ISO/DIS 22766: Plastics — Determination of the degree of disintegration of plastic materials in eulitoral and sublitoral marine habitats under real field conditions.

4.1.2.3 Biodegradation during wastewater treatment

Testing for biodegradation during wastewater treatment can be performed using:

- OECD TG 303: Simulation test - aerobic sewage treatment – A: activated sludge units
- OECD TG 314A-E: Simulation tests to assess the biodegradability of chemicals discharged in wastewater
    - OECD TG 314A: Sewer
    - OECD TG 314B: Activated sludge aeration reactor
    - OECD TG 314C: Anaerobic digester
    - OECD TG 314D: Mixing zone consisting of treated effluent and surface water
    - OECD TG 314E: Mixing zone consisting of untreated wastewater and surface water

The OECD TG 314 series allows for simulation studies in all environmental compartments that are relevant for a polymer disposed down the drain. The series consists of five simulation tests that address biodegradation in critical scenarios relevant for substances released into wastewater. These tests require the use of radiolabelled polymers or specific analytical methods which allow dosing at environmentally relevant concentrations and quantifying primary and ultimate biodegradation rates.

In addition to the OECD TG 314 series, the OECD TG 303A is applicable to polymers and provides the highest tier simulation of the fate of the polymer during secondary wastewater treatment. The OECD TG 303A can be conducted by using indirect measures of polymer concentrations (i.e. DOC for soluble polymers) or direct measurements with radiolabelled polymers or specific analytical methods.

4.1.3 Abiotic degradation

Polymers may be degraded through abiotic and/or biotic factors acting simultaneously or in sequence thereby causing the polymer matrix to breakdown. Time, weather, water, sunlight, oxidants and physical stress are mechanisms of abiotic degradation (transformation) that can alter the state of materials. These abiotic mechanisms also impact the extent to which polymers are available for biological degradation (i.e. biodegradation). The extent to which abiotic factors may transform a polymer depend on the period of time over which they are present, the properties of the material and the environment in which they are present. Although abiotic degradation has been considered a precursor to biodegradation (Gewert et al., 2015), microbial action can open up the surface area of a polymer for additional abiotic degradation. The predominant pathway for abiotic degradation of a polymer may vary depending on whether it consists solely of a carbon-carbon backbone or whether it has heteroatoms in the backbone. Two of the most important types of abiotic degradation are hydrolysis and photodegradation, which lead to breakage of the carbon-carbon bonds in the polymer chain resulting in fragmentation.

In addition, the O_2 present in the atmosphere can induce polymer degradation. Oxidative degradation can be particularly important for non-hydrolysable materials. Introduction of O_2 into the polymer leads to OH and CO formation that can aid in further breakdown processes. Ozone can also enhance the breaking of bonds. Further, physical and mechanical action can cause a polymer to be opened up revealing new sites for oxidative degradation. Unsaturated polymers are generally more susceptible to oxidative degradation.

As these deliberations show, abiotic degradation pathways are very dependent on the environmental circumstances as well as the physical and chemical properties of the material. Action from abiotic degradation alone, or in concert with biotic degradation, contribute to increasing levels of polymer degradation. Importantly, abiotic degradation can be both desirable (e.g. during end of useful life) as well as undesirable (e.g. low durability).

Hydrolysis and photodegradation are discussed in further detail below. Due to the overall paucity of published studies addressing abiotic degradation of polymers (and hence lack of generic recommendations for how to use such methods when assessing polymers), the ECETOC Polymers TF did not draw up any tables for abiotic degradation test methods.

4.1.3.1 Hydrolysis

Hydrolysis is an important abiotic degradation pathway in aquatic, sediment, and soil systems. Hydrolysis occurs when the bonds in a polymer's functional groups are cleaved by reaction with water. Moisture can diffuse into amorphous regions of a polymer matrix and cleave chemical bonds. Functional groups that are susceptible to hydrolysis are first cleaved then chains are cleaved. Hydrolysis of susceptible functional groups is partly dependent on solubility, with poorly water-soluble polymers generally having reduced susceptibility to hydrolysis. Water-insoluble polymers can undergo hydrolytic transformation by bulk erosion (i.e., hydrolysing throughout the matrix simultaneously) and surface erosion (i.e., hydrolysing from the surface only shedding off degradation products) (Murthy et al., 2012). Generally, hydrolysis rates are sensitive to pH, temperature, type of additive, if present, and the presence of hydrolysable covalent bonds (e.g. anhydride, amide, ester, ether, urea, urethane). Hydrolysis under e.g. very alkaline conditions can cause surface erosion of a polymer resulting in lower MWD. Polyanhydride, polyesters, polyamides, polyethers, poly(lactic acid), and polycarbonates are examples of polymers more prone to hydrolysis.

Various TGs are available to study hydrolysis including:

- OECD TG 111: Hydrolysis as a function of pH
- OPPTS 835.2120: Hydrolysis
- OPPTS 835.2130: Hydrolysis as a function of pH and temperature

These TGs describe laboratory methods to assess abiotic hydrolytic transformation in aquatic environments at pH 4-9. Further, the OECD TG 111 eludes to testing at pH 1.3 (gastric acids) if oral exposure to the test item is likely. The three mentioned TGs are very similar in design with minor differences in the way data are treated. Radiolabelled or non-labelled test items may be used as long as there is sufficient analytical accuracy and sensitivity to permit an acceptable mass balance. Generally speaking, these TGs are applicable to at least minimally water-soluble polymers. For poorly water-soluble polymers, solutions are prepared at half their water solubility limit and the use of 1% of a suitable co-solvent is permitted. Accurate measurements may be difficult at these levels making the study not technically feasible for some materials.

For Japanese registration of polymers, hydrolysis testing is a requisite for stability determinations (see also CF4Polymers, Appendix B, Section 2.5.2 (ECETOC, 2019)).

4.1.3.2 Photodegradation

Light can drive reactions that lead to photodegradation of polymers. Under ambient conditions, photodegradation is one of the primary mechanisms by which polymers can degrade. The main processes involved are chain scission and cross-linking reactions when exposed to UV light or visible radiation (Lemaire et al., 1995). Chain scission reactions result in the decrease of the polymer's molecular weight. The types of bonds between the atoms will impact a polymer's ability to degrade in the environment. Polymers that contain photo-reactive groups are generally more susceptible to UV light. Polymers that have removable hydrogen atoms (e.g. polypropylene) are susceptible to UV light, unless appropriate additives have been added.

Importantly, photodegradation is highly dependent on the given environment, e.g. coverage of the polymer with dust, soil particles or microorganisms already limits the potential for direct photodegradation significantly, whereas other matrix components (e.g. humic acids, nitrate) may promote indirect photodegradation effects (Wang et al., 2017).

TGs for photodegradation include:

- OECD TG 316: Phototransformation of chemicals in water – direct photolysis
- OPPTS 835.2210: Direct photolysis rate in water by sunlight
- OPPTS 835.5270: Indirect photolysis screening test: Sunlight photolysis in waters containing dissolved humic substances
- OPPTS 835.2410: Photodegradation on soil
- OECD Proposal for New Guideline of January 2002: Phototransformation of chemicals on soil surfaces; http://www.oecd.org/chemicalsafety/testing/2741541.pdf

4.1.4 Other degradation methods: Industrial composting

'Other' degradation methods include industrial composting, biodegradation of plastic mulch films, and anaerobic digestion of solid waste. This section focuses on industrial composting.

Industrial composting is a controlled process (i.e. with respect to humidity, temperature) that can be divided into two distinct phases, (1) active composting (rotting) followed by (2) curing (post-rotting). The duration of the active composting depends on the type of composting, and it may include both aerobic thermophilic processes and anaerobic processes (adapted from European Bioplastics (2015)). The industrial composting of polymers in finished articles is relevant for e.g. bags used for the collection of organic waste and single-use tableware that can be composted together with food-waste. Different standards and technical specifications are available describing test methods and assessment criteria for biodegradation, disintegration, ecotoxicity and the determination of the levels of heavy metals and other potentially hazardous substances present in the test materials (Table 4C):

Standards describing test methods

- ISO 14855: Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions: Method by analysis of evolved CO_2
    - Part 1: General method
    - Part 2: Gravimetric measurement of CO_2 evolved in a laboratory-scale test
- ISO 16929: Plastics: Determination of the degree of disintegration of plastic materials under defined composting conditions in a pilot-scale test
- ISO 20200 Plastics: Determination of the degree of disintegration of plastic materials under simulated composting conditions in a laboratory-scale test

Technical specifications describing assessment criteria

- EN 13432: Requirements for packaging recoverable through composting and biodegradation: Test scheme and evaluation criteria for the final acceptance of packaging
- EN 14995: Plastics: Evaluation of compostability – test scheme and specifications
- ISO 17088: Specifications for compostable plastics
- ISO 18606: Packaging and the environment – organic recycling

While the intrinsic ultimate biodegradability of a polymer is assessed following ISO 14855 at 58 °C for 180 days, the disintegration of finished articles and products is simulated in a pilot scale test according to ISO 16929 at mesophilic and thermophilic test conditions for a maximum of 84 days. Both of these biodegradation test methods are referred to in the specifications EN 13432, EN 14995, ISO 17088, and ISO 18606.

For example, EN 13432 describes composting under industrial conditions. EN 13432 has been approved and is mandated as harmonised standard within the framework of the EU Packaging Directive (EP and Council, 1994) to show that packaging complies with the essential requirements for organic recycling (European Commission, 2001). The endpoints included in EN 13432 comprise biodegradation, disintegration, adverse effects in plants and limits for specific constituents (e.g. heavy metals). All endpoints should be addressed. The biodegradation criterion describes inherent biodegradation of the material (e.g. powder < 500 µm) with the biodegradability range of > 90% biochemical/ThOD demand over 180 days either in absolute terms or relative to a reference material (microcrystalline cellulose). Disintegration is then determined with the final product (e.g. foil). Here, the test material is sieved on a 2-mm sieve 84 days after initiation of the test. Not more than 10% of the original material shall remain on the 2-mm sieve in order to be considered as disintegrated. The testing for adverse effects includes the OECD TG 208 (*Modified plant growth test*; Section 6.3) which forms an integral part of the testing for adverse effects. Maximum contents for heavy metals and other hazardous substances are given and shall not be exceeded in packaging material and whole packaging.

Table 4C: Test methods to evaluate industrial composting potentially suitable for the assessment of polymers

Standard or technical specification	Comments	Examples of tested polymers
Standards		
ISO 14855-1 Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions — Method by analysis of evolved carbon dioxide — Part 1: General method	Different structural and morphological descriptors of the polymer (including shape and size) may affect test result; therefore, outcomes should preferably be compared to data for (similar) reference materials	PCL, PLA/PBAT blend (Castellani et al., 2016)
ISO 14855-2 Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions — Method by analysis of evolved carbon dioxide — Part 2: Gravimetric measurement of carbon dioxide evolved in a laboratory-scale test		Cellulose, PCL, PLA, PBS (Funabashi et al., 2009)
ISO 16929 Determination of the degree of disintegration of plastic materials under defined composting conditions in a pilot-scale test		Mater-Bi (NICNAS, 2009)
ISO 20200 Plastics — Determination of the degree of disintegration of plastic materials under simulated composting conditions in a laboratory-scale test		Polyethylene, starch, starch/PCL blend (Vaverkova, 2012)
Technical specifications		
EN 13432 Requirements for packaging recoverable through composting and biodegradation – Test scheme and evaluation criteria for the final acceptance of packaging		
EN 14995 Plastics - Evaluation of compostability - Test scheme and specifications		
ISO 17088 Specifications for compostable plastics		
ISO 18606 Packaging and the environment – organic recycling		

Footnote to Table 4C:

Abbreviations: PBS: Polybutylene succinate; PBAT: Poly(butylene adipate-co-terephthalate); PCL: Polycaprolactone; PLA: Poly(lactic acid).

The technical specifications cover control of constituents, biodegradation, disintegration and ecotoxicity assessments.

See Section 2 of this Technical Report and https://www.iso.org/deliverables-all.html; for definitions of ‘standard’ versus ‘specification’.

The EN 13432 is considered equivalent to ISO 17088 which is currently being updated to include further provisions e.g. for the control of constituents and ecotoxicity testing.

As indicated, the fate-related endpoint in the various standards or specifications is not always ultimate biodegradation (i.e. development of CO_2), but the persistence of the polymer. Indeed, physical disintegration may represent the first step of polymer degradation including biodegradation, but degradation may stop after disintegration. Therefore, to ensure full compostability of a polymer, the provisions of the harmonised standards/specifications (e.g. EN 13432) should be followed.

4.1.5 Conceptual framework for polymer (bio)degradation assessment

Disclaimer: The suggested conceptual framework only provides a general outline, but does not imply that all steps are already in place for all types of polymers. It has been drawn up following the state-of-the-art in view of streamlining efforts and expenditure. Prevailing knowledge gaps and technical limitations are presented for the individual tiers, as relevant. Further, the conceptual framework should be considered a 'living proposal' that should be revisited and adapted as further evidence on approaches for polymer biodegradation assessment become available. The need for amendments might become evident as new insight on polymer hazard and risk assessment evolves. Finally, the conceptual framework is by no means considered prescriptive. Individual test methods (and their order of sequence) should be selected on a case-by-case basis as relevant for the polymer of interest (and depending on the applicable legislative framework).

As discussed in the preceding sections, polymer biodegradation assessment is complex and considerations of appropriate test methods and experimental conditions will depend on the physico-chemical properties of the polymer. This section provides a conceptual framework that can be considered for evaluation of polymer product biodegradability (Figure 1). Focus is on aerobic biodegradation testing, which is most likely the predominant form of (bio)degradation for most polymers. As necessary, the assessment may be supplemented by anaerobic biodegradation screening tests and/or abiotic degradation testing, if relevant for the polymer of interest, or for specific release and exposure patterns of those polymers.

For improved clarity, Figure 1 presents a conceptual framework for assessing (bio)degradation only. In practice, application of this conceptual framework will be closely interlinked with the conceptual frameworks for polymer bioaccumulation assessment (Section 4.2.3) and ecotoxicity assessment (Section 6.4). Figure 2 illustrates how the three conceptual frameworks can be interlinked during polymer risk assessment.

4.1.5.1 Tier 0: Identification of (bio)degradation testing needs

Commencement of the conceptual framework for (bio)degradation assessment presupposes that fit-for-purpose identification of relevant morphological and structural descriptors and physico-chemical properties of the polymer of interest has been completed. Knowledge on the physico-chemical properties of the polymer is pivotal for the selection of appropriate test methods and to establish if the testing protocol needs to be adapted to account for, e.g., limited solubility, HMW, etc.

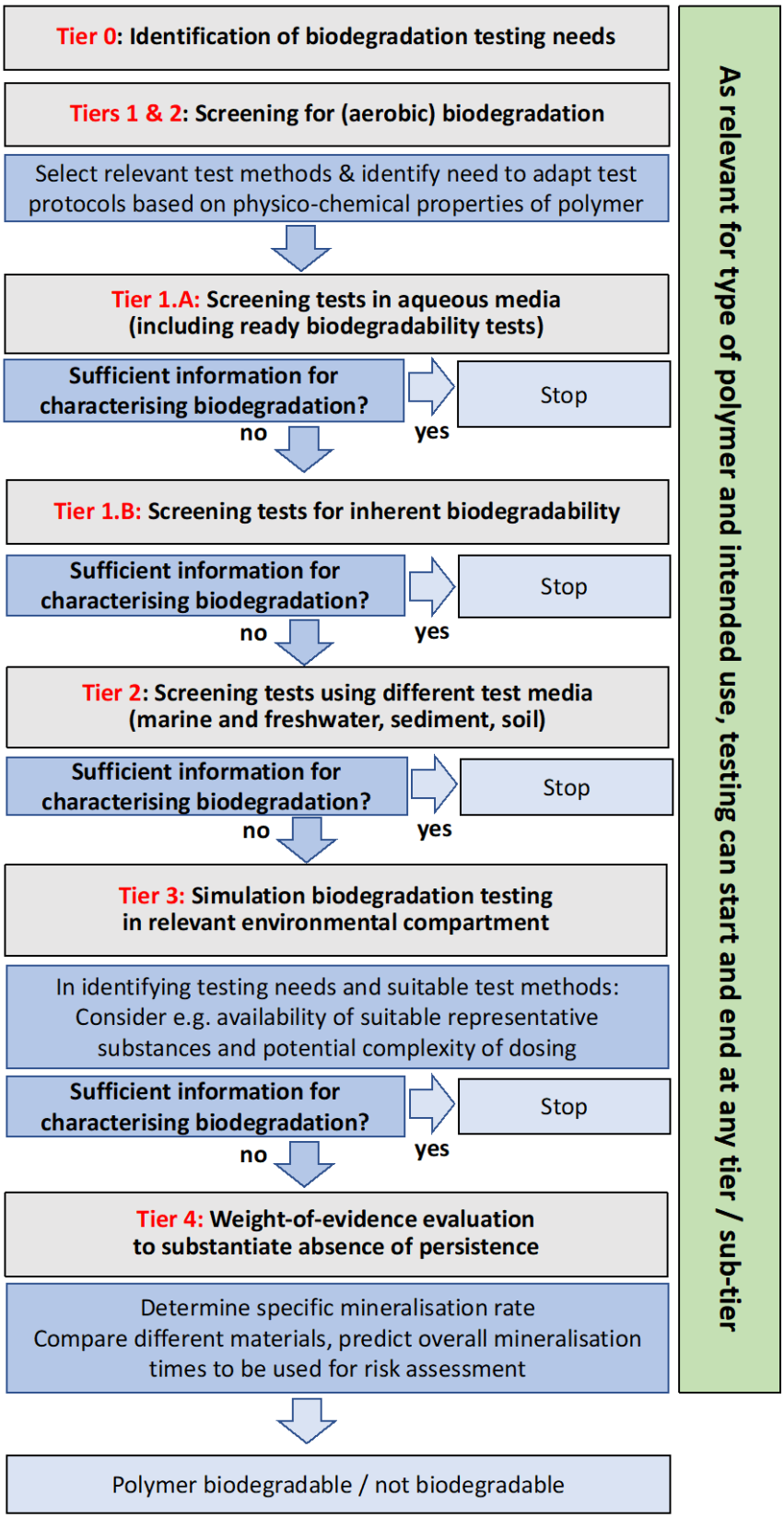

Figure 1: Conceptual framework for polymer (bio)degradation assessment

Footnote to Figure 1: See Section 4.1.5.1 for issues to consider in identifying the applicability of specific test methods for the polymer of interest and Sections 4.1.5.2-4.1.5.5 for examples of test methods that might be used in Tiers 1-3.

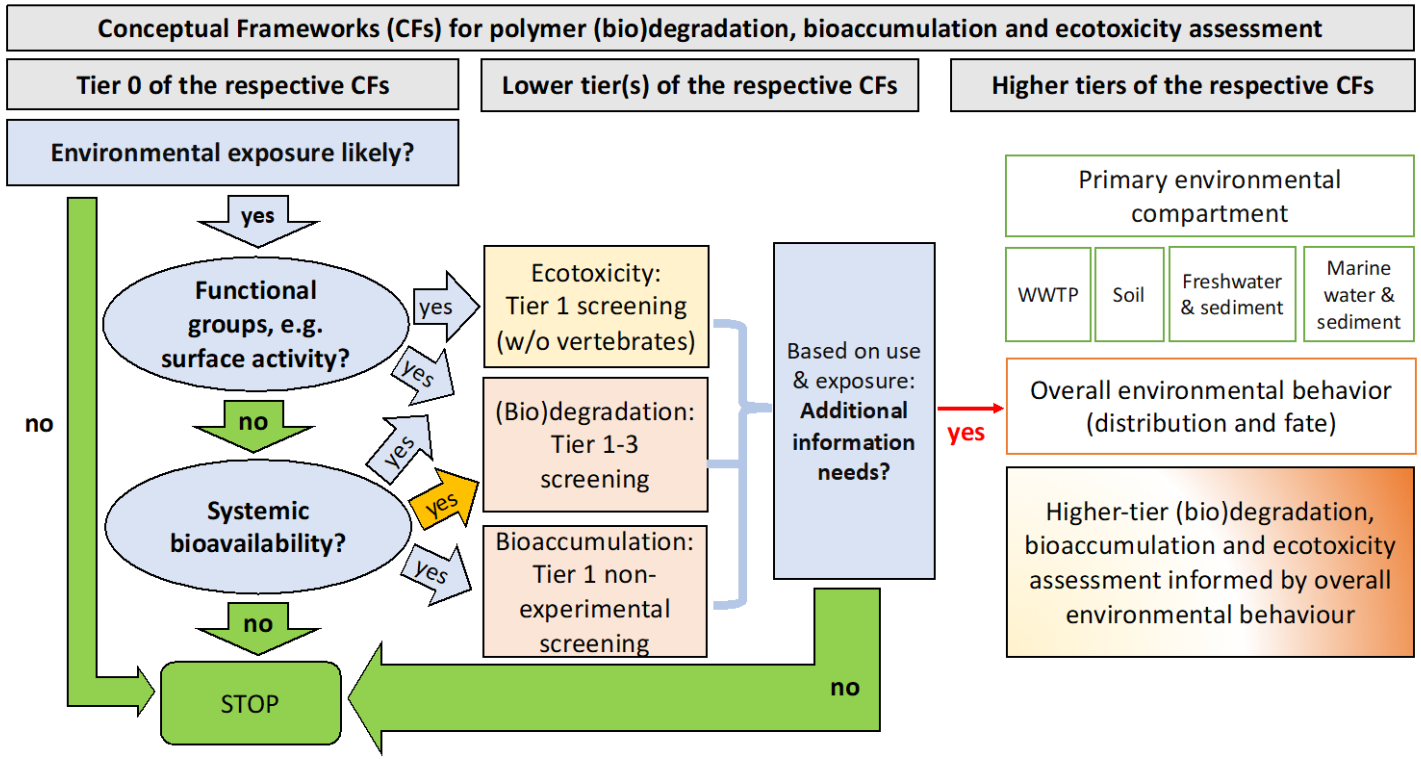

Figure 2: Overview illustrating how the conceptual frameworks for polymer (bio)degradation, bioaccumulation and ecotoxicity assessment are interlinked in practice

Footnote to Figure 2: The orange arrow indicates that absence of systemic bioavailability does not necessarily preclude the relevance of biodegradation assessment, which has to be established in a case-by-case basis for the polymer(s) under investigation. The green arrows indicate sufficient and adequate information to support the safety assessment of the polymer; hence, termination in the generation of experimental data. The red arrow indicates need for generating higher-tier information in addition to information generated at the lower-tier screening. The width of the green arrows and of the red arrow represents a not-to-scale illustration of the proportion of polymers for which the respective step is likely to be relevant.

Generally, the selection of a specific test method should include the following considerations:

1. Would the findings from this test method add knowledge that would be relevant for risk assessment?
    a. **Yes / Maybe**: Continue to question No. 2
    b. **No**: Test should not be performed
2. Is it physically / technically possible to perform the test following the formal, TG-conforming protocol?
    a. **Yes**: Proceed with testing
    b. **No / Don't know**: Continue to question No. 3
3. Can the testing protocol be adapted to enable testing of the given type of polymer (e.g. by adapting testing conditions and/or duration; or by selection of a specific approach for test item preparation that does not change key properties of the polymer)?
    a. **Yes**: Proceed with testing; clearly describe amendments to standard test protocol and provide justification for why adaptation does not change key parameters of polymer of interest
    b. **No / Don't know**: Performing the test runs the risk of yielding data that are of questionable scientific relevance thereby resulting in misleading risk characterisation. Therefore, the test should preferably not be performed. If, however, it is performed in spite of these limitations (e.g. in order to meet specific legal requirements), the technical and scientific limitations should be clearly described and the relevance and reliability of the test results established (in terms of real-world exposure scenarios)

Adaptations of testing protocols should also consider if pre-exposure of the inoculum during Tier 1 screening is indicated by use (see e.g. Stubbs et al., 2004; Itrich et al., 2015).

Beginning with the Tier 1 screening tests and following all tiers in the sequence described below will not be appropriate for all types of polymers and all intended uses. As relevant for the given type of polymer and intended use, testing can start and end at any appropriate tier / sub-tier.

Due to the conservative nature of biodegradation screening studies (low microbial biomass to test item ratio that is not environmentally relevant), a positive result for screening biodegradability studies (Tiers 1.A and 1.B) is indicative for biodegradability in all environmental compartments. As with non-polymeric substances, the Tier 2 screening tests use specific media and the Tier 3 simulation tests are compartment-specific, so degradation evaluations will need to occur (1) if environmental exposure is expected, and (2) for the environmental compartment that the material will likely reside.

Reference materials included in the assessments should mimic the test material as closely as possible in terms of physico-chemical properties as these properties will affect both bioavailability and rates of biodegradation. Natural materials may be useful as reference materials to allow for the evaluation of appropriate physical and chemical properties of the polymers. In this regard, natural polymers are considered in the ISO standards (that also include a selection of suitable polymers as positive controls) listed in Tier 2 of the conceptual framework, which makes these standards very helpful in polymer biodegradation assessment. Notably, naturally occurring polymers are derogated from the *REACH Annex XV proposal for a restriction of intentionally added microplastics* (ECHA, 2019a) as they are expected to be biodegradable.

4.1.5.2 Tier 1: Biodegradability screening tests in aqueous media and/or inherent biodegradability tests

Tier 1.A Screening tests in aqueous media (including ready biodegradability tests)

- Recommended test methods: OECD TG 301B, C, D, F, OECD TG 310
- Testing considerations:
    - Test item characterisation and establishment of accurate ThOD for manometric tests, or $ThCO_2$
    - Test extension should be employed for evaluation of polymeric substances (see discussion for bioavailability in Section 4.2.1)
    - 10-day window should not apply
    - Test vessels and volume may be enlarged
    - Inoculum may be pre-exposed if indicated by use (see e.g. Stubbs et al., 2004; Itrich et al., 2015)

Tier 1.B: Screening tests for inherent biodegradability

- Recommended test methods: OECD TG 302B, C
- Testing considerations:
    - Test item characterisation and establishment of accurate ThOD for manometric tests
    - Test extension should be employed for evaluation of polymeric substances
    - Test vessels and volume may be enlarged
    - Inoculum may be pre-exposed if indicated by use (see e.g. Stubbs et al., 2004; Itrich et al., 2015)
    - Supplementary CO_2 measurements may be considered to allow assessment of ultimate biodegradation

4.1.5.3 Tier 2: Screening tests using different test media (marine and fresh water, sediment, soil)

- Recommended test methods: OECD TG 306, ASTM 6691, ISO 14851 or ISO 14852, ISO 14853, ISO 18830 or ISO 19679, ISO 22404, ISO 23977-1 or ISO 23977-2, ISO 17556
- Testing considerations:
    - The degree of biodegradation is assessed in relation to a reference material
    - The form, size and surface area of the reference material should be comparable to that of the test material

4.1.5.4 Tier 3: Simulation biodegradation testing

Based upon its key physico-chemical properties, the potentially relevant environmental compartment(s) or steps along the exposure path (e.g. sewer water, activated sludge from wastewater treatment, etc.) are identified prior to any simulation biodegradation testing to ensure that all testing is relevant. If a polymer product does not enter a given environmental compartment, simulation biodegradation testing in the corresponding compartment is unlikely to provide relevant data that will be used for hazard or risk assessment. As discussed extensively in Section 4.1.2, for complex polymer products composed of structurally different components, representative test substances will be difficult to identify thus limiting the usability of simulation tests. In other cases where polymer products are single entities or polymeric substances are

composed of structurally similar substances, representative test substances to be used in simulation studies will be easier to identify.

- Recommended test methods: OECD TG 303A, 307, 308, 309, 314A-E
- Testing considerations:
    - Dosing of poorly soluble and non-soluble polymers may be complex
    - Synthesis of representative radiolabelled test substance will be complicated for many polymer products
    - Analytical method development to identify and follow the formation and decline of transformation products
    - Mass balances at study completion may be challenging
    - Test extension may be necessary for polymeric substances

4.1.5.5 Tier 4: Weight-of-evidence evaluation to substantiate absence of persistence

There is an inconsistency between the interpretation of test results of currently available OECD biodegradation tests in Tiers 1-3 that are potentially suitable to indicate polymer biodegradability and typical biodegradation times of natural polymers rated as 'sufficiently biodegradable' (e.g. birch leaves, pine needles; see Appendix of ISO 17556). This inconsistency can be leveraged on a case-by-case basis applying the Tier 4 weight-of-evidence (WoE) evaluation to substantiate absence of persistence.

All available physico-chemical information and (bio)degradation data (also from anaerobic screening tests and abiotic degradation testing, if relevant, for the polymer of interest) shall be used for the WoE evaluation. Specifically, polymer fragmentation and disintegration may be important processes affecting the fate of specific types of polymers in the environment (see schematics by Harrison et al. (2017) and Ehrenstein and Pongratz (2013) for further details).

The WoE evaluation might include the following aspects (not exhaustive): First, the specific mineralisation rate of the polymer is determined. This parameter can be further utilised to compare different materials, to predict overall mineralisation times, and to perform a risk assessment (ECHA, 2019a; Tosin et al., 2019). If the polymer under investigation is a natural polymer that has been modified in a manner that it no longer qualifies as natural polymer but is still structurally very similar to the original material (e.g. sulfonated lignin, methylcellulose or rayon), a direct comparison to the unmodified material should be undertaken. Technically, this could be achieved by directly comparing the biodegradation pattern of the substance and the suitable natural control in a simulation test according to OECD TG 307 or equivalent test method over 2 years including some additional evidence and methodologies such as labelling of the substance to detect residues in soil or in microorganisms to show additional degradation not only via CO_2 production or O_2 consumption.

Ongoing research activities (e.g. Cefic LRI ECO52 *Expanding the conceptual principles and applicability domain of persistence screening and prioritization frameworks, including single constituents, polymers, and UVCBs*[2]) aim at identifying options to adequately distinguish between substances with acceptable but longer degradation times (innate to polymers) and substances with unacceptable persistence.

[2] http://cefic-lri.org/request-for-proposals/lri-eco52-expanding-the-conceptual-principles-and-applicability-domain-of-persistence-screening-and-prioritization-frameworks-including-single-constituents-polymers-and-uvcbs/

4.2 Bioaccumulation, bioconcentration, biomagnification

Bioaccumulation is viewed as an inherent property of a substance that reflects its capacity to accumulate in the tissues of an organism (animal or plant). Bioaccumulation is the net result of the uptake of the substance from all environmental sources (i.e. water, food, sediment, air), its distribution and transformation in the organism and elimination therefrom (Gobas et al., 2009). As a subordinate term to bioaccumulation, **bioconcentration** relates to the net accumulation of a substance in an organism due to aqueous exposure (van Leeuwen and Vermeire, 2007; Burkhardt et al., 2011). As such, bioconcentration relates to bioaccumulation in aquatic organisms; it is usually measured in fish, and sometimes in aquatic invertebrates. Finally, **biomagnification** refers to the net accumulation of a substance in predators upon substance exposure via food ingestion and absorption in their gastrointestinal tract (Gobas et al., 2009). Concerns for bioaccumulation in the food web are greatest for substances that both bioaccumulate in organisms and biomagnify in the food chain due to predator-prey relationships (see Box 3 for definitions).

Information on bioaccumulation is an important aspect of environmental risk assessment and thus generally required for regulatory purposes i.e. to identify PBT or vPvB substances and/or POPs or for classification and labelling (see introduction to Section 4).

Testing approaches for bioaccumulation assessment of soluble or liquid polymers will generally follow those for non-polymeric substances since these materials will generally either dissolve or form stable dispersions or emulsions in aqueous media. Therefore, liquid and soluble polymers are not the main focus of this section, but rather solid, poorly soluble polymer products.

Box 3: Definition of key terms related to bioaccumulation

Bioaccumulation: "*A process in which the chemical concentration in an organism achieves a level that exceeds that in the respiratory medium (e.g. water for a fish or air for a mammal), the diet, or both."* (OECD TG 305)

Bioaccumulation Factor (BAF): *"The steady-state (equilibrium) ratio of the substance concentration in an organism to the concentration in the surrounding medium (e.g. water in natural ecosystems).*" (ECHA, 2017b).

Bioavailability: "*The rate and extent to which an agent can be absorbed by an organism and is available for metabolism or interaction with biologically significant receptors. Bioavailability involves both release from a medium (if present) and absorption by an organism.*" (WHO IPCS, 2004)

--- **External bioavailability**: The condition that some HMW polymers that are too large to cross biological barriers might nevertheless exert local toxicity in tissues (e.g. skin, eyes, respiratory tract). This toxicity may well be due to LMW components (i.e. small oligomers, IAS and NIAS, including unreacted monomers) that migrate under conditions of contact to the transitional fluid (e.g. sweat, tears, saliva), thereby being available to be absorbed and exert their toxic effect. The specific mechanisms by which such effects can occur remain to be determined (ECETOC, 2019).

--- **Internal (systemic) bioavailability** means that the polymer product is absorbed into the blood stream by an organism thereby becoming systemically available and potentially causing systemic effects (ECETOC, 2019).

--- **Physical availability** means that one or more individual components of the polymer product are released from the polymer matrix e.g. by migration or leaching (ECETOC, 2019).

Bioconcentration: The net accumulation of a substance by an organism as a result of uptake directly from its surrounding physical environment only through respiratory or dermal surfaces (Burkhardt et al., 2011).

Bioconcentration factor (BCF): *"At any time during the uptake phase of this accumulation test* [the BCF] *is the concentration of test substance in/on the fish or specified tissues thereof (Cf as mg/kg) divided by the concentration of*

the chemical in the surrounding medium (Cw as mg/L). BCF is expressed in L/kg. Corrections for growth and/or a standard lipid content are not accounted for." (OECD TG 305)

Biomagnification: *"The increase in concentration of the test substance in or on an organism (or specified tissues thereof) relative to the concentration of test substance in the food."* (OECD TG 305)

Biomagnification Factor (BMF): *"The concentration of a substance in a predator relative to the concentration in the predator's prey (or food) at steady-state."* (OECD TG 305)

Notwithstanding, while the bioaccumulation testing of solid, poorly soluble polymer products requires special considerations, it should first be decided if bioaccumulation is relevant at all for the particular polymer: As the definitions for bioaccumulation, bioconcentration and biomagnification show, these processes require uptake, i.e. internal (systemic) bioavailability (Box 3), of the substance under investigation. However, many solid, poorly soluble polymer products are too large to cross biological membranes. Accordingly, Section 4.2.1 presents and discusses internal (systemic) bioavailability and size as key determinants of the bioaccumulation potential of polymer products, and further discusses the challenges in using the K_{ow} to predict bioaccumulation properties. Based thereupon, Section 4.2.2 describes bioaccumulation test methods, and Section 4.2.3 a conceptual framework for polymer bioaccumulation assessment.

4.2.1 Systemic bioavailability as prerequisite for polymer bioaccumulation

Generally, only polymers (or components of polymer products) with internal (systemic) bioavailability are expected to bioaccumulate. To ensure unambiguous usage of the term bioavailability, the ECETOC Polymers TF distinguishes between physical availability, external bioavailability, and internal (systemic) bioavailability; see Box 3 for definitions and Section 3.7.1.1. in the CF4Polymers (ECETOC, 2019) for further discussion of these terms and the importance of internal (systemic) bioavailability for the hazard and risk assessment of polymers.

Vice versa, polymer products that are not physically available or only bioavailable externally (e.g. at the skin, eyes, lungs or gills) will have no potential for bioaccumulation, bioconcentration and/or biomagnification since they cannot be taken up into biota. Polymers that are only (reversibly) adsorbed to the external surface of an organism or that are only temporarily in transit in the gut without being absorbed are not considered as bioaccumulative (Muir et al., 1997). While a variety of poorly soluble particles have been observed to be present in the gut of different aquatic species, only few cases of relatively low systemic uptake have been reported, and these cases related to nano-sized particles (see Section 2.2.1 in the ECETOC TR No. 132 (ECETOC, 2018a) for further discussion). Notwithstanding, polymers that are only bioavailable externally may physically accumulate and e.g. cause obtrusion of the gills of fish (ECETOC, 2018a, OECD, 2019a).

For nano- and micron-sized polymer and non-polymer particles, there are indications that bioavailability may not be best described based upon the equilibrium partitioning theory since these particles might also be taken up into cells by processes other than passive diffusion, including cell surface adhesion, phagocytosis, pinocytosis, and receptor-mediated endocytosis (ECETOC, 2018a). The current database is inconsistent with some studies indicating that nano- and micron-sized polymer particles are taken up into cells or organisms, and others that they are not (reviewed in ECETOC (2018a)). Generally, the available data from studies using mammals suggest that particle uptake rates via non-diffusion processes are very low, resulting in only minimal fractions of particles becoming systemically bioavailable.

Further research work is merited to expand the database on the mechanisms by which (nano- and micron-sized) polymers may become bioavailable in environmental species. Further research work is also merited to develop (mechanistic) test methods for assessing if (nano- and micron-sized) polymers may bioaccumulate by means other than passive diffusion to allow addressing mechanisms of uptake that are not (only) based on chemical partitioning equilibrium.

4.2.1.1 Opportunities and limitations of using polymer size as indicator of bioaccumulation

The size of a polymeric substance is a key parameter determining its potential for systemic bioavailability and hence bioaccumulation potential (see also Dimitrov et al., 2002, 2005; ECETOC, 2018a). Generally, bioaccumulation is considered to be driven by passive diffusion with subsequent accumulation in lipid-rich tissues (Katayama et al., 2010), and passive diffusion is a size-dependent process.

For practicality, the size of a polymeric substance is often directly linked to its molecular weight, neglecting for example its swelling behaviour in different solvents, which is dependent on a variety of characteristics of polymer-solvent interactions. In addition to size, the systemic bioavailability of a polymer product is dependent upon further physico-chemical properties including charge, solubility and partitioning in water and/or biological media, and physical state (Figure 3).

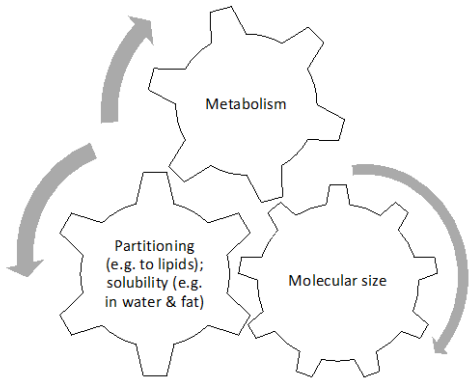

Figure 3: Schematic illustrating that systemic bioavailability is dependent upon different properties of the test item

When determining the need to assess the bioaccumulation potential of solid polymer products, it should be the goal to rule out those polymers that are too large to cross biological barriers to focus the assessment on those polymers for which bioaccumulation may be relevant. These are LMW polymers and polymer products that have a high proportion of LMW compounds. Even if the overall HMW fraction of a solid polymer product may restrict its bioaccumulation potential, its LMW fraction may still pose a concern for bioaccumulation. Therefore, assessments of polymer bioaccumulation should always reflect the behaviour of all molecular weight fractions of the given polymer product. (As discussed in Section 3, this may pose significant analytical challenges.)

These deliberations show that the bioaccumulation assessment of polymers could be facilitated by the identification of a molecular weight-related threshold (or threshold related to any other relevant property) above which no systemic bioavailability and hence no risk for bioaccumulation would be expected.

In those jurisdictions having implemented regulatory provisions on the hazard and risk assessment of polymers, a M_n threshold of ≥ 1,000 Da (and < 10% oligomers with molecular weight < 500 Da and < 25% oligomers with molecular weight < 1,000 Da) is generally applied as indicating 'polymers of low concern' (PLC;

US EPA, 1997b), i.e. polymers that are "*deemed to have insignificant environmental and human health impacts*" (OECD, 2009). However, even if a polymer undercuts the M_n 1,000 Da threshold (or has ≥ 10% or ≥ 25% oligomers with molecular weight < 500 Da or < 1,000 Da, respectively), it should not *per se* be evaluated as 'posing a hazard or risk concern' e.g. as having potential for bioaccumulation (ECETOC, 2019).

In addition to these general thresholds laid down for polymers within the PLC concept, different regulatory agencies have proposed molecular-size thresholds for the bioaccumulation assessment of substances in general (see also ECETOC (2014) *Special Report No. 18 Information to be considered in a WoE-based PBT/vPvB assessment of chemicals*). For example, ECHA (2017c) states that, *"used within a WoE approach and with expert judgment, a substance may be considered as not bioaccumulative […] using the following types of evidence"*:

1. Average maximum diameter > 1.7 nm plus molecular weight > 1,100 Da ('plus molecular weight > 700 Da' indicating 'not vB')
2. Maximum molecular length > 4.3 nm
3. Log K_{ow} > 10
4. Measured octanol solubility (mg/L) < 0.002 mmol/L × molecular weight (Da; g/mol) without observed toxicity or other indicators of bioaccumulation (ECHA, 2017c)

Nota bene: The ECHA (2017c) guidance does not clearly indicate if these four lines of evidence are linked by "and" or "or" conditions. Considering the diversity of the aspects, and the circumstance that they shall be used within a WoE approach, the ECETOC Polymers TF assumes that they are most likely linked by "or" conditions.

ECHA (2017c) further denotes that the indicator value of 1.7 nm for the average maximum diameter was derived using the descriptor from the OASIS software (http://oasis-lmc.org/products/software.aspx), but that the use of different software tools could lead to variable results for the same substance.

The overall variability and hence limited reliability of bioconcentration measurements for larger and very hydrophobic chemicals is discussed in the ECETOC (2014) Special Report. Referring to Arnot et al. (2009) and Nendza and Müller (2010), ECETOC (2014) emphasises that the regulatory molecular-size thresholds have inherent uncertainties, for which reason they are applied conservatively for regulatory purposes. The ECETOC (2014) Special Report does not address the specific bioaccumulation testing needs for polymers.

Further research work is merited to determine the suitability of general regulatory molecular-size thresholds, or any other threshold, to assess the bioaccumulation potential of polymers (further addressing how to evaluate polymer products if the M_n range of their components expands across such threshold values).

Interestingly, the molecular weight threshold reported in ECHA (2017c) for bioaccumulation potential of substances in general (1,100 Da) is similar to the M_n threshold (1,000 Da) in the PLC concept. While the scientific database substantiating these thresholds is limited (OECD, 2009), the ECETOC Polymers TF is also not aware of any scientific evidence for rejecting the PLC concept (ECETOC, 2019).

Clearly, molecular weight / MWD in itself is only a very crude descriptor to determine if a substance can be taken up by an organism and, if so, if it can also be taken up intracellularly. The three-dimensional (3D) molecular structure of a substance, described by average maximum diameter and maximum molecular length, has been suggested as a scientifically more relevant parameter (ECETOC, 2014). In the ECHA (2017c) guidance, the OASIS software module is described as suitable for modelling the 3D molecular structure of substances in general. ECETOC (2014) also refers to OASIS as a suitable model further denoting that other quantum chemical or molecular mechanics calculation tools may also be used if they are fully validated and their domain properly

defined. The ECETOC Polymers TF is unaware of investigations on the applicability of OASIS (or other models) to predict the 3D molecular structure of polymers or of the inclusion of polymers within QSAR training sets.

Further research work is merited to determine the applicability of existing models, or develop new models, as relevant, to predict the 3D molecular structure of polymers.

4.2.1.2 Opportunities and limitations of using the K_{ow} as indicator of polymer bioaccumulation

Since bioaccumulation is considered to be driven by thermodynamically controlled passive diffusion, it can generally be modelled based on knowledge of the equilibrium partitioning. Accordingly, K_{ow}-based predictions of bioaccumulation potential can be relevant for aquatic species provided that a K_{ow} value (or range) is available and relevant for the given type of polymer (see Section 3.4 for further discussion of the challenges and limitations in establishing K_{ow} values/ranges for specific types of polymers). The relevance of using K_{ow} values/ranges for predicting polymer bioaccumulation potential in aquatic species is further restricted by the circumstance that the correlation between a substance's K_{ow} and its potential to bioaccumulate was originally established for semi-volatile halogenated substances such as polychlorobiphenyls (e.g. Tracey and Hansen, 1996). Usage of the K_{ow} for screening level bioaccumulation assessment is also supported by the findings from the European Commission's Joint Research Centre (JRC, 2014) *Review of available criteria for non-aquatic organisms within PBT/vPvB frameworks log K_{ow}*, that, however, does not refer to polymers.

Despite hydrophobicity playing a role in bioaccumulation evaluation (reflected in the K_{ow}), the processes of bioaccumulation, bioconcentration, and biomagnification rely upon the relationship between the propensity of the uptake processes within the organism, and the organism's ability to metabolise (biotransform) and eliminate the chemical (see also Section 4.2.1). Being a physico-chemical endpoint, the K_{ow} does not provide any information on the potential of the substance to be metabolised and eliminated from biota. Additional properties which may mitigate a substance's potential to bioaccumulate are molecular size, water solubility, charge / acidity.

For all of these reasons, the K_{ow} may not be a reliable predictor of bioaccumulation potential for all types of polymers. Specifically, the bioaccumulation screening assessment of per- and polyfluoroalkyl substances (PFAS), as ionogenic fluorinated polymers, is very challenging due to their surfactant-like behaviour and dissociation at environmentally relevant pH values. Their partitioning behaviour in biota is not related to partitioning based on hydrophobicity (K_{ow}), but is rather a likely combination of binding to serum proteins and accumulation in phospholipids (Burkhardt et al., 2011; Armitage et al., 2012; McLachlan, 2018; Droge, 2019).

Further research work is merited to improve an understanding on how molecular weight / MWD, or other physico-chemical properties, affect polymer bioaccumulation.

4.2.2 Test methods for bioaccumulation assessment

Test methods for bioaccumulation assessment include (Section 4.2.2.1) two OECD TGs for *in vitro* biotransformation assessment; (Section 4.2.2.2) *in vivo* test methods for bioaccumulation testing using (invertebrate or vertebrate) aquatic and sediment species and (invertebrate) terrestrial species and plants.

4.2.2.1 *In vitro* biotransformation assessment

Two OECD TGs are available for *in vitro* biotransformation assessment related to aquatic species, i.e.:

- OECD TG 319A: Determination of *in vitro* intrinsic clearance using cryopreserved rainbow trout hepatocytes
- OECD TG 319B: Determination of *in vitro* intrinsic clearance using rainbow trout liver S9 sub-cellular fraction

Both the OECD TG 319A and 319B have been validated for mono-constituent substances of high purity. The general suitability of *in vitro* biotransformation assays to refine estimates of BCFs (see Section 4.2.2.2) of potentially bioaccumulative (non-polymeric) organic substances is being investigated in the Cefic LRI ECO47 programme (*Improving in vitro to in vivo extrapolation models to predict bioconcentration using in vitro biotransformation rates for bioaccumulation assessment in fish*) and other complementary Cefic LRI projects[3].

The applicability of the OECD TG 319A/B assays for the testing of a particular polymer needs to be established on a case-by-case basis. The ECETOC Polymers TF is unaware of any scientific work addressing *in vitro* biotransformation of polymers following the OECD TG 319A/B protocols or the behaviour of specific types of polymers in these assays. Generally, a prerequisite for the applicability of the *in vitro* assay using whole cells (OECD TG 319A) is that the polymer can be taken up by the cells in the suspension. Similarly, a prerequisite for the applicability of the *in vitro* assay using the S9 fraction (OECD TG 319B) is that the polymer can be suspended in a manner that it can reach the S9 fraction. (A general limitation of the S9 fraction-based assay is that it does not account for cellular uptake, i.e. cellular bioavailability.) Hence, polymer solubility and molecular weight / MWD will most likely affect test method applicability. Further, while the analytical monitoring may be challenging in either assay, it must be ensured that the dissipation of the polymer can be followed accurately.

Finally, with respect to *in vitro* biotransformation testing related to terrestrial species, the ongoing Cefic LRI research programmes ECO41 (*Enhanced screening methods to determine bioaccumulation potential of chemicals in air-breathing species*[4]) and ECO44 (*A toxicokinetic mammalian modelling framework for bioaccumulation assessment*[5]) are expected to provide useful insight. However, these research programmes do not specifically address polymers.

Further research work is merited to enhance the understanding of the applicability and limitations of *in vitro* biotransformation assays for the assessment of polymers with potential for bioaccumulation.

4.2.2.2 *In vivo* bioaccumulation testing

In vivo bioaccumulation testing includes the (experimental) determination of bioaccumulation factors (BAFs), BCFs, and/or biomagnification factors (BMFs), as relevant. By analogy to the definitions for bioconcentration versus bioaccumulation, the BAF is the ratio at equilibrium of the concentration in an organism over the

[3] http://cefic-lri.org/projects/eco47-snapfish-searching-for-refined-in-vitro-approaches-to-predict-bioconcentration-in-fish/
[4] http://cefic-lri.org/projects/eco41-improved-characterization-of-partitioning-and-biotransformation-for-screening-organic-compounds-for-the-potential-to-bioaccumulate-in-airbreathing-species/
[5] http://cefic-lri.org/projects/eco44-a-toxicokinetic-mammalian-modelling-framework-for-b-assessment/

surrounding environment and the BCF is the ratio at equilibrium (steady-state) of the concentration in an organism over the surrounding water (see also Box 3 at the top of Section 4.2). Accordingly, the BCF concept is only applicable for water-soluble polymers, whereas the BAF concept may still apply where non-water soluble or particulate polymers reach an organism via the food chain.

The BMF uses a controlled diet spiked with the test item and fed directly to laboratory animals. The OECD TG 305 includes a standard protocol for conducting laboratory BMF studies with fish exposed to the test item administered in the feed. Calculation of the BMF is particularly applicable for test items with a log K_{ow} ≥ 5 and low water solubility (approx. 0.01 – 0.1 mg/L) and, as such, may be an important consideration for some highly lipophilic polymers.

Generally, *in vivo* bioaccumulation test methods include use of a whole-body homogenate or a tissue-specific residue analysis. Whole body homogenates will capture material present in the gut as 'uptake'. This may be useful when dealing with small fish species (fatheads, zebras). By contrast, tissue-specific analyses require larger organisms with well-defined organs/tissues (widely restricting the applicability of this approach for the assessment of invertebrate species).

Further, *in vivo* bioaccumulation tests generally require comparisons of the steady-state BCF and the kinetically determined BCF. Preferably, these two values should match; divergencies elicit additional investigation. However, the determination of steady-state BCFs using whole-body samples has the potential to be highly misleading since they will also include test material present in the gut that is not necessarily taken up systemically. The consideration of gut clearance (considered in the kinetically determined BCF), reduces the potential for misinformation, but frequently requires more samplings (i.e. higher animal numbers).

***In vivo* bioaccumulation testing: Aquatic and sediment species**

The following TGs are available for *in vivo* bioaccumulation testing using aquatic and sediment species:

- OECD TG 305: Bioaccumulation in fish: aqueous and dietary exposure
- OCSPP 850.1710: Oyster BCF

The OECD TG 305 does not provide any specific guidance for the testing of polymers. Generally, bioconcentration (just as bioaccumulation in terrestrial species) can be measured for the whole organism (after grinding) or for a specific organ or tissue. While large compounds may not easily pass the respective biological barriers, attention should also be paid to the bioaccumulation behaviour of unreacted monomers or oligomeric fractions that are part of the polymer product.

In view of the aim to reduce *in vivo* bioaccumulation testing in aqueous vertebrate species (i.e. fish), a test system using the freshwater amphipod *Hyalella azteca* has been suggested as highly predictive of the BCF in fish (Schlechtriem et al., 2019); see also Cefic LRI ECO 40 Project (*Investigations on the bioconcentrations of xenobiotics in the freshwater amphipod* Hyalella azteca *and inter-laboratory comparison of a new BCF test protocol*[6]). Bioaccumulation assessment using *Hyalella* requires some level of acute and chronic toxicity to allow selecting appropriate dosages for bioaccumulation testing. Further, the ECETOC Polymers TF is unaware of any studies addressing the applicability of this test system for the assessment of polymers.

[6] http://cefic-lri.org/projects/eco-40-investigations-on-the-bioconcentrations-of-xenobiotics-in-the-freshwater-amphipod-hyalella-azteca-and-inter-laboratory-comparison-of-a-new-bcf-test-protocol/

The ECETOC Polymers TF is unaware of any scientific publication of an OECD TG 305 study with a polymer as test material. Most likely, the overall paucity of relevant published studies, which also stands true for bioaccumulation testing using terrestrial species (see below) reflects the subordinate relevance of bioaccumulation for many polymer products.

Applying a non-standardised study protocol, Muir et al. (1997) exposed rainbow trout (*Oncorhynchus mykiss*) to three different types of ^{14}C-labelled cationic polymers. The radiolabelled carbon was found associated with the fish gill tissue, while binding was reversible (Muir et al., 1997). Concentrations did not increase with repeated dose, which speaks to a sorption-based mechanism with a finite binding capacity. By contrast, Muir and co-workers do not report any systemic exposure to the cationic polymers.

For water-soluble HMW polyacrylates, the HERA project on polyacrylic acid homopolymers and their sodium salts (CAS No. 9003-04-7; HERA, 2014b) concluded that bioaccumulation is not of concern, and no experiments were performed to determine bioaccumulation potential of these anionic polycarboxylates. This was supported by negative log K_{ow} values observed for anionic polymers (see Section 3.5 for further details on the findings from the HERA (2014a, b) project).

***In vivo* bioaccumulation testing: Terrestrial animal species and plants**

The following TGs are available for *in vivo* bioaccumulation testing related to terrestrial animal species (i.e. in-soil organisms) and plants:

- OECD TG 315: Bioaccumulation in sediment-dwelling benthic oligochaetes
- OECD TG 317: Bioaccumulation in terrestrial oligochaetes
- OCSPP 850.4800: Plant uptake and translocation test

These bioaccumulation TGs should be considered for polymers that are released into the terrestrial compartment or the sediment and where systemic bioavailability is likely and the screening level data indicate potential for bioaccumulation. Expert knowledge should be applied to establish applicability of the TGs for the given type of polymer.

To the best of the ECETOC Polymer TF's knowledge, relevant published literature on the use of these TGs for the assessment of polymers is scarce. Only one single publication was retrieved addressing the bioaccumulation potential of polymers in in-soil organisms. Exposing earthworm (*Eisenia fetida*) to an insoluble anionic styrene-acrylic acid polymer (50,000-60,000 Da) and an anionic styrene-acrylic acid resin polymer (4,500-9,000 Da), very low whole-body tissue BAF (< 1) were recorded (Jop et al., 1998). Tests were performed with ^{14}C-labelled polymers to allow accurate quantification.

4.2.3 Conceptual framework for polymer bioaccumulation assessment

Disclaimer: The suggested conceptual framework only provides a general outline, but does not imply that all steps are already in place for all types of polymers. It has been drawn up following the state-of-the-art in view of streamlining efforts and expenditure, and of reducing vertebrate animal testing needs as far as possible. Prevailing knowledge gaps and technical limitations are presented for the individual tiers, as relevant. Further, the conceptual framework described below should be considered a 'living proposal' that should be revisited and adapted as further evidence on approaches for polymer bioaccumulation assessment become available. The need for amendments might become evident as new insight on polymer hazard and risk assessment

evolves. Finally, the conceptual framework is by no means considered prescriptive. Individual test methods (and their order of sequence) should be selected on a case-by-case basis as relevant for the polymer of interest (and depending on the applicable legislative framework).

As explained in Section 4.2.1, many polymers are too large to cross biological membranes. Therefore, **they are unable to become systemically bioavailable to any relevant degree, and bioaccumulation testing will not be necessary for many polymers**. If, however, bioaccumulation is considered relevant for the particular polymer, the ECETOC Polymers TF suggests following a stepwise approach for bioaccumulation assessment (Figure 4). The individual tiers are presented in further detail below with a focus on the aqueous and sediment compartments, while also referring to the terrestrial compartment.

For improved clarity, Figure 4 presents a conceptual framework for assessing bioaccumulation only. In practice, application of this conceptual framework will be closely interlinked with the conceptual frameworks for polymer biodegradation assessment (Section 4.1.5) and ecotoxicity assessment (Section 6.4). Figure 2 illustrates how the three conceptual frameworks can be interlinked during polymer risk assessment.

All available relevant information collated in the different tiers should be considered together during bioaccumulation assessment. Preferably, a (quantitative) WoE approach should be applied to derive an overall conclusion on the bioaccumulation potential of the test item (Borgert et al., 2011; ECHA, 2017c).

Integrating multiple lines of evidence for the assessment of bioaccumulation can be a challenging process. The Bioaccumulation Assessment Tool (BAT) is a recent spreadsheet-based decision-support system that can be used to systematically collect, generate, evaluate and integrate various lines of evidence relevant to bioaccumulation assessments within a WoE approach (http://cefic-lri.org/toolbox/bioaccumulation-assessment-tool-bat-a-quantitative-weight-of-evidence-qwoe-framework-to-aid-bioaccumulation-assessment/).

Further research work is merited to substantiate the practicality of the conceptual framework for bioaccumulation assessment or to identify any needs for its further refinement.

4.2.3.1 Tier 0: Identification of relevant polymer fraction and pre-selection of relevant environmental compartment(s) and species

Commencement of the conceptual framework for bioaccumulation assessment presupposes that fit-for-purpose identification of relevant morphological and structural descriptors and physico-chemical properties of the polymer of interest has been completed. Further, the physico-chemical properties of the polymer should indicate that systemic bioavailability of the polymer product or of a fraction of the polymer product is possible. If systemic bioavailability is possible, bioaccumulation assessment should focus on the relevant fraction as well as on relevant environmental compartment(s) and species.

The ECETOC Polymers TF suggests a **M_n threshold of < 1,000 Da** to determine the fraction of the polymer product that is relevant for bioaccumulation assessment (further taking into account exposure considerations).

Further research work is merited to either substantiate this threshold or indicate the need for amendment.

Second, the **potentially relevant environmental compartment(s) and hence potentially relevant species should be identified prior to any bioaccumulation screening or testing to ensure that assessments relate to relevant compartments and species**.

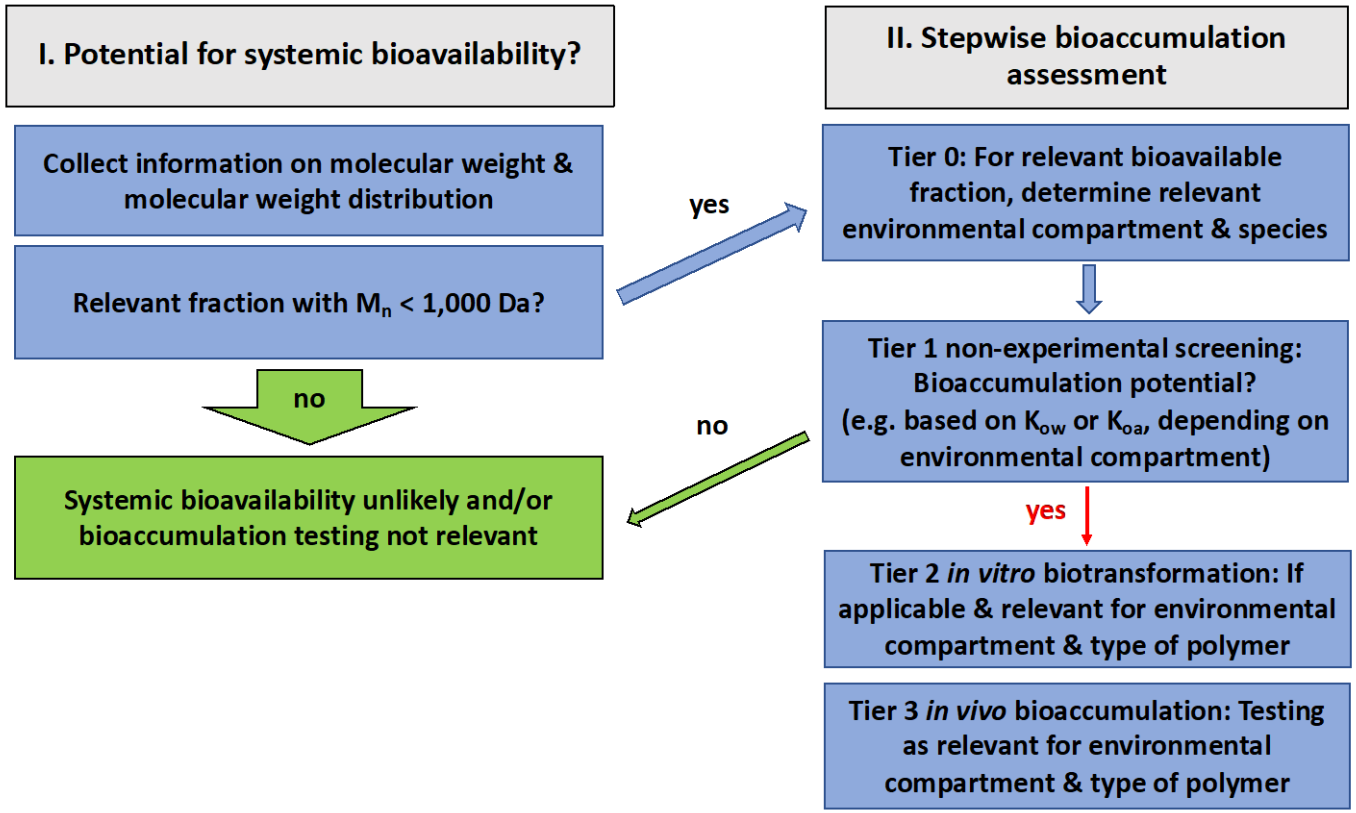

Figure 4: Conceptual framework for polymer bioaccumulation assessment

Footnote to Figure 4: See Section 4.2.3.1 for issues to consider in identifying the applicability of specific test methods for the polymer of interest. Note, the green arrows indicate absence of concern and, hence, termination of the assessment. The red arrow indicates need for additional experimental information. The width of the green arrows and of the red arrow represents a not-to-scale illustration of the proportion of polymers for which the respective step is likely to be relevant. Most likely, the potential for bioaccumulation can be excluded for the majority of polymers without experimental assessment of *in vitro* biotransformation and *in vivo* bioaccumulation potential.

If a polymer product does not enter a given environmental compartment, bioaccumulation assessments in the corresponding animal and/or plant species are unlikely to provide relevant data that will be used for hazard or risk assessment.

Once bioaccumulation testing needs and the relevant fraction of the polymer product have been identified, the selection of a specific tool or method for Tier 1, Tier 2 and/or Tier 3 assessments, as relevant, should include the following considerations:

4. Would the findings from this tool / method add knowledge that would be relevant for risk assessment?
    a. **Yes / Maybe**: Continue to question No. 2
    b. **No**: Test should not be performed
5. Is it physically / technically possible to perform the test following the formal, TG-conforming protocol?
    a. **Yes**: Proceed with testing
    b. **No / Don't know**: Continue to question No. 3
6. Can the testing protocol be adapted to enable testing of the given type of polymer (e.g. by adapting testing conditions and/or duration; or by selection of a specific approach for test item preparation that does not change key properties of the polymer)?
    a. **Yes**: Proceed with testing; clearly describe amendments to standard test protocol and provide justification for why adaptation does not change key parameters of polymer of interest
    b. **No / Don't know**: Performing the test runs the risk of yielding data that are of questionable scientific relevance thereby resulting in misleading risk characterisation. Therefore, the test should preferably not be performed. If, however, it is performed in spite of these limitations (e.g. in order to meet specific legal requirements), the technical and scientific limitations should be clearly described and the relevance and reliability of the test results established (in terms of real-world exposure scenarios)

4.2.3.2 Tier 1: Non-experimental screening for bioaccumulation potential

Assessment of K_{ow}, and/or K_{oa}, if applicable for the given type of polymer

For the Tier 1 non-experimental screening level of the bioaccumulation assessment, the (log) K_{ow} (Section 3.4) is suggested as an indicator of the polymer's potential to bioaccumulate in **aquatic species** provided that it is available and relevant for the given type of polymer (see Section 4.2.1.2 for opportunities and limitations of using the K_{ow} as indicator of polymer bioaccumulation).

For **air-breathing species** additional screening criteria were established in the ECHA (2017c) guidance (i.e. log K_{ow} > 2 and n-octanol-air partition coefficient (log K_{oa}) > 5). This subsection focusses on aquatic species and hence on usage of K_{ow} values.

***In silico* modelling of bioconcentration in aquatic organisms, as relevant**

If considered applicable for the given type of polymer, the screening level bioaccumulation assessment should not only include experimental or non-experimental log K_{ow} data, but also other *in silico* predictions of ecologically relevant parameters (further discussed in Section 5.1). When selecting *in silico* tools to predict bioaccumulation potential, it should again be considered that the assumptions underlying the predictions might not be fully applicable for polymers.

4.2.3.3 Tier 2: *In vitro* biotransformation assessment

The scientific relevance of performing (one of) the *in vitro* biotransformation assays, i.e. OECD TG 319A / 319B (Determination of *in vitro* intrinsic clearance using cryopreserved rainbow trout hepatocytes / rainbow trout liver S9 sub-cellular fraction) should be considered while taking into account the current limited experience in assessing polymer products in these assays (see Section 4.2.2.1).

4.2.3.4 Tier 3: *In vivo* bioaccumulation testing

All data gathered in Tier 1 and Tier 2 should be evaluated together applying a (quantitative) WoE approach before deciding on the need to perform *in vivo* bioaccumulation testing, further considering if the necessary information might also be derived by grouping and read-across (OECD, 2014).

If *in vivo* bioaccumulation testing is considered indispensable, it should also be considered to perform testing in non-vertebrate species before conducting *in vivo* studies using vertebrate species to comply with the legal mandate to refine, reduce and replace animal testing (see Section 1).

In vivo bioaccumulation testing should be conducted in species reflecting the respective relevant environmental compartment (i.e. aqueous and sediment species and/or terrestrial animal species and plants).

Importantly, the selection of test methods should address the current limited experience and potential technical limitations in assessing polymers (see above Section 4.2.2.2). For bioaccumulation testing in water, the procedure for dosing the polymer to the medium is also a critical issue (see Sections 6.1.2 and 6.1.3 for further details).

5. MODELS TO ASSESS POLYMER EXPOSURE POTENTIAL

5.1 Environmental exposure modelling for polymers

Provided that the use as well as the physico-chemical and fate properties of the polymer product of interest indicate likely environmental release or likely hazard potential (either at all or in a given compartment), its environmental exposure assessment follows the same guiding principles as that for non-polymers in terms of the collection and evaluation of information. In the CF4Polymers (ECETOC, 2019), exposure assessment is addressed in Step 5 (Determination of exposure scenarios) and Step 6 (Exposure characterisation).

CF4Polymers Step 5 (Determination of exposure scenarios) includes the qualitative description of the release of the polymer into relevant environmental compartments at its given life cycle stage covered by the defined risk assessment scope taking into account the intended uses and relevant routes of primary exposure. As discussed in further detail in the CF4Polymers, while the determination of exposure scenarios for polymers is not inherently different from that for non-polymers, challenges relate to complexities regarding the supply chain and intended uses of the polymer product (see Figure 5; Tiers 0 and 1).

Use codes (e.g. the use descriptor system published by ECHA (2015) with standardised descriptors for life cycles stages, sectors of use, chemical products categories, process categories, environmental release categories / specific environmental release categories (SpERCs), article categories, and technical functions) enhance comprehensiveness of the description of exposure scenarios and facilitate communication within the value chain and with authorities (ECHA, 2015). SpERCs also have utility in CF4Polymers Step 6 (exposure characterisation) further discussed below (see also Figure 5; Tier 2).

Exposure scenarios are typically developed for a given sector of industry. A series of OECD emission scenario monographs provide descriptions of sectors of industry and of the chemistries within the respective domains of applicability (http://www.oecd.org/env/ehs/risk-assessment/emissionscenariodocuments.htm). Several monographs within this series provide guidance and input on emission scenarios for polymer products. The relevance of the other monographs for further polymer products should be assessed on a case-by-case basis.

Further, industry has developed SpERCs or generic exposure scenarios (GESs) for specific classes of substances developed in the context of the REACH Regulation (EP and Council, 2006), for example:

- SpERCs from the International Association for Soaps, Detergents and Maintenance Products (AISE, 2019) that define environmental release estimates for detergents and cleaning products related to their intended uses
- SpERCs from the European Crop Protection Association (ECPA, 2017) that can be used to assess emissions of co-formulants used in plant protection products.
- GESs from the European Solvents Industry Group (https://www.esig.org/reach-ges/)
- SpERCs from the European Council of the Paint, Printing Ink and Artists Colours Industry (http://cepe.org/reach/)

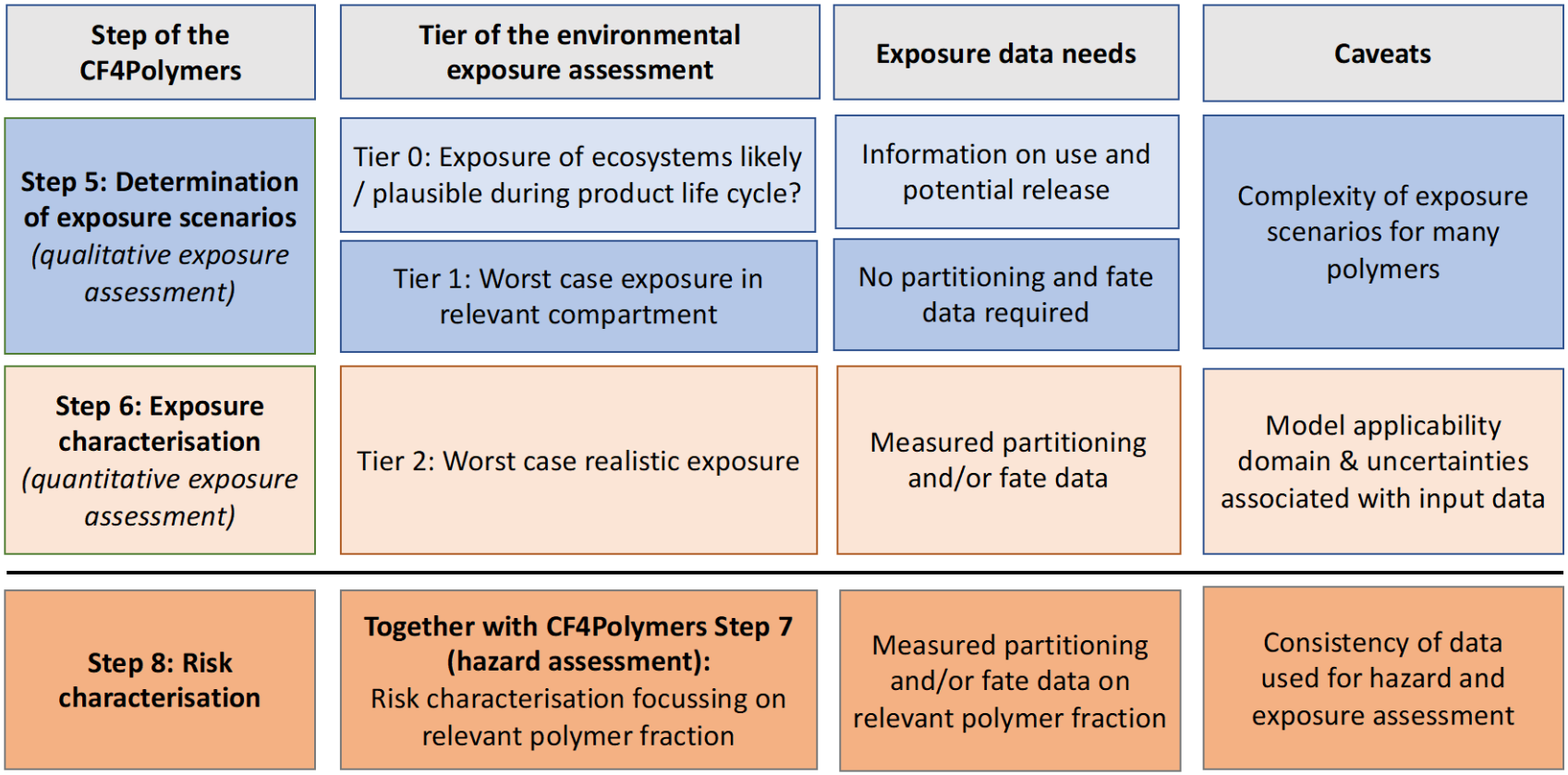

Figure 5: Tiered approach to environmental exposure assessment

Footnote to Figure 5: Illustration for how CF4Polymers Step 5 (determination of exposure scenarios) and Step 6 (exposure characterisation) are embedded in the CF4Polymers. Polymers submitted to the CF4Polymers, i.e. for which risk assessment is relevant, will generally undergo Step 5, whereas Step 6 will most likely be relevant for few polymers only. 'Relevant compartments' (see Tier 1) include water, sediment, soil, etc. The 'relevant polymer fraction' (see bottom row) may include (depending on the polymer chemistry) water extractables, the water-accommodated fraction, etc.

Cefic (2012) has published guidance on sector-specific SpERCs and their application for chemical safety assessments, supply chain communication and downstream user compliance. Information on use-mapping is also available from the website of the European Plastics Converters (https://www.plasticsconverters.eu/) and related presentations (https://echa.europa.eu/documents/10162/22848353/enes_10_1_c_dekort_plastics_en.pdf/db09ccb3-52c1-46de-8a92-2bd52f690e77).

Caution should be used when applying the available OECD emission scenario monographs and SpERCs / GESs to derive emission estimates for polymers, and their applicability for the given type of polymer has to be assessed on a case-by-case basis. For example, in a specific SpERC, the release characterisation may be based on default assumptions on e.g. solubility, that might not apply to the polymer of interest. When an exposure scenario is not available, it might be necessary to collect information on use and release of the given polymer.

CF4Polymers Step 6 (Exposure characterisation): If the exposure scenarios determined in CF4Polymers Step 5 indicate that releases requiring follow-up can occur, emissions into the relevant environmental compartments are quantified in Step 6. **By contrast, if the exposure scenarios, together with key physico-chemical and fate properties do not indicate likely environmental release or likely hazard potential (either at all or in a given compartment), quantitative exposure characterisation is unlikely to yield information that would be useful for risk assessment.** Accordingly, quantitative exposure characterisation will not be necessary for many types of polymers (Figure 5; Tier 2).

If considered necessary for the polymer of interest, the quantitative exposure characterisation considers the exposure scenario documentation, when available, or information on product use for given markets. In the CF4Polymers (ECETOC TR No. 133-1; ECETOC, 2019), the section on Step 6 (exposure characterisation) includes an in-depth discussion of how different components of the polymer product should be considered during exposure characterisation and how LMW component migration and diffusion from the polymer matrix may affect exposure potential.

Within the framework of the EU REACH Regulation (EP and Council, 2006), ECHA (2016a) provides guidance on environmental exposure assessment. Thereby, exposure characterisation includes screening level characterisation of partitioning and fate characteristics followed by an assessment of the distribution of the substance of interest between environmental compartments and the calculation of PECs covering both direct exposure to organisms and exposure via the food chain for predators (ECHA, 2016a).

Quantitative exposure characterisation is mostly performed by *in silico* modelling that considers the screening information on partitioning and fate. Generally, a number of challenges are associated with the quantitative modelling of polymer exposure potential (Di Guardo et al., 2017).

The **first challenge** relates to the **availability and appropriate use of analytical information on partitioning behaviour of the polymer** supporting the modelling, as this may introduce uncertainties to the assessment.

The approach for exposure modelling presented in the ECHA guidance (ECHA, 2016a) follows the methodology used in the *European Union System for Evaluation of Substances (EUSES*; Vermeire et al., 1997; RIVM, 2004; see also https://ec.europa.eu/jrc/en/scientific-tool/european-union-system-evaluation-substances). Notably, application of EUSES requires knowledge of the K_{ow} and K_{oc} of the substance of interest (Table 5). However, fate and exposure estimations that are based on partitioning are generally limited to the distribution of a substance in its molecular form which is only applicable to some water-soluble polymers. For other polymers, the partitioning properties upon which EUSES is based might not be relevant key parameters determining their

Table 5: Overview of exposure models that have been applied to polymers

Characteristic	EUSES	nanoDUFLOW	SimpleBox4nano	iSTREEM
Type of model	Multimedia fate model; includes transport processes in surface and marine waters and one-compartment soil model; uses SimpleBox as regional model to calculate background concentrations resulting from multiple sources across the region; local models include SimpleTreat to model dilution and fate of single substances during sewage treatment; 'local concentrations' also calculated from hypothetical default point source	Fate and transport model, calculates concentrations in river downstream of single point source such as WWTP	Multimedia box model adapted for particles; expected to replace SimpleBox in EUSES for use with nanoparticles and microplastics Calculates regional (background) concentrations resulting from multiple sources across the region	River catchment model which simulates point source discharges from WWTPs in river catchments across the USA
Suitable for which types of polymers	Water soluble polymers e.g. polycarboxylates, polyquaterniums	Water insoluble particulates e.g. styrene acrylate copolymers	Water insoluble particulates e.g. styrene acrylate copolymers	Has been applied to different types of polymers
Removal in WWTP	Not reliable; uses K_{OC} in SimpleTreat, but K_{OC} can generally only be estimated for polymers as it is difficult to derive from experimental data	Not explicit in model; would need to factor in removal when calculating WWTP effluent conc. as model input	No	No; need to specify pre-determined removal rate as model input
Transport in watercourse	No	Yes; nanoDUFLOW hydrodynamic model	No	Yes; river velocity
Settling / sedimentation / resuspension	Partitioning to sediment is modelled using K_{OC} which must generally be experimentally derived for polymers; resuspension not simulated; therefore, overestimation of sediment concentrations near the point of release	Yes; particle aggregation with suspended solids & settling to sediment simulated using Stokes law; resuspension also simulated	Yes; same particle aggregation algorithm as used in nanoDuFLOW	No; settling represented by instream decay rate which accounts for all types of losses in river (settling and degradation)
How is dilution in the watercourse modelled?	Dilution factor of 10 (ECB, 2003) for freshwater (river flow and size not accounted for); dilution factor of 100 for marine discharges	nanoDUFLOW hydrodynamic model accounts for river flow and width/depth	As for EUSES	River flow volume
Input data	Moderate complexity	High complexity	Moderate complexity	Moderate complexity

Table 5 continued

Characteristic	EUSES	nanoDUFLOW	SimpleBox4nano	iSTREEM
Type of input data	Physico-chemical properties of polymer (e.g. molecular weight, K_{ow}, K_{OC}); WWTP removal % and other WWTP parameters; emissions (tonnes)	Particle size and density; river length, width, depth, flow rate; water temperature; homo- and hetero-aggregation attachment efficiencies	Particle size and density; homo- and heteroaggregation attachment efficiencies (or use default value); density, size and conc. of natural particles in receiving waterbody	WWTP removal (sometimes available as per treatment type), emissions, instream degradation
Can different polymer types be modelled?	Yes, but only if polymer can be characterised by physio-chemical parameters such as water solubility, K_{OC}, vapor pressure, Henry constant; model cannot cope with polymer particle size, density, etc.	Yes; need density, particle size and attachment efficiencies; the latter would need to be derived experimentally or assumptions made based on polymer charge	Yes; need density, particle size and attachment efficiencies; the latter would need to be derived experimentally or assumptions made based on polymer charge	Yes, with suitable assumptions for instream losses
What does the model predict?	Annual average PEC in surface water, freshwater & sediment; marine water & sediment; agricultural and industrial soil, air; importantly, also local PEC in different compartments which is assumed to represent the concentration of a WWTP discharge plus the regional background concentration due to multiple discharges in the region	Water and sediment concentrations along the length of the river, downstream from the WWTP discharge	Background concentrations in water, sediment, soil	Freshwater concentrations across the river reaches nationwide (recent model availability for Canada); can generate nationwide concentration distributions (probabilistically) or by site
Main model limitation for modelling exposure of polymers	The physico-chemical properties of solid particulate polymers cannot be correctly modelled with EUSES since the SimpleBox model works on fluxes between compartments (partitioning) Local PECs are probably too high since transport down the river and aggregation and settling of particles over distance is not modelled	Can only do one WWTP discharge at a time, not a whole river catchment; no predictions of cumulative PECs due to multiple WWTPs Model publicly available but not user-friendly; support from provider required for usage	Simple box model; predicts fate between environmental compartments but not transport in a river; only gives estimates of background concentrations; not how concentrations would change downstream of a WWTP	"Instream losses" are estimated using a decay rate which will be highly approximate; can be overcome by generating appropriate data
Main model advantage for modelling microplastics	EUSES is publicly available with user manual; little input requirement; immediate data output	Particle aggregation with other microplastics and suspended sediment is modelled; water concentrations and sediment concentrations should be more accurate than other models	Easy to use within EUSES although not yet integrated; particle aggregation with other microplastics and suspended sediment is modelled	River catchment model; models multiple WWTP discharges across catchment, basin, or on nationwide scale (USA); predicts cumulative PECs due to successive discharges down the catchment

exposure potential and environmental behaviour. For example, for cationic polymers, partitioning does not dominate the fate interactions, but charge and viscosity (molecular weight / MWD and polydispersity) will. In such cases, EUSES will not be applicable.

Further, for many polymers that are present as multi-component substances and maybe even UVCBs, it may not be possible to determine exact K_{ow} / K_{oc} values, but only K_{ow} / K_{oc} ranges (Sections 3.3, 3.4 and 4.2.1). Also, K_{oc} values for polymers can generally not be estimated from K_{ow} values using QSARs as these rely on the *Simplified molecular input line entry system (SMILES)* to predict a specific property by the presence or absence of a specific structural alert, whereas generic SMILES cannot be determined for most polymers that are generally complex, multicomponent polymer products. While the SMILES notation allows representation of the repeating units, it does not capture the entire overlap and intertwining of the polymer chains.

Due to the versatility and complexity of polymers, it is likely that one single exposure model will not be applicable for all types of polymers. Further, to account for polymer complexity, exposure modelling may follow constituent-based approaches, where the fate of individual components is modelled separately and then added up, or block-based approaches, where the fate of groups of substances with similar properties is evaluated together. For the time being, the most appropriate model(s), and approach, need to be determined on a case-by-case basis.

Further research work is merited to determine which specific properties are key parameters governing exposure potential for specific types of polymers and how ranges of specific properties should be applied to the exposure models (e.g. should the highest, lowest or median values be used?).

The **second challenge** associated with the quantitative modelling of polymer exposure potential relates to the **applicability domain of most exposure assessment models**: Many models were developed for LMW, non-polar organic substances and are therefore not suitable for a large range of polymers due to their HMW and/or low solubility (ECETOC, 2018b). Further, many models are limited in their ability to assess charged substances or substances that only undergo partial degradation. Finally, polymer products are typically mixtures whereas many models only allow assessing mono-constituent substances.

Recently, models supporting exposure characterisation of solid polymers have been adapted from models evaluating particle transport (e.g. suspended matter) as their fate and behaviour is driven by particulate properties (e.g. size and density) (Kooi et al., 2018).

Preferably, exposure models should allow the consideration that only a specific fraction of the polymer product might be relevant for exposure assessment. For example, in the aquatic compartment, it is the water accommodated fraction (WAF) that drives the hazard potential; hence, it may also be the relevant fraction to consider during exposure modelling. Overall, care should be taken to ensure that the surrogate used for exposure assessment matches that used for the fate and hazard assessment (e.g. if ecotoxicological effects are described in terms of loading, then fate and exposure need to be described in terms of loading, too). Often, worst-case assumptions assuming no partitioning are applied for fate assessment and modelling (i.e. using separate media / models to assess fate / exposure in air, water, or soil), and often this is sufficient for exposure assessment. However, a given polymer product may also exist in multiple compartments that are further interrelated.

The quantification or modelling of polymer exposure potential in different environmental compartments is presented in further detail below, i.e. (Section 5.1.1) exposure in freshwater and freshwater sediment; (Section 5.1.2) exposure in marine water and marine sediment; (Section 5.1.3) exposure in soil; and (Section

5.1.4) exposure in WWTPs. **Whenever a given modelling tool shall be applied, expert judgement is needed to establish its applicability (and limitations) in assessing the polymer of interest.**

Significant knowledge gaps exist in the exposure assessment for polymers. These gaps range from methods to quantify sorption and degradation to issues with parameter estimation and in some cases complete lack of existing models. Significant research is needed to develop suitable quantitative exposure modelling tools that address the specific needs of different types of polymers. Given the vast differences in properties of polymer products, it is most likely that a broad variety of different approaches are required to handle different types of polymers (e.g. soluble/poorly soluble/non-soluble, charge density, molecular weight / MWD).

5.1.1 Exposure in freshwater and freshwater sediment

The fate of polymers in the freshwater compartment is determined by transport, biodegradation and partitioning to sediment. Polymers entering the aquatic compartment may partition to suspended solids and sediment, depending on their tendency to adsorb onto organic solids. Losses will be due to adsorption to, or aggregation with, suspended solids causing sedimentation, thereby reducing freshwater concentrations. A polymer's physico-chemical properties, and particularly its solubility, will govern its behaviour in the aquatic compartment.

The E-FAST (Exposure and Fate Assessment Screening Tool[7]) provides estimates of the concentrations of chemicals released to air, surface water, landfills, and from consumer products. Within the US EPA Interpretive Assistance guidance document for assessment of polymers (US EPA, 2013), E-FAST is mentioned as a tool to estimate the BCF for polymers with M_n < 1,000 Da. Notably, since E-FAST is conceived to reflect US water sheds, etc., its environmental components are hardly relevant for EU situations.

5.1.1.1 Water-soluble polymers

For certain water-soluble polymers, exposure in freshwater can be predicted using models developed for non-polymers taking into account the complexities related to polymers (see introduction to Section 5) which may increase assessment uncertainty. However, the applicability will have to be determined on a case-by-case basis to demonstrate that the given polymer is included in the applicability domain.

HERA (2014a, b) used the EUSES model to predict freshwater concentrations for a water-soluble polyacrylate. Generally, EUSES can be used for emissions from WWTPs as well as for direct discharge into water bodies such as for polymers in plant protection products carried into water bodies via run-off from agricultural land. For plant protection products, local concentrations can be predicted using the ECPA Local Environment Tool (LET; Section 5.1.3) with degradation rates in aquatic freshwater and sediment entered into the tool followed by usage of EUSES to estimate regional background concentrations. Regional environmental exposure to water-soluble polymers in the aquatic freshwater compartments can also be assessed using the *ECETOC Targeted Risk Assessment (TRA) tool* (http://www.ecetoc.org/tools/targeted-risk-assessment-tra/) or the *ECHA Chemical safety assessment and reporting tool* (CHESAR; https://chesar.echa.europa.eu).

[7] https://www.epa.gov/tsca-screening-tools/e-fast-exposure-and-fate-assessment-screening-tool-version-2014

In principle, the partitioning of water-soluble polymers to sediment can also be modelled by SimpleBox in EUSES (https://www.rivm.nl/en/soil-and-water/simplebox) by applying the K_{OC} (provided that this parameter can be measured for the given polymer; Section 3.5). SimpleBox has served as a regional distribution module in the EUSES model (Table 5, Column 'EUSES').

5.1.1.2 Water-insoluble and poorly soluble polymers

Depending on their size, water-insoluble particulate polymers will either settle by gravity (further controlled by e.g. density and surface charge or polarity) or, for smaller particles, upon aggregation with suspended solids (Besseling et al., 2017). Depending upon the physico-chemical characteristics of the given polymer, quantities entering WWTPs may be subject to efficient removal by skimming and de-greasing/de-oiling stage (Murphy et al., 2016). This fraction is normally recovered and treated as toxic waste. For greater accuracy in exposure predictions, both settling by gravity and aggregation as well as resuspension into the water column should be simulated during modelling. Besseling et al. (2017) developed the NanoDUFLOW model which simulates this aggregation behaviour of particulate polymers while predicting their transport and fate in rivers (Table 5). Besseling et al. (2017) ran a number of different model scenarios varying particle size and density and found that these two parameters significantly affect the particle fate in freshwater rivers. Generally, smaller particles were transported much further downstream. Notably, NanoDUFLOW currently does not account for polymer charge, and, being the intellectual property of Wageningen University (NL), it is currently not available for public use. Notwithstanding, this type of model could be used for higher-tier exposure and risk assessment of polymers where information on the location of polymer particle accumulation in sediments along rivers is required.

A further model to assess exposure in freshwater and freshwater sediment is SimpleBox4nano (https://www.rivm.nl/en/soil-and-water/simplebox4nano; Table 5), which was developed for modelling the fate of colloidal nanoparticles. Just as NanoDUFLOW, SimpleBox4nano simulates particle aggregation behaviour but using a simple box model (in which the compartments air, soil, water and sediment are each represented as a box). SimpleBox4nano estimates steady-state environmental background concentrations for the colloids in the 'boxes' of the environmental system, and it should also be applicable to polymer nanoparticles with a primary particle size up to 100 nm. SimpleBox4nano is a modified version of SimpleBox (Section 5.1.1.1), and it adds first order rate constants for transport- and transformation processes of colloids, where SimpleBox only does so for molecular processes of substances dissolved in water. Unlike SimpleBox, SimpleBox4nano treats partitioning between dissolved and particulate forms of the chemical not as equilibrium speciation but as nonequilibrium colloidal behaviour. Therefore, within each compartment, nanoparticles can occur in different physico−chemical forms: (1) freely dispersed, (2) hetero-aggregated with natural colloidal particles (< 450 nm), or (3) attached to larger natural particles (> 450 nm) that are prone to gravitational forces in aqueous media.

The iSTREEM® model (https://www.istreem.org; Table 5) is an exposure model sponsored by the American Cleaning Institute that allows predicting the concentration of substances used in down-the-drain products in the watersheds of continental USA and several watersheds in Canada. iSTREEM® is comprised of 18,613 river reaches, which are further segmented based on spatial integration of WWTP locations, drinking water intakes. iStreem® depends upon user-based input parameters, i.e. *per-capita* loading, treatment process efficacy and in-stream loss for the substance of interest, to estimate its concentrations in influent, effluent and receiving waters at mean annual flow and low flow (10 years, seven days) conditions. iSTREEM® utilises established modelling for computing in-stream concentrations of point source discharges based on linkage to a river

network, allowing the incorporation of cumulative upstream discharges in concentration estimates (Kapo et al., 2015; Holmes et al., 2019). While the iSTREEM model has mostly been used for the assessment of LMW compounds, Sanderson et al. (2013) report its usage to screen for the occurrence of alcohol ethoxylate surfactants in three US river sediments while highlighting that the database used to validate this model did not include particles with primary sizes below 60-65 µm.

Finally, Cefic LRI is currently funding the research project ECO48 *Develop fate and transport model for microplastics in the aquatic environment*. The results of this project are likely be highly valuable for the aquatic exposure assessment of water-insoluble particulate polymers (Box 6).

Box 6: Cefic LRI ECO48; http://cefic-lri.org/projects/eco48-nano2plast-extending-nanoparticle-models-to-open-source-models-of-the-fate-and-transport-of-microplastic-in-aquatic-systems/

In recognition of the gaps in exposure modelling for microplastics (and water insoluble particulate polymers), ECO48, that took up its work in 2019, aims at developing a regional and global scale fate and transport model for microplastics in the aquatic environment. The model shall

- Take into account and leverage existing fate and transport modelling frameworks for similar particulate matter (i.e., nanoparticles, sediment transport models);
- Identify physico-chemical properties of microplastics that are useful for informing environmental fate and transport of microplastics (e.g. size, density, mechanical durability, aging);
- Determine environmental characteristics that are useful for informing environmental fate and transport of microplastics (i.e., effects of water chemistry on agglomeration or dispersion of microplastic particles);
- Inform on the expected environmental concentrations of microplastics in different compartments to help define realistic exposure scenarios for future ecotoxicity studies, thereby better informing the risk assessment of microplastics.
- Be validated with existing data, with one or more regions selected for which detailed fate and transport data has been previously collected for microplastics.

Follow-up studies are also proposed to further investigate the most important factors affecting fate and transport of microplastics, based on the preliminary sensitivity analysis from the modelling exercise.

Further research work is merited to develop an open source model that can be used to predict local and regional concentrations in freshwater and sediment compartments for water insoluble polymers. While Cefic LRI ECO48 addresses microplastics, and so should be applicable to solid particulate polymers, a model applicable to liquid or semi-solid polymers and/or waxes (which may have different fate behaviour) also needs to be considered. Also, further research work is merited to establish the applicability of SimpleBox4nano, that was developed for nanoparticles, to assess the exposure potential of larger polymer microparticles (primary particle size > 100 nm).

5.1.2 Exposure in marine surface water and marine sediment

EUSES allows generating a marine PEC for discharges entering the sea directly from land sources (e.g. WWTPs) by applying a dilution factor of 100 to account for the increased dilution in the sea. This approach can be applied to water-soluble polymers to generate a steady-state marine PEC for lower-tier risk assessment. Again, a K_{OC} value (Section 3.5) is required to predict the sediment PEC.

Polymers in drilling fluids used in the offshore oil and gas industry which are released into the sea will be dispersed locally and may settle to marine sediments depending on their physico-chemical properties. Water-soluble polymers will dissolve in the water column and be dispersed around the drilling area by ocean currents, while water-insoluble particulate polymers are likely to attach to other solid particles in the drill cuttings discharges and settle to sediment along with drill cuttings and drilling mud solids.

The Dose Related Risk and Effects (DREAM) / ParTrack models developed by SINTEF (https://www.sintef.no/en/software/dream/) have been developed to model the settling and deposition of drill cuttings discharges in the open ocean environment. These models allow the prediction of aquatic concentrations of water-soluble polymers. These models would need to be adapted to account for the aggregation and attachment behaviour of water-insoluble liquid and/or particulate polymers in order to be able to predict sediment concentrations.

Further research work is merited to develop an open source model that can be used to predict local and regional concentrations in marine surface water and marine sediment compartments for water insoluble polymers. While the Cefic LRI ECO48 programme addresses microplastics, and so should be applicable to solid particulate polymers, a model applicable to liquid or semi-solid polymers and/or waxes (which may have different fate behaviour) also needs to be considered.

5.1.3 Exposure in soil

Adsorption to solid surfaces can be an important partitioning process that drives distribution of polymer products in soil, surface waters, and sediments (Section 3.5). The adsorption of a polymer product to soil, sediment, suspended matter and sludge can be obtained or estimated from:

- Direct or indirect measurement e.g. using OECD TG 106, OECD TG 121, OPPTS 835.1110 or ISO 18749 (Section 3.5)
- Adsorption control via inherent biodegradability testing (mostly relevant for soluble polymers) e.g. using OECD TG 302A-C; Section 4.1.1.1)
- Simulation biodegradation testing e.g. using OECD TG 303A-B, 307-309 or 314B (Section 4.1.2)

For soluble polymers, the standard *in silico* models to assess exposure in soil, e.g. EUSES, will generally be applicable. Local concentrations of water-soluble polymers in plant protection products in soil can also be modelled using the LET (ECPA, 2018). Conceptually, a treated 1-hectare agricultural field with an adjacent shallow waterbody is simulated. Tool input parameters include water solubility, soil adsorption (K_{OC}) and fate parameters (e.g. degradation rates in soil). The LET employs equilibrium partitioning calculations performed following the European Chemical Bureau's Technical Guidance Document (ECB, 2003). Based upon the LET modelling, EUSES can then be used to predict regional background soil concentrations of the water-soluble polymers present in plant protection products.

For water-insoluble polymers that will be distributed in the environment as particles, extrapolation based on partitioning coefficients may not be relevant since the partitioning method may underestimate exposure in soil and sediment and overestimate exposure in water. For such water-insoluble polymers, it may be preferable to conduct simulation biodegradation tests or monitoring studies, e.g. in WWTPs (if feasible), to inform on the amount of polymer present in the sludge which would then be applied to land. Extensive field

monitoring studies are available for water-insoluble particulate polymers showing high removal rates (Magnusson and Norén, 2014; Carr et al., 2016; Murphy et al., 2016).

5.1.4 Exposure via wastewater treatment plants

For polymers entering the sewer system e.g. via industrial or professional processes or down-the-drain personal care and laundry products, removal in the sewer and during wastewater treatment must be estimated (Section 4.1.2.3). This will allow determining the mass of polymer entering the soil compartment via sewage sludge application to agricultural land and the mass remaining in the WWTP effluent entering freshwater or marine aquatic compartments.

The SimpleTreat model developed by the Dutch National Institute for Public Health and the Environment (RIVM, 2018) predicts the fate of neutral or organic chemicals in a WWTP (Table 5; see column 'EUSES'). Adsorption to sludge during primary and secondary wastewater treatment is simulated using the K_{oc} as model input if it can be experimentally derived or derived from read-across for other types of polymers (Section 3.5). If a measured or derived K_{oc} value is available, SimpleTreat can be used to predict the mass of a polar soluble polymer which is removed and adsorbed to sludge and the mass remaining in the effluent that will be discharged into freshwater. Biodegradation rates (default from screening studies or measured from simulation studies; Sections 4.1.1 and 4.1.2) can be used to estimate removal during secondary wastewater treatment.

By contrast, SimpleTreat cannot be used for water insoluble particulate polymers, and it is also less suitable to assess ionic polymers. Their WWTP removal rate would need to be determined by higher-tier testing (using e.g. OECD TG 303A and 314B) or monitoring studies from WWTPs (skimmers, settlement tanks, etc.). Generally, such data can be used to simulate WWTP removal where K_{oc} values are unavailable.

Further research work is merited to develop better knowledge on the functioning of WWTPs and to establish removal rates and distribution within the different matrices (effluent water, sludge, de-oiling residue) for different types of polymers.

5.2 Human health exposure assessment

As further discussed in the CF4Polymers Step 6, potentially relevant human exposures include occupational and consumer exposures.

Occupational exposure can occur within industry during polymer manufacture and processing as well as during professional use. Such occupational exposures include potential for dermal and inhalation exposure, whereas oral exposure is generally not considered relevant for occupational scenarios. Industrial occupational exposure focuses on the polymeric substance and/or the polymer product, whereas professional occupational exposure mostly focuses on polymer products (e.g. floor sealants or concrete additives) and polymeric articles.

Consumer exposure can occur by use of goods and articles containing polymer products, and it includes the following potential routes of exposure: dermal and inhalation (e.g. household products, cosmetics, glues, paints) as well as oral (e.g. mouth/lip cosmetics, excipients, food additives).

Further, humans can be exposed indirectly via the environment, and both humans and the environment can be exposed to LMW components migrating from solid polymer matrices.

Exposure potential to polymers in medical devices must be addressed in compliance with the respective applicable legislation. Similarly, the use of additives for food applications must follow e.g. the European Food Safety Authority (EFSA) notes for guidance for exposure assessment. These examples are considered outside the scope of this report.

Many available human exposure modelling tools (e.g. those that characterise exposure associated with different activities or operations or processes related to mass transfer such as evaporation or diffusion) will only be applicable to specific types of polymer products and their components, depending on their physico-chemical properties. The models discussed below can be recommended for human exposure assessment of polymers, unless available evidence indicates that a given model is not applicable for the particular polymer.

Further research work is merited to address the lack of data and methods for estimation of within-polymer diffusion coefficients and the partition coefficients of polymer (constituents) to different media in order to improve predictive power of available exposure models.

As with risk assessment of mono-constituent substances, it may be prudent to assess exposure to polymers and their constituents following a tiered approach. Initial assessments may rely on conservative assumptions and (more) readily available physico-chemical property information to estimate exposure. The intent of such screening approaches is to overestimate actual exposure. If the conservative screening estimates are deemed unlikely to indicate a health risk, then additional effort to characterise exposure may not be warranted. However, if margins of safety (or other appropriate metrics of risk) based on screening estimates are unfavourable, additional work can serve to refine the initial estimates, i.e. by invoking more sophisticated models or measurements to characterise exposure more accurately. Lower-tier exposure assessments generally present point estimates, e.g. as upper bound estimates or as 'typical' and 'high end' estimates. More sophisticated risk assessments may progress to developing probabilistic distributions of exposure.

Initial estimates of exposure to polymers and their constituents rely on exposure factor defaults (i.e. "*factors related to human behaviour and characteristics that help determine an individual's exposure to an agent*" (US EPA, 2011)) since they provide initial estimates of the 'contact rate' between humans and different 'media', such as product use, or environmental media (air, water, diet, etc.). Zaleski et al. (2016) published a global comprehensive resource on exposure factor data and information of relevance for consumer exposure assessments. Further, a number of governmental agencies have published handbooks or compilations of exposure factors that address many different sources of exposure, for example Canada (Richardson and Stantec Consulting Ltd., 2013), China (Duan, 2015), the EU (https://expofacts.jrc.ec.europa.eu), Japan (https://unit.aist.go.jp/riss/crm/exposurefactors/english_summary.html), Korea (Jang et al., 2014), and the USA (US EPA, 2011). In addition to human behaviour related factors, the characteristics of a polymer product itself (e.g. composition, MWD, physico-chemical properties) as well as environmental conditions of use (e.g. ambient temperature, other release-promoting conditions) will govern human exposure potential to polymer products.

5.2.1 Inhalation exposure modelling

Due to their usually higher molecular weight, inhalation exposure to polymers is more likely to arise from applications that involve generation of aerosol particles, such as spray applications, grinding, abrasion, etc. Potential inhalation exposure to polymers from spray applications can be evaluated using tools generally developed to model particle or aerosol deposition in the respiratory tract (see e.g. Corley et al. (2012)). These models are designed for computational fluid dynamics approaches such as the Multi-Particle Pathway Distribution (MPPD) model (https://www.ara.com/products/multiple-path-particle-dosimetry-model-mppd-v-304).

Other publicly available aerosol exposure models, that can be recommended for human exposure assessment of polymers, unless the available evidence indicates otherwise, include:

SprayExpo: This Microsoft Excel-based model was developed by the German Federal Institute for Occupational Safety and Health to predict exposure to aerosols during the spray application of non-evaporating biocidal substances. SprayExpo calculates the airborne concentrations of the various health-relevant particle size fractions. It takes into account turbulent diffusion, droplet evaporation, as well as sedimentation movements and also includes an improved module for calculating the overspray during spraying onto a surface. Notably, for dermal exposure assessment, SprayExpo should be used with caution: While it provides reliable predictions of dermal exposure in room spraying scenarios (relevant for consumer uses), occupational exposure has shown to be underestimated in most cases (https://www.baua.de/EN/Topics/Work-design/Hazardous-substances/Assessment-unit-biocides/Sprayexpo.html).

ConsExpo web (spray model); https://www.rivm.nl/en/consexpo: This model, developed by the RIVM, describes human (consumer) exposure to formed non-volatile aerosols. Key features are the option to account for a cloud volume, to provide experimentally derived particle size distributions, factor in gravitational settling of aerosols, variate mass generation rates for different types of spray devices (e.g. can spray, trigger spray), and to assess oral exposure to non-respirable fraction following spraying.

ConsExpo web (emissions from solid material model); RIVM (2010): This model includes a (one-dimensional) diffusion model to predict substance diffusion in a solid matrix and transfer into air. The model requires a number of input parameters including diffusion and partition coefficients and transport velocity (from the boundary layer to bulk medium) that are dependent on a combination of properties of the substance of interest and article matrix type, concentration of the substance in the matrix as well as article dimensions. The model neglects indoor sinks other than ventilation. Therefore, it will likely overestimate the real air concentrations for less volatile substances for which other sinks, e.g. sorption to dust, will play a significant role. The model is based on limited measured data for plasticisers and flame retardants from a few matrices (including non-polymeric ones). Therefore, the defaults-based assessment results obtained for substances outside of the model's applicability domain will inevitably be of limited accuracy. If this model is used for the evaluation of polymers, the boundaries of applicability should be respected and the results interpreted with caution.

ChemSTEER model: This model is used by the US EPA to estimate occupational exposures for industrial and some professional scenarios. The model allows the user to address built-in scenarios, such as sampling, loading and unloading liquids, and filter media changeout, and also allows the user to define a custom scenario. ChemSTEER includes existing generic spray exposure scenarios that could be used to assess inhalation exposure to polymers in certain applications (US EPA, 2015). Generally, ChemSTEER would also be relevant

for estimating dermal exposure during industrial handling of polymers (during manufacture and processing) (https://www.epa.gov/tsca-screening-tools/chemsteer-chemical-screening-tool-exposures-and-environmental-releases).

Stoffenmanager (https://stoffenmanager.com/): Now available as version 8, Stoffenmanager was developed as a refined tool for estimating inhalation exposure in occupational settings. Stoffenmanager can be used to predict inhalation exposure to non-volatile liquids - including polymers - and powders; however, it is openly recognized that the tool likely underestimates inhalation exposure (van Tongeren et al., 2017). Therefore, the developers recommend application of a higher percentile of the predicted value (i.e. 95%) or meeting a lower risk characterisation ratio (RCR; i.e. 0.5) to compensate for the underestimation. The tool does not address exposure to substances in solid matrices/articles.

Advanced REACH Tool (ART) (https://www.advancedreachtool.com/): ART is a higher-tier inhalation worker exposure model. Polymers fall within its applicability domain when spray applications can generate aerosol or mist. The tool incorporates a database of exposure measurements that may be relevant for the handling of polymers, including data from the handling of low-volatility liquids, solid objects, and powders, granules, or pelletised material. Within ART, these data can be used in conjunction with mechanistically derived exposure estimates. The development of dermal Advanced REACH Tool (dART) is ongoing (Goede et al., 2019; McNally et al., 2019). The beta-version of dART is capable of predicting hand exposure to low volatile liquids (vapour pressure ≤ 10 Pa at 20°C), including solids-in-liquid products, based on the three key processes involved in dermal mass transport, i.e. deposition, direct emission and contact, and transfer. Notably, however, to date dART has an overall poorer precision than the (inhalation) ART for dusts and vapours. Hence, reliability of its predictions will depend largely on the competence of the user and the quality of contextual information available for the particular exposure scenario.

Additionally, the US EPA has compiled a database of exposure-related information (not specifically for polymers) including models, data sets, and guidance (https://www.epa.gov/expobox). The US EPA document on *Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry* (US EPA, 1994) provides a detailed explanation of approaches to estimate inhalation exposure to vapours, aerosols and particles.

Further, different European industry sectors have developed exposure modelling tools specifically for polymers:

The Worker GES for Polymer Processing (https://www.esig.org/reach-ges/workers/) in industrial and professional settings is based on the ECETOC TRA v.3.0 for workers. The GES was originally developed for chemical safety assessment of low volatility solvents (with vapour pressures ranging from 0.01 Pa to 0.5 kPa) under the provisions of REACH (EP and Council, 2006) aiming to cover occupational inhalation and dermal exposure from aerosol and non-aerosol generating activities. Exposure predictions from non-aerosol generating scenarios, however, will represent exposure to polymer components rather than to the polymeric substance itself.

The PESTOOL (https://www.polymercomplyeurope.eu/pce-services/pestool-service), developed by the European Plastics Converters association, is another REACH chemical safety assessment tool. It provides exposure scenarios that are specific to plastic manufacturing and address exposure to hazardous additives. The worker module takes the ECETOC TRA v.2.0 default estimates as a basis and refines them using the 'matrix effect' model, which is based on the diffusion solution from the US EPA AMEM-ADL Polymer Migration

Estimation Model (US EPA, 1990). The environmental module employs environmental emission factors developed by the OECD (https://www.oecd.org/env/plastic-additives-9789264221291-en.htm).

5.2.2 Dermal and oral exposure modelling

For LMW constituents including monomers, oligomers and additives, diffusion through a polymer matrix to the surface and subsequent migration to skin or saliva upon dermal contact or ingestion may be a relevant exposure pathway. A number of screening models exist to predict reasonably conservative dermal and oral (external) exposure to substances incorporated into polymeric matrices.

For example, the ECETOC TRA Consumer v.3.1 for Article Category AC13 (Plastic articles) employs dermal and oral exposure models that are based on the article surface layer thickness parameter. This parameter determines the layer from which a substance incorporated into the polymer matrix can transfer to skin/saliva upon direct contact within the timeframe of an exposure event. Such an approach is considered to be reasonably conservative for estimating human exposure to substances in articles. For plastic articles, the lower default of article surface layer thickness of 0.001 cm (compared to 0.01 cm for liquid mixtures) accounts for the reduced mobility of substances in the article matrix.

Delmaar et al. (2013) applied a simplified mechanistic infinite diffusion model to estimate dermal exposure to substances in consumer articles including those made of plastic. Briefly, the model makes use of the average distance that a diffusing molecule will travel in the article matrix within a given time. The diffusion coefficient and the travel time need to be pre-determined. The main limitation of this model is that it assumes no material-skin transfer resistance (i.e. all of the substance diffused to the article surface will be transferred to skin and become available for dermal absorption). As a result, the model predictions are on average 100-fold higher than the ECETOC TRA estimates for plastic article scenarios. The ECETOC Polymers TF disagrees with the conclusion by Delmaar et al. (2013) on insufficient conservatism of the ECETOC TRA consumer for articles scenarios and considers it unfounded for the following reasons:

- The study shows that designing of even more conservative dermal exposure models than in the ECETOC TRA Consumer tool is possible if important exposure mechanisms are neglected;
- The study shows no evidence that the ECETOC TRA exposure predictions are underestimations of real exposures;
- The exposure values obtained with the model described in Delmaar et al. (2013) go far beyond the realm of reality (e.g. emissions are close to 1 kg of substance per event of contact with clothing).

Hence, the ECETOC Polymers TF concludes that the algorithm described by Delmaar et al. (2013) is unreasonably conservative and cannot be used for semi-volatile organic compounds, for which emissions are mass-transfer limited.

Scenarios from the Consumer Exposure Model (CEM; US EPA, 2019b) can be used to model dermal and oral exposure to substances in articles from handling and mouthing. The model can also account for exposure via inhalation and ingestion of particles emitted from articles (i.e. due to wear of a surface from friction such as flooring). Therefore, at least certain scenarios within CEM can be applied for polymers.

An important use of the US EPA E-FAST tool (see Section 5.1.1 for further details) is to estimate environmental concentrations of substances. E-FAST also predicts the general population exposures to the substance of

interest resulting from contact with environmental media. E-FAST is a screening model that requires a limited number of inputs many of which can be known or estimated for polymers. Since the model relies on mass balance, it can generally be used to screen polymers. However, even though consumer exposure scenarios are also included in E-FAST, it is now preferable to access these scenarios via the CEM platform.

Higher-tier exposure models usually provide capabilities to quantitatively predict diffusion and partitioning of substances from plastic matrices to contact medium. These models are widely used for risk assessment in the area of food contact materials (Limm and Hollifield, 1996; Baner et al., 1996), but might also be applied to estimate dermal (and oral) exposures to chemicals in non-food applications. An older model developed for the US EPA for exploring migration of additives through plastics includes the above-mentioned AMEM-ADL Polymer Migration Model (US EPA, 1990).

In the EU, guidance has been developed to facilitate exposure assessment of components migrating through plastics intended for contact with food (JRC, 2015). The most common modelling approach for exposure to substances in food contact materials via food is based on the diffusion model for chemicals within a solid matrix where the transient diffusion through the material is given by Fick's 2nd Law (Fick, 1855). The key parameters in those models are the diffusion coefficient of the migrant through the food contact material and the partition coefficient between the food contact material and the food, both of which are chemical- and product-specific.

Diffusion coefficients of substances in polymers can be estimated by generally recognised estimation procedures like the Piringer model (Piringer, 2008) based on polymer-specific constants which are available for a limited number of polymers. Estimation of partition coefficients between the contact layer and the contact medium (e.g. polymer and food simulant or water) is based on the migrant's polarity expressed by the octanol/water partition coefficient.

Commercially available software, e.g. Advanced Kinetics and Technology Solutions-SML (https://www.akts.com) and FABES MIGRATEST EXP (https://www.fabes-online.de/en/software-en/migratest-exp/) can predict migration of organic substances including residual monomers, additives, contaminants, reaction products, and NIAS from plastic multi-layer or multi-material multi-layer products into the packaged food or other contact media like pharmaceuticals, cosmetics, drinking water.

The Stochastic Human Exposure and Dose Simulation high throughput model (SHEDS-HT; Isaacs et al., 2014) is primarily relevant to (discrete) organic chemicals. In principle, it should also be applicable for polymers in scenarios where there may be spray exposure or migration to dust, presence in food and exposure to polymer components (i.e. additives) that can migrate from articles.

5.2.3 Exposure measurements

If the default-based predicted exposure evaluated with any of the tools mentioned above does not enable demonstration of safe use of a polymer product or its components, measured data from emission/migration/leaching experiments could be obtained. Different kinds of tests under controlled conditions can be employed to experimentally evaluate the releases and subsequent exposure to a polymer product and its constituents:

Emissions into air can be determined with atmospheric test chamber or microchamber experiments that simulate indoor concentration arising from the emissions of a substance from a product/article specimen

under standard conditions (i.e. at 23°C, 50% relative humidity, 0.1 m/s air velocity, varying air change rates depending on chamber type). Short-term emissions are based on 3-day observations; long-term emissions are determined using the 28-day concentration data.

Given the diversity of global regulatory risk management landscape for polymers, as well as due to the diverse (compositional) variety and complexity of polymer products placed on the market, the primary focus has been on developing methods for testing of migration/release of (potentially hazardous) constituents, and not polymer products *per se*. Methods have been and continue to be developed and applied to estimate migration rate to saliva (to estimate oral exposure from mouthing and teething) and migration to skin through dermal contact (see e.g. US EPA (2017) and DIN 53160 *Testing of coloured toys DIN for resistance to saliva and perspiration*). The migration rate to saliva, as a function of the substance's physico-chemical properties and the article material characteristics, governs oral exposure along with mouthing area, frequency and duration of contact (Steiner et al., 1998; Rijk and Ehlert, 1999; Fiala et al., 2000; Simoneau et al., 2001, Simoneau and Rijk, 2001). Testing methods to estimate migration to skin are less standardised than those for saliva. *Ex vivo* dermal contact tests (Bartsch et al., 2016, 2018) can be performed with (plastic) article specimens to estimate the migration of plastic additives and their degradation products to skin surface (bioaccessibility) or to measure dermal absorption (bioavailability). Migration testing to artificial sweat can also be used to evaluate the releases from plastic material (e.g. JRC (2018)).

Occupational exposure to synthetic organic particles and fibres need to be controlled below the low toxicity dust limits applicable in the given jurisdiction. For example, the US *National Institute for Occupational Health and Safety* and the *American Conference of Governmental Industrial Hygienists* (ACGIH®) published occupational exposure limits of 10 mg/m^3 and 3 mg/m^3 for inhalable and respirable dust, respectively (https://www.cdc.gov/niosh/topics/flavorings/limits.html), and the German Research Foundation's *Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area* set general threshold values for dust of 4 mg/m^3 and 0.3 mg/m^3, for the inhalable and respirable fractions, respectively (DFG, 2019). However, in addition to particle toxicity caused by the plastic dust itself, other agents (e.g. residual monomers, thermal degradation products, processing aids, additives, finishing agents) may be partly responsible for observed adverse health effects (Washko et al., 1998; Burkhart et al., 1999; Porter et al., 1999) and may have to be considered in exposure assessment.

Occupational exposure monitoring data for (total and/or respirable) plastic dust is widely available. For example, the ECETOC Technical Report No. 69 (ECETOC, 1996) summarises the occupational exposure data to respirable fibre-shaped particulates during production, use and disposal of man-made organic fibres in the early 1990s. Thereby, industrial exposure is of the same magnitude as in the general population and ranges typically between 0.01 and 0.1 fibres/cm^3 for commodity fibres and < 0.5 fibres/cm^3 for *p*-aramids[8] (ECETOC, 1996). The 1985 report from the *Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area* of the German Research Foundation (DFG, 1985) indicates that occupational exposure to fine polyvinylchloride (PVC) dust at workplaces in the PVC industry varied between 0.1 and 1.0 mg/m^3. However, PVC dust exposure levels exceeding 10 mg/m^3 occasionally occur even nowadays

[8] *p*-aramid is a widely known asbestos substitute material, and is a type of polyamide fibre similar to nylon fibres. This material is mostly used to improve the strength, durability, and heat resistance of synthetic materials in aviation, automotive and sports industries; it is also used to reinforce fibre for synthetic materials, thermoplastic materials, tires, and rubber products.

(Süyür et al., 2012). More recent data on occupational exposure to chemical agents at thermoplastic processing and PVC production plants in the EMEA region (Europe, Middle East and Africa) indicates that exposure to total respirable particulate is from 0.1 to 4.24 mg/m^3 (HSE, 2010; Abdel-Rasoul et al., 2016) depending on the operation (e.g. grinding, mixing, extrusion). In the synthetic textile industry, the maximum personal exposure to respirable dust can be as low as 0.06 mg/m^3, but the total dust maximum air concentrations may exceed the ACGIH guideline values (Malakouti et al., 2015).

A range of chemicals that are used in the production and finishing of polymers may be hazardous. Biomonitoring (i.e. measuring concentration of indicators of exposure in biological samples) provides an integrated measure of human exposure to contaminants from multiple sources and is often considered a gold standard approach to exposure estimation. Several human biomonitoring campaigns have determined body burdens of chemicals used in plastic manufacture. Some studies have also correlated the levels of those chemicals in blood and urine samples with adverse effects in the human population (e.g. Swan et al., 2005; Lang et al., 2008). Importantly, biomonitoring provides a snapshot in time estimate of an individual or population aggregate exposure to a chemical. Combined with reverse dosimetry using an appropriate physiologically-based kinetic model, such measurements can be used to estimate e.g. equivalent oral doses. However, since biomonitoring data are agnostic with respect to exposure source, such data are difficult to use to identify sources of exposure when a chemical may be present in multiple products or media. Additionally, care must be taken when collecting, analysing, and interpreting biomonitoring information for substances that are rapidly excreted from the body.

6. TEST METHODS TO ASSESS ECOTOXICITY POTENTIAL OF POLYMERS

In the CF4Polymers (ECETOC TR No. 133-1; ECETOC, 2019), the ecotoxicity potential of polymers is addressed in Step 7 (hazard assessment).

A range of TGs is available for determining the ecotoxicity potential of chemicals, and hence potentially also of polymers, e.g., the OECD TG 200 series and the US EPA OCSPP/OPPTS 850 series. This section further discusses the applicability of ecotoxicity test methods using (Section 6.1) aquatic organisms; (Section 6.2) sediment-dwelling organisms; and (Section 6.3) terrestrial organisms. Based thereupon, Section 6.4 describes a conceptual framework for polymer ecotoxicity assessment that follows the overall outline described in Step 7 (hazard assessment) of the CF4Polymers (ECETOC TR No. 133-1; ECETOC, 2019).

Considering the complexity and versatility of different types of polymers, this section only presents general principles to be considered during ecotoxicity testing of polymers. Specific aspects to be considered during ecotoxicity testing are presented in detail in Section 6.1 related to aquatic toxicity testing and then referred to in Sections 6.2 and 6.3 for assessments using sediment-dwelling and terrestrial organisms, as applicable:

- Section 6.1.1: Fit-for-purpose polymer identification prior to aquatic toxicity testing
- Section 6.1.2: Selection of polymer concentrations for aquatic toxicity testing
- Section 6.1.3: Preparation of polymers for aquatic toxicity testing
- Section 6.1.4: Verification of polymer concentrations during aquatic toxicity testing
- (followed by Section 6.1.5: Aquatic toxicity test methods)

For an in-depth discussion of how migration and diffusion of LMW components of the polymer product from the polymer matrix may affect exposure potential, and hence also hazard potential, please refer to the ECETOC TR No. 133-1 CF4Polymers Step 6 (exposure characterisation; ECETOC, 2019).

6.1 Ecotoxicological assessments using aquatic organisms

Generally, aquatic toxicity testing is relevant for polymers that are both likely to reach aquatic compartments and that are at least poorly to moderately soluble in aqueous media and/or that (bio)degrade into water-soluble degradation products. However, also particulate polymers have been observed to be ingested by aquatic invertebrates and vertebrates including coral, barnacles, sea cucumbers, polychaete worms, rotifers, ciliates, crustaceans, amphipods, molluscs and fish (Anderson et al., 2016).

The aquatic hazard assessment of polymers includes identifying the potential for adverse effects via:

- Intrinsic toxicity (E_{iT}) upon exposure; and
- Direct and indirect, physical hazards (E_{Pint}), including physical obstruction of gills, gastrointestinal tract, or carapaces and reduction of light penetration (Besseling et al., 2014; ECETOC, 2018a).
- Further, during (long-term) aquatic toxicity testing, polymer particles may sorb onto food thereby potentially impairing food quality (e.g. by diminishing the nutrient content).

Effects observed in aquatic toxicity tests are generally expressed in terms of concentrations of the dissolved test substance to prevent underestimating the intrinsic toxicity potential. However, *"for some charged polymers, exposure concentrations should be expressed in terms of the whole test substance dispersed in dilution water*" (OECD, 2019a). Depending on the type of polymer under investigations, exposure metrics may include particle counts or surface area, etc., per unit volume of exposure media in addition to common mass-based metrics (e.g. mg/L).

6.1.1 Fit-for-purpose polymer identification prior to aquatic toxicity testing

To enhance the relevance of test results, aquatic toxicity studies (and all other ecotoxicity studies) should always be preceded by fit-for-purpose identification of the polymer of interest (Sections 3.1-3.6).

Water solubility is a key parameter affecting the ready availability of polymers in aqueous media and hence aquatic toxicity potential. Therefore, the preparation of an aquatic toxicity study (or any other ecotoxicity study) should always address whether the polymer of interest is (at least) poorly water soluble, not soluble, or particulate. However, it may be difficult to precisely determine the water solubility of a complex polymer product, so that it may rather be denoted as a range of water solubilities of its different constituents (Section 3.3). Poor water solubility of substances in general may indicate the need for longer exposure periods before aquatic effects may evolve (ECHA, 2017d), and most likely the same applies for polymers. If time to equilibrium between the insoluble fraction and the fraction in solution is decisive, longer-term aquatic toxicity tests may be required to observe manifestation of chronic and sublethal effects. However, the ecotoxicological potential of a polymer may not only depend upon its water solubility, but also on further properties, e.g. molecular weight and charge density. The interrelationships between specific properties of polymers and their aquatic toxicity potential are being addressed in the Cefic LRI ECO46 iTAP programme (first introduced in Section 3.7.1).

The ECETOC TR No. 132 (ECETOC, 2018a) discusses challenges associated with the aquatic toxicity and bioaccumulation testing of sparingly soluble and particulate substances. Therein, the importance of the physico-chemical properties of the test item and how they can affect the bio-physical interactions (E_{pint}) and biological uptake (E_{iT}) in an aquatic toxicity test are highlighted. The morphological attributes of a particulate (size and shape) as well as surface properties (porosity, specific surface area, surface charge, and zeta potential) should be taken in to account when designing the study set-up as well as during the interpretation of the results (ECETOC, 2018a). Ogonowski et al. (2016) compared the effects of spherical polyethylene plastic particles and irregularly shaped polyethylene plastic particles in short- and long-term *Daphnia* studies. Kaolin clay was used as a reference material for comparison of effects. The observed adverse effects were similar for the spherical polyethylene and kaolin, whereas the irregularly shaped polyethylene particles were observed to exert a negative response to reproduction and survival, albeit at very high particle concentrations. The authors concluded that the irregularly shaped polyethylene may have formed aggregates and caused internal damage while passing through the gut of daphnids (Ogonowski et al., 2016). Particle surface charge and zeta potential are key parameters influencing the stability of dispersions (Zhang et al., 2009).

Further research work is merited to develop in-depth knowledge of the significance of morphological and surface properties of polymers with respect to aquatic toxicity intrinsic and physical effects, and to provide standardised methods to allow measuring these properties for a given polymer.

6.1.2 Selection of polymer concentrations for aquatic toxicity testing

Whenever the aquatic toxicity or other ecotoxicity potential of polymers (or any other substance) is assessed, dose levels need to be selected and adjusted to include concentrations that are relevant in the environment. There are various factors to consider before selecting relevant and reliable concentrations of polymers to test for aquatic toxicity testing. These factors include: physico-chemical properties of the polymer (e.g., water solubility and solubility in the testing media), environmental relevance (i.e., based on intended uses and environmental exposure potential), technical feasibility for analytically measuring exposure concentrations, as well as potential regulatory acceptance and classification and labelling needs. For soluble polymers, it is justified to test up to the limit of solubility, whereas for poorly soluble polymers different approaches (e.g., WAF, water-soluble fraction, passive dosing) may need to be employed to avoid confounding effects of undissolved material. If test concentrations are significantly higher than environmentally relevant exposures, care should be taken to ensure the mode-of-action at the higher concentration is not different than the mode-of-action at relevant concentrations, otherwise artefacts can be introduced that are irrelevant to hazard and risk evaluations. Furthermore, for very poorly soluble polymers, consideration should be given to whether aqueous exposures are even the most relevant route to evaluate toxicity versus sediment or dietary-based tests.

While an increasing number of studies addressing the aquatic toxicity potential of solid polymers (specifically, microplastics) is being published, many of these have failed to adequately characterise the test materials or to further evaluate concentrations or mass loadings to derive adverse effects concentrations (Connors et al., 2017). Similarly, very few studies verified if the particles remained stable and homogenous in the test solutions or related the applied test material concentrations to environmentally relevant concentrations (extensively reviewed by Connors et al. (2017)). Indeed, in numerous studies assessing the aquatic toxicity potential of microplastics, the aquatic species were exposed to particle loadings that by far exceeded those observed in the environment. If unrealistically high dosages are applied, the elicitation of adverse physical effects (E_{pint}) may be overestimated and the mechanism of toxicity may not be relevant for typical environmental exposures (Koelmans et al., 2016; Burton, 2017; Beer et al., 2018; Scherer et al., 2018). Burns and Boxall (2018) addressed this mismatch of concentrations of microplastics used in laboratory aquatic toxicity studies compared with maximum environmental concentrations by building a species sensitivity distribution using published no- and lowest-observed effect concentrations for a broad variety of aquatic species tested with different loadings of microplastics particles per litre of exposure medium. Burns and Boxall concluded that the 95% level of the maximum measured environmental concentration of microplastics is 3- to 4-orders of magnitude lower than the hazardous concentration for the most sensitive 5% of species derived from the species sensitivity distribution. However, the authors also identified a number of knowledge gaps that need to be addressed to improve the robustness and relevance of the species sensitivity distributions to permit sound application to probabilistic risk assessments, including testing of relevant particle size and shape fractions and standardisation of testing approaches (Burns and Boxall, 2018). This review also underlines the importance of adopting environmentally relevant dosing rates in aquatic toxicity studies which would, presumably, permit the identification of a cut-off between the derivation of E_{iT} and E_{pint}.

6.1.3 Preparation of polymers for aquatic toxicity testing

Following fit-for-purpose polymer characterisation and selection of appropriate test item concentrations, the adequate preparation of the polymer of interest is of utmost importance to ensure the relevance and reliability of results from aquatic toxicity studies. It may be technically and logistically challenging to adequately prepare poorly soluble or insoluble polymers for aquatic toxicity testing, due to the difficulty in obtaining homogeneous test media (ECETOC, 2018a; OECD, 2019a). This technical limitation may also stand true if the polymers are present as small particles or droplets.

The OECD Guidance Document (GD) 23 (OECD, 2019a) provides general guidance o*n "aqueous phase aquatic toxicity testing of difficult test chemicals"* including polymers. Therein, different approaches to prepare test solutions for aquatic toxicity testing are being described. For solid test materials, usage of appropriate solvents in conjunction with physical mixing is suggested to facilitate the preparation of homogenous test solutions. This can be effective for crystalline materials that have some aqueous solubility. However, added solvents may affect the structure and hence also ecotoxicological properties of different types of polymers (e.g. by swelling or dissolution of the polymer matrix). Solvents may not help distribute insoluble polymers in the exposure media. Preferably, solvents should only be added to the polymer preparations if it is ensured that they do not affect the physico-chemical properties of the polymer of interest or act as a passive dosing source of solvent in the final testing environment. If this appears unattainable, it should be established to which extent the added solvent may affect the properties of the polymer.

While stable dispersions or emulsions can sometimes be produced by the simple expedient of physically mixing the test substance with the aqueous phase, the testing of dispersions and emulsions is not generally advocated for difficult test chemicals (OECD, 2019a). Nevertheless, it may be acceptable for polycationic polymers with a silicone polymer backbone (OECD, 2019a). The ECETOC Polymers TF concurs with this view that polymer dispersions and emulsions should only be applied in exceptional circumstances if actual solubilities of the polymer of interest are not exceeded (which, however, can be difficult to evaluate). Similarly, the use of chemical dispersants or emulsifying agents is not generally advocated because they may physico-chemically interact with the test item thereby potentially affecting the apparent aquatic toxicity. The potential for any method of dispersion or emulsification to cause breakdown of the test item should be verified before using it in the toxicity test (OECD, 2019a). For super absorbent polymers (e.g. acrylamide reticulated polymers), which have a high water-retention capacity due to their pronounced water solubility, no specific testing conditions are recommended (OECD, 2019a).

In the OECD GD 23, 'passive dosing' is described as further technique for establishing and maintaining concentrations of poorly soluble substances (i.e. hydrophobic organic chemicals) in aquatic toxicity tests (OECD, 2019a). Thereby, a biocompatible donor is first loaded with the test item and then included in the test system where it acts as a partitioning donor that controls exposure concentrations throughout the test (Mayer et al. (1999). Passive dosing has been used to control the level and composition of increasingly complex mixtures including UVCBs (Schmidt et al., 2013; Birch et al., 2018; Bera et al., 2018).

If (e.g. HMW) polymers cannot be maintained under stable aqueous exposure conditions, aquatic toxicity testing via dietary test item application might be more appropriate. However, there are currently no formally adopted test protocols for this route of application and hence also no harmonised approaches for how to use the test results for hazard assessment in a regulatory context.

In summary, there is no 'quick and easy fix' to the aquatic toxicity testing of sparingly soluble and particulate test items, and considerations are needed to adapt existing methodology (ECETOC, 2018a). When testing poorly soluble and particulate polymer products, it needs to be considered that particle intrinsic and extrinsic (system-dependent) properties and test system extrinsic properties might influence aggregation, agglomeration, sedimentation and dissolution. For particulates, the relative importance of the physical hazard might decrease with ongoing sedimentation and study duration (depending on the tested species), whereas effects induced by dissolved fractions might become more prominent, due to the inhomogeneity of the test solution.

Further research work is merited to develop protocols for creating and maintaining homogenous preparations of different types of polymers and ensuring the stability of the polymer in the preparations to minimise test artefacts and strengthen reproducibility and interpretability of the test results.

Further research work is also merited to establish for which types of polymers passive dosing might be appropriate.

6.1.4 Verification of polymer concentrations during aquatic toxicity testing

To enhance the relevance of test results, all aquatic toxicity or other ecotoxicity testing of polymers should include verification of the exposure concentration (Section 3.7). This serves to identify the amount of test item that is maintained in the test system over the course of the study, i.e. the proportion of the test item that the organisms may be exposed to.

Whenever possible, chemical-specific methods should be used for exposure verification. Alternatively, techniques capable of detecting changes in total mass of substance, e.g. DOC or TOC, prior to and during testing may be used (OECD, 2019a). However, the TOC and DOC also include organic carbon from constituents of the test media, and such constituents may affect the external and systemic bioavailability of hydrophobic and cationic polymers (or non-polymeric substances) (OECD, 2019a). Therefore, standard test media for aquatic toxicity tests usually have low TOC (< 2 mg/L; see OECD (2019a), Annex 3) even though such low concentrations of TOC in the media may not reflect environmentally relevant exposure conditions.

It may be necessary to assess the extent to which the bioavailability and toxicity of the test item is affected by the TOC or DOC present in the test media, as outlined in Annex 3 of the OECD GD 23 (OECD, 2019a). The OPPTS 850.1085 humic acid mitigation study allows acquiring data on the acute toxicity of substances to fish with and without the presence of naturally occurring dissolved organic substances (e.g. humic acids and their salts). In principle, similar testing can be applied using other biota (e.g. daphnids, algae) to be informative for the respective effects of polymers as well.

For polymers such as polyanionic polymers with carboxylic and phosphoric acids, within-study exposure verification should also address potential for complexation since such chemical reactions may significantly affect their bioavailability and hence also intrinsic toxicity potential. Complexation of e.g. polycarboxylates may also reduce the availability of salts (such as calcium and magnesium) and trace elements in the test medium which are essential for supporting healthy test organisms (OECD, 2019a).

6.1.5 Aquatic toxicity test methods

Table 6 summarises available OECD and OPPTS/OCSPP TGs describing aquatic toxicity test methods and provides an overview of their applicability and technical limitations for the aquatic toxicity testing of specific types of polymers. Generally, aquatic toxicity test methods describing the direct addition of water-soluble test substances to the test system are appropriate for polymer products whose components fully dissolve within the proposed range of test concentrations. The methods are also used to assess potential hazards associated with the dissolved fraction of a polymer product and may be used to characterise physical effects associated with particulates, providing additional controls are in place.

If UVCBs (and hence also some polymer products) are only partially soluble in water, the OECD GD 23 (OECD, 2019a) recommends using the WAF for aquatic toxicity testing since it is prepared individually and not by serial dilution of a single stock solution. The WAF is defined as the "*aqueous fraction containing the dissolved and/or suspended and/or emulsified fraction of a multicomponent substance*" (OECD, 2019a). To prepare the WAF, defined measured amounts of the polymer product are added directly to water and mixed until an equilibrated concentration of dissolved components is achieved in the aqueous phase. Following cessation of mixing and a period of settling to allow phase separation, the aqueous phase, i.e. the WAF, is drawn off for testing with the assumption that the WAF contains the relevant ecotoxicological components (OECD, 2019a). Notwithstanding, a hazard assessment associated with the WAF may only be representative of the soluble fraction of the polymer product, whereas less soluble components in the non-aqueous phase may require testing in a different context (e.g. sediment; addressed in Section 6.2). It is further important to note that the available guidance for how to prepare WAFs (e.g. by extended physical agitation followed by separation in separatory funnels) often specifically addresses petroleum UVCBs (see e.g. Singer et al. (2000)).

Acute aquatic toxicity testing using brackish water (e.g. following the protocol of the OPPTS 850.1075; fish acute toxicity test) may serve to enhance the environmental relevance of the test results. However, such testing is currently not supported by the OECD TGs for aquatic toxicity testing. Within the Cefic LRI ECO46 iTAP programme (first described in Section 3.7.1), it is currently being investigated how environmental exposures should be described and experimental exposure concentrations should be controlled, when DOC compounds such as humic acids reduce the apparent toxicity of cationic polymers (by one or two orders of magnitude).

Sound knowledge regarding the physico-chemical properties of a poorly soluble and/or particulate substance is required prior to preparing and justifying the choice of a particular test system. Furthermore, the properties of the test system are key in understanding and controlling the exposure of the test item within the test system in order to permit an accurate evaluation of the E_{iT} for given test item and/or the E_{Pint} (ECETOC, 2018a). These factors clearly apply to a significant number of polymers and suggests that currently available test guidelines for aquatic toxicity require modification to incorporate additional elements of assessment to accommodate, in particular, the testing of particulate materials. Similarly, the results from studies addressing poorly soluble polymers should be interpreted with caution when the results are used as points-of-departure to derive safety parameters during hazard and risk assessment. Depending on the dosing strategy in the test systems, the effects could inform on intrinsic toxicity and/or physical hazard of polymer products, while dosing with WAF would rather reflect the intrinsic toxicity potential from the dissolved fraction. Importantly, such evaluations should include sound exposure verification.

Table 6: Test methods potentially suitable for assessing the aquatic toxicity potential of polymers

Test method / guideline	Comments	Examples for tested polymers
Toxicity to aerobic and anaerobic bacteria and protozoans		
OECD TG 209: Activated sludge, respiration inhibition test, carbon and ammonium oxidation OPPTS 850.3300: Modified activated sludge, respiration inhibition test OECD TG 224: Inhibition of activity of anaerobic bacteria OECD TG 244: Aerobic protozoan activated sludge	Current test methods generally appear appropriate for safety testing of polymers; polymers discharged to aquatic environments may inhibit microorganism activity both in aerobic (e.g. supernatant liquid in sewage treatment plants) and anaerobic (e.g. subnatant sludge) zones; it is essential that chemicals do not inhibit microorganism activities in either zone	A variety of polymers have been tested in the OECD TG 209, 224 and/or 244, including halogenated linear polyamines, aromatic, cycloaliphatic, linear aliphatic polyacrylates, polymercaptans, reaction products of waxes and fatty acids, linear, cycloaliphatic polyamides [a]
Toxicity to algae and aquatic plants		
OECD TG 201 (OPPTS 850.4500 & 850.4550): Freshwater algae and cyanobacteria, growth inhibition test	All three TGs generally appear to be suitable for the assessment of polymers; test protocol modifications may be required e.g. depending on polymer charge and solubility (to be determined on a case-by-case basis); for coloured – or other light-scattering – polymers, testing using algae may not be suitable Relationship between OPPTS TG 96-hour final cell density and OECD TG 201 72-hour growth rate tests requires further evaluation; fundamentally r (OECD TG 201) is higher than y-based endpoints (secondary in OECD TG 201, primary in OPPTS TGs)	A variety of polymers have been tested in the OECD TG 201, including halogenated linear polyamines, aromatic, cycloaliphatic, linear aliphatic polyacrylates, reaction products of waxes and fatty acids, iinear, cycloaliphatic polyamides, alkoxylated linear polyelectrolytes [a]
OECD TG 238: Aquatic plants		
OECD TG 221 (OPPTS 850.4400): *Lemna* sp. growth inhibition test		
Short-term toxicity to invertebrates (e.g. crustaceans, molluscs)		
OECD TG 202 (OPPTS 850.1010): *Daphnia* sp. acute immobilisation test OPPTS 850.1020: Gammarid amphipod acute toxicity OPPTS 850.1025: Oyster acute toxicity OPPTS 850.1035: Mysid acute toxicity OPPTS 850.1045: Penaeid acute toxicity OPPTS 850.1055: Bivalve acute toxicity ISO 20665 / EPA 1002.0: *Ceriodaphnia*	OECD TG 202 might be appropriate for water soluble polymers, however, insoluble or poorly-soluble polymers exerting particulate effects (e.g. biological surface coating potentially inhibiting moulting or inducing gut clogging) will not be considered OPPTS TGs cover a wider array of species and there is a more complete view on applicable marine species (fish and daphnia)	A variety of polymers have been tested in the OECD TG 202, including halogenated linear polyamines, aromatic, cycloaliphatic, linear aliphatic polyacrylates, reaction products of waxes and fatty acids, linear, cycloaliphatic polyamides, alkoxylated linear polyelectrolytes [a]

Table 6 continued

Test method / guideline	Comments	Examples for tested polymers
Long-term toxicity to invertebrates		
OECD TG 211 (OPPTS 850.1300): *Daphnia magna* reproduction test OECD TG 242: *Potamopyrgus antipodarum* reproduction test OECD TG 243: *Lymnaea stagnalis* reproduction test	Exposure verification may require labelling of compounds (^{14}C, ^{3}H, SI) until cold, specific analytical techniques become more widespread Effects driven by dissolved and particulate fractions need to be distinguished during long-term exposure periods and the ratio will change over time	
Short-term toxicity to fish		
OECD TG 203 (OPPTS 850.1075): Fish, acute toxicity test OECD TG 212: Fish, short-term toxicity on embryo and sac-fry stages OECD TG 236: Fish embryo acute toxicity test [b]	These test methods investigate aqueous exposure (albeit at very low concentrations of the test items in the aqueous media) and generally appear appropriate for polymers Test methods also consider indirect physical effects; relevant e.g. for cationic polymers, known for the external interactions with organisms	A variety of polymers have been tested in the OECD TG 203, including halogenated linear polyamines, aromatic, cycloaliphatic, linear aliphatic polyacrylates, reaction products of waxes and fatty acids, and linear, cycloaliphatic polyamides [a] Polymers addressed in the validation of the OECD TG 236 included Luviquat HM552 and Merquat 100 (OECD, 2012); since, many more polymers have been tested in the OECD TG 236
OPPTS 850.1085: Fish acute toxicity mitigated by humic acid	Added value of this test is that it allows acquiring data on acute toxicity to fish with and without the presence of naturally occurring dissolved organic substances	
Long-term / early development stages (vertebrates) [c]		
OECD TG 210 (OPPTS 850.1400): Fish, early life-stage toxicity test		

Footnote to Table 6:

[a] As per information that is freely available on the ECHA dissemination portal; https://echa.europa.eu.

[b] Further, a fish embryo sediment contact assay has been developed (Rocha et al., 2011).

[c] If invoked by special circumstances, testing using further aquatic long-term / developmental toxicity studies using vertebrates may be considered for specific types of polymers (depending on their intended uses in combination with the outcomes of short-term studies), e.g. OECD TG 204 (Fish, prolonged toxicity test (14-days); OECD TG 215: Fish, juvenile growth; OECD TG 230 (21-day fish assay); OECD TG 231 (Amphibian metamorphosis assay); OECD TG 234 (Fish sexual development test); OECD TG 240: (Medaka extended one generation reproduction test); and OECD TG 241 (Larval amphibian growth and development assay).

For the assessment of short-term aquatic toxicity in vertebrates, the fish embryo toxicity test (OECD TG 236) should be considered as replacement to the acute aquatic toxicity test using fish (OECD TG 203) giving attention to the duration of post-hatch exposure to ensure the eleutheroembryo has indeed been exposed. Busquet et al. (2014) included testing of moderately soluble polymers in the validation program for the OECD TG 236. Further, application of the threshold approach for acute fish toxicity (OECD, 2010) should be considered; preferably in combination with the fish embryo test (Section 6.4.2.1). In the vast majority of cases evaluated where all sets of acute toxicity data (algae, daphnids, fish embryos, fish) are available, Rawlings et al. (2019) demonstrated that data from the fish embryo test could indeed replace the use of fish acute toxicity. Further development and validation are required to determine whether or not the Rainbow Trout Gill Cell Assay (ISO 21115), that has been included in the work plan for the OECD TG Programme in June 2019 (OECD, 2019b), can be used as a non-animal approach to determine the acute aquatic toxicity of polymers.

In the absence of aquatic data, a critical body burden approach (ECETOC, 2011b) could be used for read-across between compartments and species (unless the hazard is related to disruption of diet). However, the applicability of such an approach needs to be determined on a case-by-case basis, since the currently available critical body burden approaches (reviewed in ECETOC (2011b)) were not established for water-insoluble particulate polymers (but may be applicable for other types of polymers).

Notably, additional guidance on testing methods to evaluate effects of microplastic particles including quality assurance criteria and the determination of microplastic effect thresholds for aquatic species are being evaluated in the ongoing Cefic LRI project ECO49 *Microplastic effect thresholds for aquatic species* (http://cefic-lri.org/projects/eco49-microplastic-effect-thresholds-for-aquatic-species-metas/).

Further research work is merited to identify the need to adapt aquatic toxicity test methods for the assessment of specific types of polymers. For example, if the goal is to address the water-soluble fraction of these materials, such research work should aim at identifying specific physico-chemical properties of polymers that indicate the need to perform testing with the WAF (instead of the test item as such). As far as possible, guidance should be developed for how such parameters should be applied in a practicable manner to determine appropriate testing conditions. Also, new preparation techniques may need to be developed for the preparation of WAFs appropriate for different types of polymers. Similar research work is merited to enhance the understanding and to develop guidance on the appropriate use of solvents and humic acid amendments.

While polymer aquatic toxicity testing using marine water most likely generally necessitates the same considerations as aquatic toxicity testing using freshwater, the current knowledge base on specific items to be considered when testing different types of polymers merits further consideration.

Further research work is merited to develop a critical body burden approach for different types of polymers to facilitate read-across between polymers with similar physical, chemical and/or biological properties.

Finally, further discussion is merited to determine the relevance of test results and to seek agreement on how they should be used as points-of-departure to derive safety parameters for hazard and risk assessment. Further work is also merited to incorporate all new evidence into formal TGs and to modify the TGs to account for more relevant exposure conditions.

With respect to publications addressing the aquatic toxicity potential of polymers, an increasing amount of studies are available addressing the environmental fate and effects of microplastics (e.g. as reviewed by Desforges et al. (2015) and De Sá et al. (2018)). De Sá et al. (2018) concluded that the majority of such studies

were conducted on marine species, and when freshwater organisms were evaluated, *Daphnia magna* and fish were the most common species.

Finally, while many polymers may be too large to cross biological membranes or to be taken up into cells (see also Section 4.2.1) so that testing for ecotoxicity potential will not be relevant, nano-sized polymers (that are generally not the focus of the present report) have been observed to negatively affect primary producers (e.g. algae) and also be transferred to higher trophic levels: Exposure to water-insoluble nano-sized polystyrene reduced population growth and chlorophyll concentrations in the green alga *Scenedesmus obliquus*, and *Daphnia magna* fed with algae exposed to this polymer exhibited reduced body size and altered reproduction, including smaller and less numerous offspring and an increased rate of malformations (Besseling et al., 2014). When *Daphnia magna* fed with algae mixed with nano-sized polystyrene particles were fed to carp (*Carassius carassius*), repeating this 3-day feeding cycle for 61 days, the carp exhibited reduced feeding activity, behavioural changes, metabolites in liver and muscle, and brain histology abnormalities (Mattsson et al., 2015).

6.2 Ecotoxicity test methods using sediment-dwelling organisms

Sediment-dwelling (benthic) organisms might be exposed to different types of polymers via, e.g., consumer down-the-drain products that enter receiving water bodies in effluent (in the dissolved phase or sorbed to solids) and then partition to the sediment, or via agricultural applications and run-off.

Table 7 summarises available TGs describing ecotoxicity test methods using sediment-dwelling organisms and indicates their applicability and/or technical limitations for the hazard assessment of different types of polymers. The table lists commonly used test methods that may be useful for assessing the sediment toxicity potential of polymers and has a focus on OECD TGs; further sediment toxicity test methods using a broad spectrum of sediment-dwelling organisms are listed in Table R.7.8-5 in ECHA (2017d). Generally, testing for chronic effects in sediment dwelling organisms is expected to be the most relevant exposure scenario, unless punctual short-term exposure can be justified.

The available TGs to study sediment effects are generally appropriate for polymers. Current TGs allow for the use of polar and non-polar solvents to deliver the test item to the sediment matrix. However, added solvents may modify the structure and/or systemic bioavailability of the polymer of interest and hence, possibly, also its fate and ecotoxicological properties (Section 6.1.3). Preferably, the use of solvents should be avoided, but depending on the type of polymer under investigation and the type of test system, their use may be indispensable. In such cases, it should be aimed to establish the extent to which the selected solvent modifies the structure and/or bioavailability of the polymer of interest. Direct addition of the polymer test item by precise weighing followed by thorough homogenisation can also be performed.

All sediment toxicity studies should be preceded by fit-for-purpose analytical characterisation of the polymer of interest (Section 6.1.1). Dose ranges should be selected to include the effects that are anticipated in the environment. For example, testing only at high concentration levels may induce effects for mechanisms that are not relevant at ambient concentrations (Section 6.1.2). Further, verification of the exposure concentrations in sediment (and soil) prior to and during testing should be ensured (Sections 6.1.4). Generally, the TGs describing sediment toxicity studies include exposure verification, i.e. assessments of the different fractions of the test preparations at different time-points.

Table 7: Test methods potentially suitable for assessing the sediment toxicity potential of polymers

Test method/ guideline	Comments	Examples for tested polymers
Sediment Compartment		
OECD TG 218: Sediment-water chironomid toxicity using spiked sediment	Current test methods generally appear applicable for the assessment of polymers; they allow for organic solvent (e.g. hexane, acetone) for preparing poorly soluble polymers (see text for caveats related to the addition of solvents)	Polyacrylate homopolymer (HERA, 2014a) PDMS (Putt, 1994)
OECD TG 219: Sediment-water chironomid toxicity using spiked water		
OECD TG 225: Sediment-water *Lumbriculus* sediment toxicity test using spiked sediment		
OECD TG 233: Sediment-water chironomid life-cycle toxicity test using spiked water or spiked sediment		
OECD TG 235: *Chironomus sp*., acute immobilisation test		
OECD TG 239: Water-sediment *Myriophyllum spicatum* toxicity test;		
OECD TG 238: Sediment-free *Myriophyllum spicatum* toxicity test		
Test for survival and growth in sediment and water using the freshwater amphipod *Hyalella azteca* (Environment Canada, 2013)	Merits further investigation to enhance understanding on how to best deploy a sound aqueous exposure protocol and sediment exposure protocol for *Hyalella*	
ASTM E1611-00: Standard guide for conducting sediment toxicity tests with polychaetous annelids	Current test methods generally appear applicable for the assessment of polymers; they allow for organic solvent (e.g. hexane, acetone) for preparing poorly soluble polymers (see text for caveats related to the addition of solvents)	
OSPAR Commission sediment bioassay using amphipod *Corophium* species (OSPAR Commission, 2006)		

Footnote to Table 7: This table lists commonly used test methods that may be useful for assessing the sediment toxicity potential of polymers and has a focus on OECD TGs; further sediment toxicity test methods using a broad spectrum of sediment-dwelling species are listed in Table R.7.8-5 in ECHA (2017d). Their applicability for the assessment of polymers should be assessed on a case-by-case basis.

Different polymers have been studied for effects on sediment-dwelling organisms using TG-conforming test protocols (HERA, 2014b; Putt, 2014). Further, due to the intense interest on microplastics over the last couple of decades, numerous studies have focussed on the potential for contact or ingestion of microplastics by benthic organisms (reviewed by Desforges et al. (2015) and De Sá et al. (2018)). Such studies provide additional evidence for method applicability and feasibility (with respect to particulate polymers).

Further discussion is merited to determine the relevance of test results and to seek agreement on how they should be used as points-of-departure to derive safety parameters for hazard and risk assessment. Further work is also merited to incorporate all new evidence into formal TGs and to modify the TGs to account for more relevant exposure conditions.

6.3 Ecotoxicity test methods using terrestrial organisms

Table 8 summarises ecotoxicity test methods using terrestrial organisms that are potentially suitable for polymer hazard assessment. Terrestrial organisms include (1) organisms that live in or on the ground (in-soil and terrestrial organisms, e.g. earthworm (*Eisenia fetida*)); and (2) those that live above the ground (pollinators (honeybees, bumblebees), birds); and (3) plants (European Commission, 2002; EFSA PPR Panel, 2017). Organisms living in the soil or on the ground can be exposed to polymers via, e.g., consumer down-the-drain products or agricultural applications and run-off. Terrestrial exposure can also occur when polymers are sorbed to activated sludge that is land-applied. Organisms living above the ground can potentially come into contact with polymers by ingestion or inhalation, and plants can either come into contact with polymers when they are sorbed to the seed in the ground or when they are sorbed to plant stems and leaves.

The majority of TGs for terrestrial toxicity testing were originally developed for and are currently in use by the agrochemical industry. Therefore, the default dosing approaches in these TGs are often more relevant for the typical routes of exposure of agrochemicals, such as direct application of the test item to plant stems and leaves (OECD TG 227: *Vegetative vigour test*). Further, the TGs for terrestrial toxicity testing are commonly used for the assessment of consumer down-the-drain products (e.g. PDMS (Fendinger, 2000; ECETOC, 2011a)) since these materials may reach the soil via land application of sludge from WWTPs. Soil exposure may be direct (by spraying on fields) or indirect (sewage sludge application). For terrestrial exposure from land application of biosolids, there is a need to consider regional differences with sewage sludge disposal procedures and agricultural practices. Specifically, terrestrial testing may be waived for certain scenarios and/or local use criteria in countries where sewage sludge is not applied to agricultural land. Alternative disposal techniques that are commonly employed are via incineration or controlled landfill sites. Statistics obtained from the European Commission's EUROSTAT data-base (https://ec.europa.eu/eurostat/data/database) clearly indicate that a number of European countries incinerate the grand majority, if not the entirety, of their national sewage sludge production.

These considerations highlight the need to identify potential routes of exposure prior to initiating ecotoxicity testing using terrestrial organisms as this may help in defining the relevance of a given TG (further taking into account the given legal/regulatory framework). Ecotoxicity testing using plants that assumes direct contact to plant stems and leaves and ecotoxicity testing using organisms living above the ground (i.e. assuming potential for ingestion or inhalation by bees and birds) will generally only be relevant for very specific types of polymers and types of intended uses. Therefore, this section focusses on terrestrial studies using in-soil organisms.

Table 8: Test methods potentially suitable for assessing the terrestrial toxicity potential of polymers

Test method/ guideline	Comments	Examples for tested polymers
Terrestrial compartment; in-soil organisms		
In-soil microorganisms		
OECD TG 216: Soil microorganisms: nitrogen transformation test	Current test methods generally appear applicable for the assessment of polymers; they allow for water, organic solvent (e.g. hexane, acetone, chloroform), or sand + artificial soil as delivery vehicle If relevant for the given exposure scenario, activated sludge should also be considered as substrate	Polyacrylate homopolymer (HERA, 2014a) Polyacrylic/maleic acid copolymers (HERA, 2014b) PDMS (Forster (1997); referenced in ECETOC (2011))
OECD TG 217: Soil microorganisms: carbon transformation test		
In-soil invertebrates (short-term tests)		
OECD TG 207: Earthworm, acute toxicity	Current test methods generally appear applicable for the assessment of polymers; they allow for water, organic solvent (e.g. hexane, acetone, chloroform), or sand and artificial soil as delivery vehicle If relevant for the given exposure scenario, activated sludge should also be considered as substrate	Polyacrylate homopolymer (HERA, 2014a) Polyacrylic/maleic acid copolymers (HERA, 2014b) UVCBs: reaction products of waxes, fatty acids (ECHA, 2019b)
In-soil and terrestrial invertebrates (long-term / reproduction tests)		
OECD TG 222 (ISO 11268-2): Earthworm reproduction test	Current test methods generally appear applicable for the assessment of polymers; they allow for water, organic solvent (e.g. hexane, acetone, chloroform), or sand and artificial soil as delivery vehicle If relevant for the given exposure scenario, activated sludge should also be considered as substrate	Polyacrylate homopolymer (HERA, 2014a) Polyacrylic/maleic acid copolymers (HERA, 2014b) PDMS (Garvey (1997); referenced in Fendinger (2000) and ECETOC (2011)) 1,2,3-Propanetriol, homopolymer, diisooctadecanoate, reaction products of waxes, fatty acids (ECHA, 2019c)
OECD TG 220 (ISO 16387): *Enchytraied* reproduction test		
OECD TG 232: Collembolan reproduction test in soil		PDMS (Collins (1998); referenced in Fendinger (2000) and ECETOC (2011))
OECD TG 226: Predatory mite reproduction test		
OECD TG 228: Dipteran dung fly developmental toxicity test	Current test methods generally appear applicable for the assessment of polymers; they allow for water, organic solvent (e.g. ethanol, acetone), or dung as a delivery vehicle	

Table 8 continued

Test method/ guideline	Comments	Examples for tested polymers
Terrestrial compartment; plants (application via soil or directly onto plant stems and leaves)		
OECD TG 208 (OPPTS 850.4225): Terrestrial plant test; seedling emergence and seedling growth test	May be relevant if presence in soil may reasonably be anticipated; allows for water, acetone, ethanol, polyethylene glycol, gum arabic, sand; time-course of exposure (in life) currently not required but would be relevant for biodegradable or partially labile substances	Polyacrylate homopolymer (HERA, 2014a) Polyacrylic/maleic acid copolymers (HERA, 2014b) PDMS (Tolle et al., 1995)
OECD TG 227 (OCSPP 850.4150, 850.4250): Terrestrial plant test; vegetative vigour	Test material applied to plant stems, leaves, not soil; traditionally used to study agrochemicals	
Terrestrial compartment; above-ground organisms		
Above-ground invertebrates (short-term tests)		
OECD TG 213: Honeybees, acute toxicity	Traditionally used to study agrochemicals, scant experience with polymer testing; presumably applicable to soluble polymers, but not solid polymers; use may be considered if presence of polymer in compartment of interest can reasonably be anticipated	
OECD TG 214 (OCSPP 850.3020): Honeybees, acute contact toxicity		
OECD TG 246: Bumblebees, acute contact toxicity		
OECD TG 247: Bumblebees, acute oral toxicity test		
Birds (short-term tests)		
OECD TG 223 (OCSPP 850.2100): Avian acute oral toxicity test	Traditionally used to study agrochemicals, scant experience with polymer testing; presumably applicable to different types of polymers (application via feed or gavage, as relevant depending on the physico-chemical properties of the test item); importantly, use should only be considered in exceptional cases if exposure can reasonably be anticipated and potential for effects indicated in studies using invertebrates	
OECD TG 205 (OCSPP 850.2200): Avian dietary toxicity test		
Birds (long-term tests)		
OECD TG 206 (OCSPP 850.2300): Avian reproduction test	Scant experience with polymer testing; presumably applicable to different types of polymers (application via feed or gavage, as relevant depending on the physico-chemical properties of the test item); importantly, use should only be considered in exceptional cases, if exposure can reasonably be anticipated and potential for effects indicated in short-term studies	

Generally, terrestrial ecotoxicity testing follows a tiered approach (European Commission, 2002; EFSA PPR Panel, 2017). Lower-tier tests are usually single species tests assessing the acute and chronic toxicity potential of the test item. They can be considered laboratory tests (using artificial soil) or extended laboratory tests (using natural soil). For terrestrial ecotoxicity testing of consumer products from WWTP, spiking the soil with sludge may also be relevant.

Following the provisions of chemicals legislation (e.g. the EU REACH Regulation; EP and Council (2006)), terrestrial ecotoxicity testing is usually restricted to laboratory and extended laboratory tests, whereas following the provisions of plant protection products legislation (e.g. the EU Regulation (EC) 1107/2009; EP and Council (2009)), higher tier studies involving micro- and/or mesocosms and semi-field studies or field studies may also be required. If the polymer of interest is used in a plant protection product, testing aligned with the route of exposure (soil) is typically carried out using the formulated product that includes the polymer product and also e.g. non-polymeric surfactants. Such testing needs to consider how the different components of the formulated product may affect the structure, systemic bioavailability, and hence also ecotoxicity potential of the polymer product.

In Table 8, the test methods using in-soil and terrestrial organisms are laboratory or extended laboratory studies. Most of these test methods refer to artificial soil as standard substrate, with the exception of the OECD TG 208 (Plant toxicity – seedling emergence) and OECD TG 227 (*Vegetative vigour*) test methods where the TGs indicate that either artificial or natural soil may be used. Further, the OECD TG 228 (*Dipteran dung fly developmental toxicity*) uses natural dung as substrate, and the OECD TG 216 (*Soil microorganisms nitrogen transformation*) and OECD TG 217 (*Soil microorganisms carbon transformation*) both include natural soil as substrate. While the OECD TG 216 and 217 are lower-tier studies, they are not single-species studies, and they measure a biological function (e.g. soil nitrification) instead of the toxicity to a given species of microorganism. Tests using microorganisms are very sensitive, and may indeed be decisive for hazard and risk assessment. Plant studies are also not single-species studies, but often conducted with several species of crops, which are tested individually. Notably, Table 8 only includes commonly used lower-tier laboratory and extended laboratory studies involving in-soil and terrestrial organisms. The need to assess a polymer's (acute) ecotoxicity potential in further species, such as *Pardosa* (wolf-spider) and *Poecilus* (carabid beetle) (ESCORT, 2002), should be assessed on a case-by-case basis.

Higher-tier ecotoxicity tests using in-soil organisms, that are also not listed in Table 8, include e.g. assessments of the microcosm (usually laboratory tests, using a few species) or mesocosm studies (usually outdoor tests, using a higher number of species, thereby enabling the assessment of effects on populations and communities). Further, litter bag studies, semi-field studies and large-scale field studies measuring effects on e.g. earthworm or collembolan communities may be relevant in single cases for persistent polymers regulated under the plant protection products legislation for which high ecotoxicity was recorded in the lower-tier studies. However, since the majority of polymers are unlikely to exhibit pronounced effects in the lower-tier studies, such testing should only be relevant for polymers in exceptional cases.

Existing TGs to study effects on in-soil and terrestrial organisms are generally appropriate for polymers. Information on the partitioning of the polymer in the environmental matrix is instrumental in identifying the relevant species for testing, as exposure routes differ among traits (oral versus dermal). Challenges in determining single K_{ow} and/or K_{oc} values for the polymer of interest are described in Sections 3.3 and 3.4. Further, it may be challenging to prepare and maintain homogeneously distributed test material preparations, e.g. when fortifying soils, and to ensure equal spread throughout the prepared volume. While current TGs allow for the use of polar and non-polar solvents to deliver the test items to the soil matrix, usage of solvents

should consider how these may affect the structure, bioavailability and ecotoxicity potential of the polymer of interest (Section 6.1.3). Dose preparations (application procedures to exposure media) should generally follow the same guiding principles as described in Section 6.2 for test methods using sediment-dwelling organisms.

These considerations underline the importance of verifying exposure both prior to and during terrestrial toxicity studies. In contrast to studies using sediment-dwelling species (Section 6.2), such analytical assessments of the different fractions of the test preparations at different time-points are not included in the TGs for ecotoxicity testing using terrestrial species. Indeed, it may be challenging to adequately retrace the polymer product in the soil preparations.

Different types of polymers have been studied for effects on terrestrial species using TG-conforming test protocols (see references in Table 8). This provides additional evidence for method applicability and feasibility. Further, numerous studies have investigated if (and how) microplastics may come into contact or be ingested by organisms living in the soil or above the ground (reviewed by Desforges et al. (2015) and De Sá et al. (2018)). As concluded by De Sá and co-workers, birds only comprised approximately 2% of all microplastic ecotoxicological effects studies (whereas the vast majority of studies comprised aquatic species; Section 6.1.5). These avian studies generally did not follow standard TGs and involved the use of solid polymer products which could theoretically be dosed with the basal diet (De Sá et al., 2018). However, acute oral dose studies involving liquid diets are more appropriate for polymers that are soluble in water or organic solvents, dispersants and emulsifiers of low toxicity.

Further research work is merited to develop approaches of relevance for different industry sectors to allow integrating polymer products (e.g. from consumer and personal care products) into activated sludge matrices followed by dosing into soil. Such investigations should also address needs and/or means to use inactivated sludge. In addition, approaches should be developed to ensure that relevant fractions of the polymer product are identified, extracted and assessed during terrestrial ecotoxicity studies and to identify and address background components that may confound test results.

Finally, further discussion is merited to determine the relevance of test results and to seek agreement on how they should be used as points-of-departure to derive safety parameters for hazard and risk assessment. Further work is also merited to incorporate all new evidence into formal TGs and to modify the TGs to account for more relevant exposure conditions.

6.4 Conceptual framework for polymer ecotoxicity assessment

Disclaimer: The suggested conceptual framework only provides a general outline, but does not imply that all steps are already in place for all types of polymers. It has been drawn up following the state-of-the-art in view of streamlining efforts and expenditure, and of reducing vertebrate animal testing needs as far as possible. Prevailing knowledge gaps and technical limitations are presented for the individual tiers, as relevant. Further, the conceptual framework described below should be considered a 'living proposal'. The need for amendments might become evident as new insight on polymer hazard and risk assessment evolves. Finally, the conceptual framework is by no means considered prescriptive. Individual test methods (and their order of sequence) should be selected on a case-by-case basis as relevant for the polymer of interest (and depending on the applicable legislative framework).

This section provides a conceptual framework that can be considered for polymer ecotoxicity assessment (Figure 6). For improved clarity, Figure 6 presents a conceptual framework for assessing ecotoxicity potential only. In practice, application of this conceptual framework will be closely interlinked with the conceptual frameworks for polymer biodegradation assessment (Section 4.1.5) and bioaccumulation assessment (Section 4.2.3). Figure 2 illustrates how the three conceptual frameworks can be interlinked during polymer risk assessment.

6.4.1 Tier 0: Identification of ecotoxicity testing needs and of relevant environmental compartment(s)

Commencement of the conceptual framework for polymer ecotoxicity assessment (Figure 6) presupposes that fit-for-purpose identification of relevant morphological and structural descriptors and physico-chemical properties of the polymer of interest has been completed. Collation of information on physico-chemical properties of polymers should also serve to explore means to apply grouping and read-across for ecotoxicity assessment (see ECETOC TR No. 133-1 for further details on grouping and read-across).

Second, commencement of the conceptual framework presupposes that the physico-chemical properties of the polymer of interest indicate that external and/or systemic bioavailability of the polymer product or of a fraction of the polymer product is possible.

Third, relevant environmental compartment(s) should be identified.

Environmental screening assessments to characterise intrinsic hazards of non-polymeric substances are typically performed in aquatic species. Similarly, the aquatic compartment may be relevant for water-soluble polymers, e.g. polymeric surfactants used in down-the-drain consumer products. By contrast, the aquatic compartment may not be appropriate for most water-insoluble polymers since they do not partition to the water compartment. Instead, the sediment and terrestrial compartments will be relevant for water-insoluble polymers. Nevertheless, poorly soluble polymers used in down-the-drain consumer products merit an in-depth review to characterise the likelihood of exposure of the aquatic compartment following wastewater treatment (and the relevance of the outcome of the aquatic toxicity testing to support risk assessment). Hence, polymers with other routes of primary exposure may still be screened for aquatic toxicity potential if the test method is appropriate. When the primary routes of exposure do not include aquatic systems and/or aquatic screening is not technically representative or appropriate, a review of the level of adsorption may inform on the route of exposure to soil species (oral, dermal) and associated representative species (e.g. plant versus earthworm) (ECETOC, 2019).

Further research work is merited to enhance the understanding of how specific physico-chemical properties of polymers inform on the likely relevant environmental compartments and to prepare guidance for how such information should be used in a comprehensive, flexible manner to design ecotoxicity testing strategies for different types of polymers.

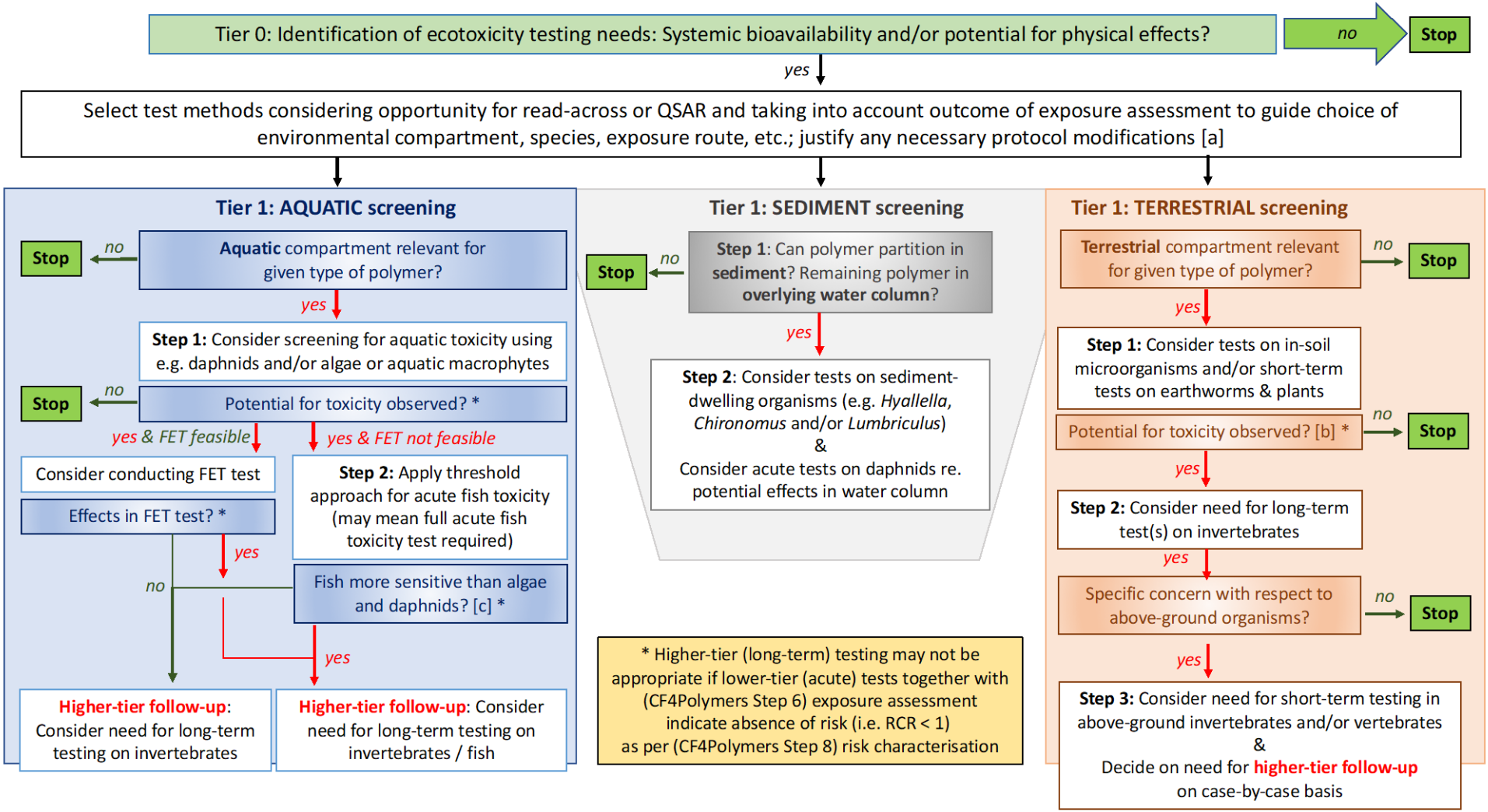

Figure 6: Conceptual framework for polymer ecotoxicity assessment (see Footnote on next page)

Footnote to Figure 6: Abbreviations: FET: Fish embryo toxicity; RCR: Risk characterisation ratio (see Section 6.4.3).

[a] See Section 6.4.2 for issues to consider in identifying the applicability of specific test methods for the polymer of interest. Selection of the appropriate test method(s) should also consider the relevant polymer fraction and the need to assess the extent to which the bioavailability and toxicity of the test item is affected by the TOC or DOC present in the test media. For the vast majority of polymers, higher-tier follow-up of ecotoxicological screening is unlikely to be relevant.

[b] For polymers to be used in down-the-drain articles, activated sludge should be considered as environmental matrix.

[c] If the threshold approach indicates that fish lethality is elicited at the threshold concentration, a full test design (OECD TG 203) may be required to ascertain if fish are more or less sensitive than algae or daphnids (OECD, 2010)

6.4.2 Tier 1: Screening for acute ecotoxicological effects

Once testing needs and the relevant environmental compartment have been identified (see Tier 0), the selection of a specific test method should include the following considerations:

1. Would the findings from this test method add knowledge that would be relevant for risk assessment?
    a. **Yes / Maybe**: Continue to question No. 2
    b. **No**: Test should not be performed
2. Is it physically / technically possible to perform the test following the formal, TG-conforming protocol?
    a. **Yes**: Proceed with testing
    b. **No / Don't know**: Continue to question No. 3
3. Can the testing protocol be adapted to enable testing of the given type of polymer (e.g. by adapting testing conditions and/or duration; or by selection of a specific approach for test item preparation that does not change key properties of the polymer)?
    a. **Yes**: Proceed with testing; clearly describe amendments to standard test protocol and provide justification for why adaptation does not change key parameters of polymer of interest
    b. **No / Don't know**: Performing the test runs the risk of yielding data that are of questionable scientific relevance thereby resulting in misleading risk characterisation. Therefore, the test should preferably not be performed. If, however, it is performed in spite of these limitations (e.g. to meet specific legal requirements), the technical and scientific limitations should be clearly described and the relevance and reliability of the test results established (in terms of real-world exposure scenarios)

Regardless of the identified relevant environmental compartment (aquatic / sediment / terrestrial), all ecotoxicological assessments should aim at identifying both the potential for physical hazard and intrinsic toxicity. Hence, certain endpoints, such as gut contents, should generally be included in the respective selected test methods (see also Section 4.2; bioaccumulation).

6.4.2.1 Aquatic compartment

Step 1: If aquatic toxicity testing is deemed relevant for the polymer of interest, it should begin by screening for acute aquatic effects. Such screening should preferably include short-term test methods using algae or aquatic plants and non-vertebrate species (e.g. *Daphnia magna*). By contrast, bacteria (OECD TG 209, OECD TG 224) are rarely used to inform aquatic toxicity. Usage of protozoans (OECD TG 244) might be considered depending on species sensitivity considerations.

In some situations, where algal inhibition testing may be considered less reliable (e.g. when cationic polymers elicit clumping of algae by physical external interactions thereby influencing growth rate potential), aquatic macrophyte tests (using e.g. *Lemna* species in OECD 223 TG) should be considered for the assessment of short-term toxicity to plants.

With respect to short-term aquatic toxicity testing using vertebrates, the fish embryo toxicity test (OECD TG 236) should be considered giving attention to the duration of post-hatch exposure (Section 6.1.5).

Step 2: If aquatic toxicity testing using fish embryos is not feasible (which should be an exception based on experience of members of the ECETOC Polymers TF), the threshold approach for acute fish toxicity (OECD, 2010) should be applied. If the threshold approach indicates that fish lethality is elicited at the threshold concentration, a full test design (OECD TG 203) may be required to ascertain if fish are more or less sensitive than algae or daphnids (OECD, 2010).

Higher-tier follow-up (see also Section 6.4.3): If the short-term toxicity data indicate the need to refine the hazard assessment, long-term aquatic toxicity should be further investigated. Preferably, such long-term testing should begin by using invertebrates (e.g. *Daphnia*), whereas the need for long-term testing using fish should be determined on a case-by-case basis taking into account the outcome of the threshold approach (Step 2). For low-solubility polymers, long-term toxicity may be indicated in the absence of short term-toxicity data. Appropriate exposures for long-term toxicity should be carefully selected and may range from typical dosing of fully soluble polymers to the usage of WAFs.

Notably, higher-tier (long-term) testing may not be appropriate if lower-tier (acute) tests, together with (CF4Polymers Step 6) exposure assessment, indicate absence of risk (i.e. RCR < 1) as per (CF4Polymers Step 8) risk characterisation (see also Section 6.4.3).

6.4.2.2 Sediment compartment

Step 1: Evaluate where the polymer partitions in the sediment and water system. Even if the polymer adsorbs onto the sediment, there may be remaining toxicity in the water column. Consider additionally performing tests using *Daphnia magna* to address potential for remaining aquatic toxicity in the overlying water column.

Step 2: Select test methods to address a representative spectrum of sediment-dwelling species. For example, amphipods (*Hyallela azteca*), midges (*Chironomus* species) and oligochaetes (*Lumbriculus variegatus*) feed differently in sediment (Environment Canada, 2013; Ingersoll et al., 2014). Therefore, a testing strategy should identify the relevant route of uptake in selecting the species to be submitted to testing as well as different aspects of toxicity on sediment-dwelling species.

6.4.2.3 Terrestrial compartment

Step 1: If terrestrial toxicity testing is deemed relevant for the given polymer, test for effects in in-soil microorganisms (in natural soil) and short-term effects in earthworm (using artificial soil) and/or plants; further considering if an environmentally more appropriate exposure matrix (e.g. activated sludge) is appropriate.

Step 2: If Step 1 tests indicate potential for toxicity in in-soil microorganisms, earthworm and/or plants, consider performing long-term and/or reproduction tests in invertebrates.

Step 3: Only if there is a specific concern for the given polymer and if exposure to organisms living above the ground is likely, testing using above-ground organisms should be considered. In this case, testing should begin with short-term assessments using invertebrates (pollinators (honeybees, bumblebees)). Short-term avian toxicity testing should only be considered if there is a specific, justifiable concern for avian toxicity.

Notably, higher-tier (long-term) testing may not be appropriate if lower-tier (acute) tests, together with (CF4Polymers Step 6) exposure assessment, indicate absence of risk (i.e. RCR < 1) as per (CF4Polymers Step 8) risk characterisation (see also Section 6.4.3).

6.4.3 Higher-tier follow-up of ecotoxicological screening

Even for the most likely small proportion of polymers that are submitted to ecotoxicity testing (because physico-chemical properties indicate likelihood of systemic bioavailability and/or ecotoxicity potential), most of the polymers submitted to Tier 1 screening will most likely not reveal any effects. **Hence, for the vast majority of polymers, higher-tier follow-up of ecotoxicological screening is unlikely to be relevant.**

Importantly, higher-tier (long-term) testing may not be appropriate if lower-tier (acute) tests, together with (CF4Polymers Step 6) exposure assessment, indicate absence of risk (i.e. RCR < 1) as per (CF4Polymers Step 8) risk characterisation. The RCR is defined as the ratio of the PEC and the predicted no-effect concentration (PNEC) (ECETOC, 2003; ECHA, 2016b). For example, following Annex IX, Section 9.1, Column 2 of the REACH Regulation (EP and Council, 2006), long-term aquatic toxicity testing shall be proposed if the chemical safety assessment indicates the need to investigate further the effects on aquatic organisms. In this regard, the ECHA (2017d) *endpoint-specific guidance on information requirements and chemical safety assessment* indicates an RCR < 1 (also effectuated by refining exposure) as an appropriate risk reduction measure.

If deemed necessary following risk assessment, the need for higher-tier assessment of endpoints indicating long-term effects and effects on early developmental stages should be considered and relevant test methods selected on a case-by-case further considering the appropriate environmental compartments and species.

7. TEST METHODS TO ASSESS HUMAN HEALTH TOXICITY POTENTIAL OF POLYMERS

The OECD TG 400 series present *in chemico*, *in vitro* and *in vivo* test methods for predicting the human health toxicity potential of chemicals.

In the CF4Polymers (ECETOC TR No. 133-1 (ECETOC, 2019)), human health hazard assessment is addressed in Step 7. Therein, it is highlighted that polymer hazard assessment should be science-driven. Accordingly, Step 7 hazard assessment is performed for the given life cycle stage of the polymer taking into account its intended uses (identified in Step 1 – problem formulation) and reflecting the exposed human population (identified in Step 5 – determination of exposure scenarios). Further, as determined during Step 3 (polymer component strategy), hazard assessment may be performed for the polymer product (i.e. including its LMW compounds – oligomers, IAS and NIAS), for the polymeric substance (polymeric macromolecules), and/or for all or selected NIAS or IAS, as relevant. For example, when the physical state and/or composition of the polymer product trigger a concern for potential leaching of such low molecular weight compounds with subsequent relevant exposure. Finally, hazard assessment may consider the hazard potential of breakdown products of the polymer, if relevant. (Notably, while the CF4Polymers included general considerations for how to address components of the polymer product in isolation, the present appraisal of the applicability of test methods for human health hazard assessment of polymers generally assumes the polymer product as such is submitted to the evaluation.)

Many of the TGs included in the OECD TG 400 series are generally applicable for the testing of polymers. Nevertheless, their relevance and utility for determining the human health hazard potential of a given polymer product taking into account its intended form and function has to be determined on a case-by-case basis. In order to generate toxicity data that can be meaningfully applied for polymer hazard and risk assessment, all existing information on the polymer under investigation and on similar polymers, including their physico-chemical properties, should be considered. New toxicity testing is not needed if missing data can be extrapolated applying grouping and read-across (see CF4Polymers Step 4 – grouping approach evaluation). If new toxicity testing is deemed necessary, the physico-chemical key parameters for a given (type of) polymer allow determining which specific toxicological endpoints, end hence test methods, are appropriate. Important aspects to consider in determining the need for toxicity testing include the external and internal bioavailability of the polymer and the presence of structural alerts indicating reactivity.

Against this background, this section on test methods to address human toxicity potential of polymers covers:

- Section 7.1: The purpose of the assessment
    - Section 7.1.1: Intended form and function of the polymer
- Section 7.2: Potential for exposure to LMW compounds
- Section 7.3: Bioavailability
- Section 7.4: Reactivity and surface activity
- Section 7.5: Endpoint-specific testing
- Section 7.6: Exposure route considerations

7.1 The purpose of the human health hazard assessment

The human health hazard assessment of a polymer should consider its intended uses and exposure potential. The CF4Polymers has been designed to minimise the need for extensive animal testing. Its Step 1 (problem formulation) includes definition of the risk assessment scope and protection goal. This includes identifying the life cycle stages and intended uses of the polymer. Further, Step 5 (determination of exposure scenarios) and Step 6 (exposure characterisation) that precede Step 7 (hazard assessment) serve to ensure that exposure potential (including physical availability; Section 7.3) have been comprehensively assessed before deciding on toxicity testing needs. This approach serves to prevent the testing of polymers where such data would not inform a risk assessment and to drive a more detailed toxicological assessment on those polymers where exposure potential is greatest. Similarly, it allows targeting testing to the relevant routes of exposure and exposure levels reflective of the final use.

7.1.1 Intended form and function of the polymer

Polymers in their originally manufactured ('as produced') form may not represent their final form and/or composition on the market. Therefore, the hazard assessment of an 'as produced' polymer might not be suitable to inform on the safety of the end use of the polymer. The choice of appropriate endpoints and respective methods for hazard characterisation will partly depend on what form of the polymer is most relevant for exposure – i.e. is the population in scope exposed to pellets, dust, emulsions, suspensions, solutions or articles of a solid or semi-solid polymer or is the polymer liquid? Particularly, many toxicological methods are not suitable for testing insoluble materials, and testing performed on a form of the polymer which will not be present in the life cycle of the polymer is not meaningful. In some cases, it may be useful to remove solvents from the polymer to enable testing e.g. in cell line-based methods, but this is only meaningful if the polymeric substance itself will not be altered by removal of some components. Specifically, emulsion polymeric substances may change their form when other components are removed from the polymer product.

Some polymers are manufactured to have a specific function e.g. as surfactant, water repellent, or water adsorbent. Such information on function is often a good indicator for physico-chemical properties, reactivity potential and exposure scenarios, and as such helpful in selecting relevant and suitable methods.

7.2 Potential for human exposure to LMW compounds

For specific types of polymer products, LMW compounds (i.e. oligomers, IAS and NIAS) may migrate or diffuse, i.e. leach, from the polymer matrix during the intended use or at the end-of-life stages. If LMW compound leaching may occur such that exposure to LMW compounds is plausible, it may be necessary to separately assess their hazard potential. In the CF4Polymers, the relevant components of the polymer product to be included in the further hazard and risk assessment are selected in Step 3 (polymer component strategy). Further, Step 6 (exposure characterisation) provides details on the relevance of LMW compound migration and diffusion for the hazard and risk assessment of specific types of polymer products – predominantly poorly soluble, solid polymer products.

Generally, LMW compounds only make up a minor fraction of the polymer product, leading to very low exposures even if released from the matrix. Therefore, the ECETOC Polymers TF does not recommend to perform *in vivo* oral toxicity testing using the polymer products to assess the toxicity potential of leachable LMW compounds even when oral exposure is a relevant route of exposure. Such testing is unlikely to lead to sufficient exposure to the potential leachate to give the study the power necessary to assess if effects can be elicited, or not. Also, it is likely that the leachate will be variable in composition and as such any effects observed may be difficult to assign to a particular substance or substances coming out of the polymer.

In some cases, however, it is common to apply extracts or migrates of materials when performing testing (typically when performing *in vitro* assays, but in some cases also during *in vivo* studies). Specifically, the standard methods for biocompatibility testing of medical devices (e.g. the ISO 10993 standards) include the standardised generation of extracts and testing of extracts in *in vitro* and *in vivo* experiments. The use of extracts or migrate concentrates for testing of DNA reactivity is also being proposed for the assessment of complex mixtures for which it is not possible or practicable to identify and/or synthesise all components for separate testing (Schilter et al., 2019).

7.3 Bioavailability

As highlighted in the CF4Polymers Step 7 (hazard assessment), bioavailability characterisation is of utmost importance for human health (and ecological) hazard assessment. In the CF4Polymers (ECETOC TR No. 133-1), Section 3.7.1.1 provides a detailed discussion on the role of bioavailability in ecological and human health hazard assessment. In brief, the ECETOC Polymers TF distinguishes between physical availability (as an indicator of exposure potential) and external and internal bioavailability (see Section 4.2.1 of the present review for further details and the ECETOC Polymers TF working definitions for these terms).

After considerations on exposure potential and routes (Section 7.1; purpose of the assessment), the polymer product's potential for external or internal bioavailability should be addressed when designing the human health hazard assessment procedure and to identify necessary and/or relevant test methods. External bioavailability of LMW compounds will also depend on the use scenario and polymer product in scope, e.g. whether an article is expected to come into contact with food, sweat or saliva, or whether a polymer powder, solution or suspension will come into contact with skin, eyes or digestive fluids.

For those components of the polymer product that can become physically available (see Section 7.2; potential for exposure to LMW compounds), the next step of human health hazard assessment includes determining if only external bioavailability or also internal bioavailability are of relevance: With respect to the polymeric substance, available information on the MWD and other physical properties (water and lipid solubility, potential for degradation/hydrolysis to smaller subunits, surface activity) will inform the assessment of whether external or internal bioavailability are likely or not.

If the polymeric substance or polymer product is not capable of crossing biological membranes so that internal bioavailability is unlikely e.g. due to HMW (M_n > 10,000 Da), any attempt to assess systemic toxicity or genotoxicity by the oral and dermal routes of exposure or upon inhalation exposure is irrelevant. For example, in the case of *in vivo* genotoxicity testing, the polymer product must be able to reach the target tissue (blood cells, bone marrow). If it cannot, such a study would be invalid according to the guideline.

However, if it is concluded that the polymer product, a particular LMW fraction, or a known degradation / hydrolysis product could become systemically available, the use of *in silico and in vitro* methods to further clarify the potential bioavailability by the identified relevant routes of exposure should be considered. *In vivo* bioavailability testing would only be considered under specific circumstances, as instead of such testing, *in vitro* or *in vivo* toxicity testing would typically be performed. If only a small fraction of the polymer product were expected to be bioavailable, it may be possible to rely on Thresholds of Toxicological Concern (EFSA and WHO, 2016) for the assessment of the fraction assumed to become systemically available. Alternatively, if there is sufficient evidence to conclude that the polymer product would become bioavailable, further assessment of bioavailability can be set aside and data be gathered to assess the potential for systemic toxicity via relevant routes of exposure.

7.4 Reactivity and surface activity

Irrespective of the potential for internal bioavailability, human health hazard assessment should also consider assessing potential local effects at relevant sites of exposure such as the respiratory tract (upon inhalation exposure), skin, or eyes. The need for this assessment will depend on the potential reactivity and surface activity of the substance.

Reactive and surface-active polymers are more likely to produce local effects such as irritation/corrosion, skin sensitisation, and if bioavailable internally, systemic toxicity or genotoxicity. Reactivity and surface activity of a polymer is typically linked to the presence of reactive or cationic functional groups, water solubility and charge, and it is influenced by molecular weight. These key elements of polymer hazard and risk assessment are presented and discussed in Section 4 of the CF4Polymers (ECETOC TR No. 133-1 (ECETOC, 2019)); see also Figure 7 of the present report, adapted from the CF4Polymers.

Importantly, reactive or cationic functional groups constitute structural alerts, but do not by themselves indicate hazard potential. Therefore, the identification of functional groups (or other structural alerts, such as specific chemical elements) should be followed up by *in vitro* or *in chemico* lower-tier screening together with an evaluation of all available data for the given polymer and similar ones as well as *in silico* modelling as applicable. Testing unreactive, non-bioavailable polymers using e.g. OECD TG toxicity studies is unlikely to provide any meaningful information for a human health hazard and risk assessment. Only if the lower-tier screening indicates hazard potential, the TF considers higher-tier *in vivo* testing justifiable. The identification of reactive functional groups also serves to hypothesise a mode-of-action and hence to identify relevant toxicological endpoints and applicable test methods (see also Section 7.5 below).

7.5 Endpoint-specific testing

Table 9 summarises OECD TGs that are potentially suitable to assess the human health hazard potential. It includes the toxicological endpoints acute toxicity, skin corrosion/irritation, skin sensitisation, eye irritation/corrosion, *in vitro* and *in vivo* genotoxicity, carcinogenicity, developmental and reproductive toxicity, target organ toxicity (repeated dose toxicity), neurotoxicity, and other (toxicokinetics including skin absorption and phototoxicity).

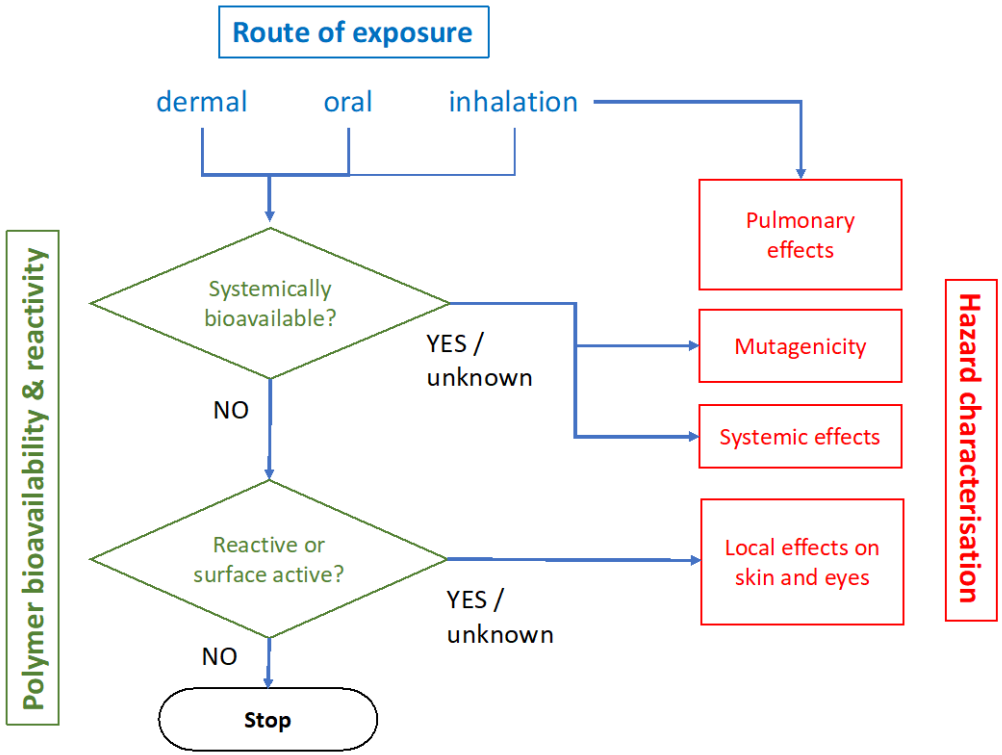

Figure 7: Workflow: Polymer human health hazard assessment; adapted with modification from ECETOC (2019)

Footnotes to Figure 7: This workflow can be applied to the polymer product or to physically available LMW components.

Note 1: The ECETOC Polymers TF members are unaware of the existence of genotoxic polymers. If genotoxic polymers were to exist, the TF members hold the view that they would most likely also have to be (a) bioavailable and (b) "reactive", i.e. contain some form of electrophilic functionality capable of reacting with nucleophilic sites on DNA (as indicated in the original figure published in the CF4Polymers (ECETOC, 2019)). Regarding bioavailability, the TF recognises that it is likely that only the lower molecular weight fractions of the polymer product, such as oligomers with molecular weight < 500 Da, would be bioavailable in practice to the cellular DNA at the site of contact or distant target tissues. For precautionary reasons to afford a high level of protection, the workflow has been modified from the original figure with regard to reactivity to indicate that mutagenicity testing should be considered for polymers that are likely to become systemically bioavailable (or for which the absence of bioavailability has not been established) regardless of whether they are reactive or not.

Note 2: Hazard characterisation for skin and eye irritation will also inform on the test item's potential to irritate the gastrointestinal tract and respiratory epithelium.

Table 9: OECD Test Guidelines (TGs) potentially suitable for assessing the human health toxicity potential of polymers

Endpoint	Test method (OECD TG)	Comments
Acute toxicity	TG 420: Acute oral toxicity – fixed dose procedure TG 423: Acute oral toxicity – acute toxic class method TG 425: Acute oral toxicity – up-and-down procedure	Only relevant for specific cases where chemistry triggers a concern, or high dose oral exposures are intended and the polymer or a relevant fraction thereof is estimated to be systemically available
Skin corrosion / irritation	TG 439: *In vitro* skin irritation: reconstructed human epidermis test method TG 430: *In vitro* skin corrosion: transcutaneous electrical resistance test TG 431: *In vitro* skin corrosion: reconstructed human epidermis test method TG 435: *In vitro* membrane barrier test method for skin corrosion TG 404: Acute dermal irritation / corrosion	Only useful for polymers with reactive functional groups or surface tension activity Most relevant assays are *in vitro* skin irritation tests, as polymers typically are not corrosive Some *in vitro* methods are not suitable for insoluble polymers Accuracy of the *in vitro* methods for different types of polymers needs to be evaluated
Skin sensitisation	TG 406: Skin sensitisation (guinea pig tests) TG 429: Skin sensitisation: Local lymph node assay TG 442A: Skin sensitisation: Local lymph node assay: DA TG 442B: Skin sensitisation: Local lymph node assay: BrdU-ELISA TG 442C: *In chemico* skin sensitisation (Direct peptide reactivity assay) TG 442D: *In vitro* skin sensitisation (ARE-Nrf2 luciferase test method) TG 442E: *In vitro* skin sensitisation (activation of dendritic cells)	Only useful for polymers with reactive functional groups Some *in chemico*/*in vitro* methods are not suitable for insoluble polymers, and accuracy of the in vitro methods for different polymer types needs to be evaluated. Not yet adopted as OECD TG: Sens-IS assay; suitable for insoluble test materials
Eye irritation / corrosion	TG 492: Reconstructed human cornea-like epithelium test method [a] TG 405: Acute eye irritation / corrosion TG 437: Bovine corneal opacity and permeability test method [b] TG 438: Isolated chicken eye test method [b] TG 460: Fluorescein leakage test method [c]	Only useful for polymers with reactive functional groups or surface tension activity Most relevant assays are *in vitro* eye irritation tests, as polymers typically are not corrosive Some *in vitro* methods are not suitable for insoluble polymers
In vitro genotoxicity [d]	TG 471: Bacterial reverse mutation test TG 473: *In vitro* mammalian chromosome aberration test TG 476: *In vitro* mammalian cell gene mutation test TG 481 Saccharomyces cerevisiae, miotic recombination assay TG 487: *In vitro* mammalian cell micronucleus test	Only useful for polymers with internal bioavailability and reactive functional groups, or structures expected to be metabolised to reactive groups Running *in vitro* genotoxicity assays should be considered in cases where bioavailability and reactivity of metabolites cannot be ruled out via modelling or available data with reasonable certainty

Table 9 continued

Endpoint	Test method (OECD TG)	Comments
In vivo genotoxicity [d]	TG 474: Mammalian erythrocyte micronucleus test TG 475: Mammalian bone marrow chromosome aberration test TG 478: Rodent dominant lethal test TG 483: Mammalian spermatogonial chromosome aberration test TG 485: Mouse heritable translocation assay TG 486: Unscheduled DNA synthesis test with mammalian liver cells *in vivo* TG 488: Transgenic rodent somatic and germ cell gene mutation assays TG 489: *In vivo* mammalian alkaline Comet assay	Only useful for polymers with internal bioavailability and reactive functional groups, or structures expected to be metabolised to reactive groups
Carcinogenicity	TG 451: Carcinogenicity study TG 453: Combined chronic toxicity / carcinogenicity study Numerous non-guideline cell transformation assays	Only informative for polymers with internal bioavailability and significant exposure to humans
Developmental and reproductive toxicity	TG 414: Prenatal developmental toxicity study TG 415: One-generation reproduction toxicity study TG 416: Two-generation reproduction toxicity study TG 421: Reproduction/developmental toxicity screening test TG 422: Combined repeated dose and reprod./develop. toxicity screening test TG 443: Extended one-generation reproduction toxicity study	Only informative for polymers with internal bioavailability and significant exposure to humans
Target organ toxicity	TG 407: Repeated dose 28-day oral toxicity study in rodents TG 408: Repeated dose 90-day oral toxicity study in rodents TG 409: Repeated dose 90-day oral toxicity study in non-rodents TG 410: Repeated dose dermal toxicity: 21/28-day study TG 411: Subchronic dermal toxicity: 90-day study TG 412: Subacute inhalation toxicity: 28-day study TG 413: Subchronic inhalation toxicity: 90-day study TG 452: Chronic toxicity study TG 453: Combined chronic toxicity / carcinogenicity study	Only informative for polymers with internal bioavailability and significant exposure to humans

Table 9 continued

Endpoint	Test method (OECD TG)	Comments
Neurotoxicity	TG 418 / 419: Delayed neurotoxicity of organophosphorus substances following acute / 28-day repeated dose exposure TG 424: Neurotoxicity study in rodents TG 426: Developmental neurotoxicity study TG 443: Extended one-generation reproduction toxicity study	Only informative for polymers with internal bioavailability and specific reason for concern
Other	TG 417: Toxicokinetics TG 427: Skin absorption: *In vivo* method TG 428: Skin absorption: *In vitro* method TG 432: *In vitro* 3T3 neutral-red-uptake phototoxicity test	TG 417, TG 427 and TG 428: Only informative for polymers with expected internal bioavailability and significant exposure to humans. Those study types pose significant analytical challenges and will typically require radiolabelled test material. TG 432: only relevant for specific cases where chemistry triggers a concern – the ECETOC Polymers TF is not aware of examples of phototoxic polymers / this TG may not be suitable for insoluble polymers.

Footnote to Table 9:

[a] Adopted for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage

[b] Adopted for identifying (i) chemicals inducing serious eye damage and (ii) not requiring classification for eye irritation or serious eye damage

[c] Adopted for identifying ocular corrosives and severe irritants

[d] Further OECD TGs for *in vitro* and *in vivo* genotoxicity testing were deleted on 2 April 2014, i.e.

- TG 477: Sex-linked recessive lethal test in drosophila melanogaster
- TG 479: *In vitro* sister chromatid exchange assay in mammalian cells
- TG 480: Saccharomyces cerevisiae, gene mutation assay
- TG 482: DNA damage and repair, unscheduled DNA synthesis in mammalian cells *in vitro*
- TG 484: Mouse spot test

If toxicity studies need to be performed to assess the potential reactivity of a polymer product (see Section 7.4 above), *in vitro* cytotoxicity assays (including *in vitro* skin and eye irritation assays) should be considered first. Generally, with regard to the applicability of *in vitro* (or *in chemico*) test methods for polymer hazard assessment, the focus lies on considerations on the applicability domains of the respective methods. Further, the polymer's potential internal (*in vivo*) bioavailability must also be taken into account when utilising *in vitro* assays to assess cytotoxicity or potential systemic toxicity and reactivity and interpreting the findings from these assays. For example, the evaluation of a positive finding from a receptor-based assay should include an *in vitro* to in *vivo* extrapolation to address whether the polymer is capable of becoming systemically available and reaching the receptor in question. Or rather, *in vitro* to *in vivo* extrapolations should be performed before running any assays to evaluate whether meaningful results are expected to be obtained.

With regard to higher-tier *in vivo* testing, the need for such testing should be determined on a case-by-case basis considering the physico-chemical properties of the polymer under investigation (e.g. the presence of reactive or cationic functional groups), its exposure potential and internal bioavailability. New *in vivo* testing should only be performed if the necessary data cannot be obtained by other means, e.g., grouping and read-across (addressed in CF4Polymers Step 4 – grouping approach evaluation; see also OECD (2014) and ECHA (2017e)). Further, new *in vivo* testing should only be performed if it is expected to have an impact on risk assessment outcome and risk management, and only on relevant toxicological endpoints, taking into account the physico-chemical key parameters and potentially relevant modes-of-action of the polymer under investigation.

7.6 Exposure route considerations

Most polymeric substances and polymer products only have potential for dermal exposure throughout their lifecycle. Hence, local toxicological endpoints relevant for the dermal route should be the primary consideration, such as skin and eye irritation and skin sensitisation. However, also for these endpoints, local tissue bioavailability will determine if effects can be expected at all, and compatibility of the different *in vitro* and *in chemico* models with insoluble materials will have to be considered.

Significant oral exposure may occur from uses such as excipient, food additive and lip and oral care uses. If significant fractions of the polymeric substance are expected to become systemically available, repeated dose information becomes necessary for risk assessment of such uses, and will typically be obtained by standard *in vivo* studies.

Any materials, polymeric or not, to be used in respirable aerosols at significant concentrations require information on pulmonary toxicity. However, because the actual concentration of respirable aerosol depends on the spray device and formulation, any *in vivo* testing of aerosols should only be considered when particle size distributions of the actual aerosols formed demonstrate the necessity of toxicity data.

Additionally, it is important to understand if and how the intended functionality of the polymer could impact lung function, i.e. if it has the potential to elicit substance-specific intrinsic toxicity or unspecific pulmonary overload. For example, strongly irritating materials and reactive materials can be predicted to have local toxicity in the pulmonary tract and are poor candidates for aerosol applications.

Similar to other particulate materials such as wood dust, HMW particulate polymers (M_n > 10,000 Da) which are poorly soluble and of low intrinsic toxicity nevertheless have the potential to overload the lung if exposure

is sufficiently high and/or prolonged to overwhelm the pulmonary clearance mechanism (ECETOC, 2013). Notably, however, while the evolvement of pulmonary overload conditions has been observed in rat studies, the human health relevance of such effects has been questioned (ECETOC, 2013).

For poorly soluble polymers without specific reactivity, inhalation toxicity testing may not be necessary as long as the appropriate exposure controls can be implemented to minimise inhalation exposure to adhere to the incumbent occupational exposure limits such as the Maximum Concentrations at the Workplace and low toxicity dust limits published by the German Research Foundation (DFG, 2019), (see also Section 5.2.3).

At present, organotypic *in vitro* models for respiratory tract toxicology testing are under development and are promising to become valuable tools in screening chemicals (including natural chemicals) for the potential to exert specific pulmonary toxicity (Wiemann et al., 2016; Zavala et al., 2016; Metz et al., 2018). Further research will have to investigate if and for which materials such methods can also provide quantitative information for hazard characterisation.

Further research work is merited to enhance the understanding of how specific chemistries and physico-chemical properties of polymers impact the accuracy of different *in vitro* assays for the detection of specific toxicity endpoints. This applies to, and beyond, the *in vitro* OECD TGs for skin and eye irritation and corrosion, skin sensitisation and genotoxicity.

8. CONCLUSIONS AND RECOMMENDATIONS

This second part of the ECETOC TR No. 133 series has provided a detailed review of the applicability of standard analytical tools, *in vitro* and *in vivo* test methods and *in silico* models to assess the physico-chemical, fate, exposure-related, ecotoxicological, and toxicological properties of polymers. However, this report should not be misunderstood as a check list for a 'tick-box approach' to hazard assessment (Combes and Balls, 2005). Therefore, it also includes general guidance on how to identify if a specific test method might be relevant for specific types of polymers and on how relevant test methods might be structured in a tiered approach. Specifically, three conceptual frameworks for polymer (bio)degradation, bioaccumulation and ecotoxicity assessment, respectively, are presented that complement the ECETOC TR No. 133-1 CF4Polymers by 'zooming into' specific steps of the CF4Polymers to show how potential for (bio)degradation, bioaccumulation and ecotoxicity might be addressed within the CF4Polymers. (The CF4Polymers already provided a similar 'zoom in' for human health hazard assessment, so that this approach is referred to in the present report.)

Generally, while the hazard, exposure and risk assessment (as well as grouping) of many polymers is more complex than that of many mono-constituent substances, both the ECETOC TR No. 133-1 CF4Polymers and the ECETOC TR No. 133-2 review show that following a transparent, structured approach will enable more reliable hazard and risk assessment thereby ensuring the safe use of polymers. Indeed, many polymers can be reasonably assumed not to pose environmental or human health concerns, as also indicated by the internationally applied concepts for PLCs (US EPA, 1997b; OECD, 2009). Nevertheless, knowledge gaps prevail in the hazard and exposure assessment for polymers. These gaps range from the lack of specific methods to quantify specific parameters to issues related to test item preparation and opportunities to adapt existing testing protocols. Given the versatility and complexity of polymer products, a variety of different approaches are required to handle different types of polymers (depending on solubility, charge density, molecular weight / molecular weight distribution, etc.). Opportunities to address such knowledge gaps by future research work have been identified in the present report.

In the ECETOC TR No. 133-1, five recommendations were spelled out for how to advance the scientific knowledge base underlying polymer hazard and risk assessment. These five recommendations are revisited below to incorporate the findings from the present ECETOC TR No. 133-2:

ECETOC TR No. 133-1 Recommendation 1: *Identify sets of structural and/or morphological descriptors as well as physico-chemical and fate properties that are key parameters for different types of polymer products*.

ECETOC TR No. 133-2 provides further details on research that is merited to identify which specific properties are relevant key parameters for fit-for-purpose identification and grouping of specific types of polymers. Specific key parameters might generally be relevant across different types of polymers, or they might be unique to specific types of polymers. Knowledge on such key parameters will also facilitate the identification of data needs during exposure and hazard assessment. Please refer to the respective sections for details.

ECETOC TR No. 133-1 Recommendation 2: *Consider prevailing technical limitations of available tools, test methods and models for polymer risk assessment*.

ECETOC TR No. 133-2 provides details on the specific technical limitations that currently impair the applicability of specific tools, test methods and models for polymer risk assessment. It also indicates further research that is merited to contribute to overcoming such technical limitations when assessing polymers and to permit standardisation of testing. Please refer to the respective sections for details.

ECETOC TR No. 133-1 Recommendation 3: *Maintain the CF4Polymers as a 'living', flexible framework, and review and update it in line with emerging knowledge on how it can efficiently and effectively support polymer risk assessment.*

ECETOC TR No. 133-2 complements the ECETOC TR No. 133-1 by providing further evidence to support the general outline of the CF4Polymers and more detailed guidance on how to pass through its steps (specifically: CF4Polymers Step 2: Polymer identification; Step 5: Determination of exposure scenarios; Step 6: Exposure characterisation; Step 7: Hazard assessment). Importantly, the information evaluated for the present report did not indicate any need to amend the CF4Polymers.

ECETOC TR No. 133 Series Recommendation 4: *Expand the knowledge base to (1) substantiate the PLC concept and (2) to identify under which conditions the presence of specific structural alerts or physico-chemical properties poses environmental or human health hazard concerns*. Particularly, there is only weak evidence that anionic or amphoteric and water absorbing polymers might generally have a relevant hazard potential.

The information collated in ECETOC TR No. 133-2 supports this goal in an indirect manner by showing how specific parameters related to the PLC concept can be measured. While the PLC concept has been implemented in different non-EU jurisdictions for many years, or even decades, without indications to disprove its validity, the recommended research work shall serve to eventually extend the criteria, if sufficient experimental justification becomes available. For example, there is some evidence indicating that the current thresholds for relative fractions of oligomers in the PLC concept might be too conservative (ECETOC, 2019).

It is expected that the planned case studies, which shall be published in ECETOC TR No. 133-3, will serve to advance the evidence collated in ECETOC TR No. 133-1 and 133-2 that is relevant to advancing Recommendation 4. Further, it is expected that the planned case studies shall serve to enhance an understanding on the opportunities to group polymers by common physical, chemical and/or biological properties.

ECETOC TR No. 133 Series Recommendation 5: *Develop environmentally relevant models, methods and/or criteria to assess (bio)degradation to improve the reliability of exposure and fate assessments important to the risk assessment of polymers*.

ECETOC TR No. 133-2 provides further details on the specific types models and/or criteria to assess (bio)degradation (or other key parameters for polymer risk assessment) taking into account the type of (bio)degradation, its duration (i.e. half-lives), and whether it is intended during the given life cycle stage of the polymer, or not.

To complement the CF4Polymers (ECETOC TR No. 133-1) and the present ECETOC TR No. 133-2, a selection of case studies is planned as ECETOC TR No. 133-3. These case studies shall address different components of polymer grouping and risk assessment to put the CF4Polymers into practice and to further substantiate the need to address specific physico-chemical properties, ecological and/or human health-related parameters for polymer risk assessment. Further, the case studies shall serve to enhance the understanding on the applicability and/or technical limitations of the corresponding tools, test methods, and models. It is expected that these case studies will not only serve to identify opportunities and/or the need to refine specific parts of the CF4Polymers, but that they will also provide important insight to advance the understanding on the applicability of standardised tools, methods, and models for polymer hazard and risk assessment. ECETOC has

mandated an *ad-hoc* committee to follow up such new insight and proactively update the TR No. 133 series to keep abreast of the state-of-the-art within this domain.

GLOSSARY

Adsorption: The process of the binding of a chemical to surfaces of soils. Adsorption processes can be physical and chemical adsorption as well as surface catalysed degradation, bulk adsorption or chemical reaction. (adapted from: OECD TG 106)

Article: "*An object which during production is given a special shape, surface or design which determines its function to a greater degree than does its chemical composition."* (EP and Council, 2006; Article 3(3))

Bioaccumulation: "*A process in which the chemical concentration in an organism achieves a level that exceeds that in the respiratory medium (e.g. water for a fish or air for a mammal), the diet, or both."* (OECD TG 305)

Bioaccumulation Factor (BAF): *"The steady-state (equilibrium) ratio of the substance concentration in an organism to the concentration in the surrounding medium (e.g. water in natural ecosystems).*" (ECHA, 2017b).

Bioavailability: "*The rate and extent to which an agent can be absorbed by an organism and is available for metabolism or interaction with biologically significant receptors. Bioavailability involves both release from a medium (if present) and absorption by an organism.*" (WHO IPCS, 2004)

- **External bioavailability**: The condition that some HMW polymers that are too large to cross biological barriers might nevertheless exert local toxicity in tissues (e.g. skin, eyes, respiratory tract). This toxicity may well be due to LMW components (i.e. small oligomers, IAS and NIAS, including unreacted monomers) that migrate under conditions of contact to the transitional fluid (e.g. sweat, tears, saliva), thereby being available to be absorbed and exert their toxic effect. The specific mechanisms by which such effects can occur remain to be determined. (ECETOC Polymers TF working definition)
- **Internal (systemic) bioavailability** means that the polymer product is absorbed into the blood stream by an organism thereby becoming systemically available and potentially causing systemic effects. (ECETOC Polymers TF working definition)
- **Physical availability** means that one or more individual components of the polymer product are released from the polymer matrix e.g. by migration or leaching. (ECETOC Polymers TF working definition)

Bioconcentration: The net accumulation of a chemical by an organism as a result of uptake directly from its surrounding physical environment only through respiratory or dermal surfaces. (Burkhardt et al., 2011)

Bioconcentration Factor (BCF): *"At any time during the uptake phase of this accumulation test* [the BCF] *is the concentration of test substance in/on the fish or specified tissues thereof (Cf as mg/kg) divided by the concentration of the chemical in the surrounding medium (Cw as mg/L). BCF is expressed in L/kg. Corrections for growth and/or a standard lipid content are not accounted for."* (OECD TG 305)

Biodegradability: "*The ability of a material to decompose after interactions with biological elements.*" (Goswami and O'Haire, 2016)

Biodegradation: "*The process by which organic substances are decomposed by micro-organisms (mainly aerobic bacteria) into simpler substances such as carbon dioxide, water and ammonia.*" (OECD Glossary of Statistical Terms; https://stats.oecd.org/glossary/detail.asp?ID=203)

- **Primary biodegradation**: Biotransformation resulting in the loss of a specific property of the original substance. (OECD, 2006)
- **Ultimate biodegradation**: Mineralisation by microorganisms to CO_2, water, new microbial cellular constituents (biomass), and other inorganic substances (e.g. NH_3). (OECD, 2006)

Biomagnification: *"The increase in concentration of the test substance in or on an organism (or specified tissues thereof) relative to the concentration of test substance in the food."* (OECD TG 305)

Biomagnification Factor (BMF): *"The concentration of a substance in a predator relative to the concentration in the predator's prey (or food) at steady-state."* (OECD TG 305)

Biotransformation: see biodegradation, primary.

Breakdown product (degradation product): "*A metabolite / a chemical derived from a parent molecule that has been altered e.g. by heat, light, or enzymes*." (https://medical-dictionary.thefreedictionary.com/Breakdown+Product)

Cationic polymer: A polymer that has "*one or more monomer units that are covalently bound and bear a net positive charge.*" (Government of Canada, 2005)

Composting: A controlled process (i.e. with respect to humidity, temperature) that can be divided into two distinct phases i.e. active composting (rotting) followed by curing (post-rotting). The duration of the active composting phase depends on the type of composting, and it may include both aerobic thermophilic processes, but also anaerobic processes. (adapted from: European Bioplastics (2015))

Degradation, decomposition, or depolymerisation: *"A type of chemical change in which a polymeric substance breaks down into simpler, smaller weight substances as the result of (for example) oxidation, hydrolysis, heat, sunlight, attack by solvents or microbial action."* (US EPA, 1997b)

- **Physical degradation,** induced by e.g. heat, irradiation;
- **Chemical degradation**, induced by e.g. the presence of specific acids, bases, or oxidative agents, as applicable;
- **Biological degradation** (biodegradation), induced by specific microorganisms. (adapted from: Doyle et al. (1982))

Desorption: The reversibility of adsorption. (see definition)

Disintegration: The physical breakdown of a material into fragments. (ISO 24513)

Dissociation: The reversible splitting of a substance into two or more chemical species which may be ionic. (OECD TG 112)

Environmental fate: The destiny of a substance after release into the environment. (adapted from https://www.informea.org/en/terms/environmental-fate)

Exposure assessment: *"The process of estimating or measuring the magnitude, frequency, and duration of exposure to an agent, along with the number and characteristics of the population exposed. Ideally, it describes the sources, pathways, routes, and the uncertainties in the assessment."* (WHO IPCS, 2004)

Exposure estimation: "*The exposure estimation entails three elements: (1) emission estimation; (2) assessment of chemical fate and pathways; and (3) estimation of exposure levels.*" (EP and Council, 2006; Annex I (5.2.1))

Exposure factors: "*Factors related to human behaviour and characteristics that help determine an individual's exposure to an agent.*" (US EPA, 2011)

Exposure scenario: "*The set of conditions, including operational conditions and risk management measures, that describe how the substance is manufactured or used during its life-cycle and how the manufacturer or importer controls, or recommends downstream users to control, exposures of humans and the environment. These exposure scenarios may cover one specific process or use or several processes or uses as appropriate*." (EP and Council, 2006)

Grouping (of chemicals): The general approach for considering more than one chemical at the same time. It can include formation of a chemical category or identification of chemical analogue(s) with the aim of filling data gaps as appropriate. (OECD, 2014)

Hazard: "*Inherent property of an agent or situation having the potential to cause adverse effects when an organism, system, or (sub)population is exposed to that agent*." (WHO IPCS, 2004) (Hence, the term hazard is used in its general meaning that is not specific to polymers.)

Hazard identification: *"The identification of the type and nature of adverse effects that an agent has an inherent capacity to cause in an organism, system, or (sub)population. Hazard identification is the first stage in hazard assessment."* (WHO IPCS, 2004)

Hazard characterisation (dose-response assessment): *"The qualitative and, wherever possible, quantitative description of the inherent property of an agent or situation having the potential to cause adverse effects. This should, where possible, include a dose–response assessment and its attendant uncertainties. Hazard characterization is the second stage in the process of hazard assessment."* (WHO IPCS, 2004)

Hydrolysis: A chemical process of decomposition involving the splitting of a bond and the addition of the hydrogen cation and the hydroxide anion of water. (https://www.merriam-webster.com/dictionary/hydrolysis)

Inherent biodegradability tests: "*Aerobic tests that possess a high capacity for degradation to take place, and in which biodegradation rate or extent is measured. The test procedures allow prolonged exposure of the test substance to microorganisms and a low ratio of test substance to biomass, which offers a better chance to obtain a positive result compared to tests for ready biodegradability.*" (OECD, 2006)

Intentionally added substances: A "*substance which is intentionally added to plastics to achieve a physical or chemical effect during processing of the plastic or in the final material or article; it is intended to be present in the final material or article."* (Definition for 'additive' in European Commission (2011))

Life cycle (of a product): The entire lifespan of a product, i.e. all stages from raw material extraction through materials processing, manufacturing, distribution, use, repair and maintenance, and eventual disposal or recycling. (adapted from: ICCA (2016))

LMW (low molecular weight) compound: Small oligomers, IAS, and NIAS, including unreacted monomers.

Macromolecule: "*A molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetitions of units derived, actually or conceptually, from molecules of low relative molecular mass.*" (IUPAC, 1997)

Mineralisation: See biodegradation, ultimate.

Mixture: "*A mix or solution of two or more substances. Under the EU chemicals legislation, mixtures are not considered substances*." (https://echa.europa.eu/support/substance-identification/what-is-not-a-substance)

Monomer:

OECD: *"A molecule which is capable of forming covalent bonds with two or more like or unlike molecules under the conditions of the relevant polymer-forming reaction used for the particular process*." (http://www.oecd.org/env/ehs/oecddefinitionofpolymer.htm)

REACH (Article 3(6)): "*A substance which is capable of forming covalent bonds with a sequence of additional like or unlike molecules under the conditions of the relevant polymer-forming reaction used for the particular process.*" (EP and Council, 2006)

Monomer, unreacted: Depending on the manufacturing process and intended use of the polymer product, unreacted monomers can either be intentionally added substances or non-intentionally added substances.

Non-intentionally added substance (NIAS): *"An impurity in the substances used or a reaction intermediate formed during the production process or a decomposition or reaction product."* (European Commission, 2011)

Number average molecular weight (M_n): The arithmetic average (mean) of the molecular weights of all molecules in a polymer. $M_n = \frac{\sum_{i=1}^{n} H_i}{\sum_{i=1}^{n} \frac{H_i}{M_i}}$ (US EPA, 1997b)

Oligomer: *"A compound of relatively low molecular weight containing up to five monomer units."* (https://www.collinsdictionary.com/dictionary/english/oligomer); oligomers can be part of the polymeric substance (at the low end of its molecular weight range); in some contexts, they are also referred to as non-intentionally added substances.

Persistence: *"Length of time a substance stays in the environment (or body organs) after its introduction."* (http://www.businessdictionary.com/definition/persistence.html)

Polymer:

IUPAC: "*Substances composed of macromolecules, very large molecules with molecular weights ranging from a few thousand to as high as millions of grams/mole."* (https://iupac.org/polymer-edu/what-are-polymers)

OECD: "*A polymer means a substance consisting of molecules characterized by the sequence of one or more types of monomer units and comprising a simple weight majority of molecules containing at least three monomer units which are covalently bound to at least one other monomer unit or other reactant and consists of less than a simple weight majority of molecules of the same molecular weight. Such molecules must be distributed over a range of molecular weights wherein differences in the molecular weight are primarily attributable to differences in the number of monomer units. In the context of this definition a 'monomer unit' means the reacted form of a monomer in a polymer.*" (http://www.oecd.org/env/ehs/oecddefinitionofpolymer.htm)

REACH (Article 3(5)): *"A substance consisting of molecules characterised by the sequence of one or more types of monomer units. Such molecules must be distributed over a range of molecular weights wherein differences in the molecular weight are primarily attributable to differences in the number of monomer units. A polymer comprises the following:*

a) a simple weight majority of molecules containing at least three monomer units which are covalently bound to at least one other monomer unit or another reactant;
b) less than a simple weight majority of molecules of the same molecular weight.

In the context of this definition a 'monomer unit' means the reacted form of a monomer substance in a polymer." (EP and Council, 2006)

Polymer matrix: The continuous phase in multi-constituent or multi-phase (composite) systems. (adapted from: Wang et al. (2011))

Polymer product: A chemical product with a polymeric substance as main component, and NIAS and sometimes IAS as other components. (ECETOC Polymers TF working definition) Polymer products are only in some cases finished articles.

Polymeric substance (polymeric macromolecules): The chemical (co)polymer and possibly present oligomers (both are composed of the same monomeric units). (ECETOC Polymers TF working definition)

Reactive functional group: *"An atom or associated group of atoms in a chemical substance that is intended or can be reasonably anticipated to undergo facile chemical reaction."* (US EPA, 1997b)

Read-across: A technique for predicting endpoint information for the target substance by using available data from the same endpoint from the source substance(s). The read-across approach encompasses (i) elements addressing the structural similarity; (ii) a read-across hypothesis; (iii) a read-across justification; and (iv) the prediction of the property (properties) of the target substance(s). (ECHA, 2017e)

Ready biodegradability tests: "*Stringent screening tests, conducted under aerobic conditions, in which a high concentration of the test substance (in the range of 2 to 100 mg/L) is used and biodegradation is measured by non-specific parameters like Dissolved Organic Carbon (DOC), Biochemical Oxygen Demand and CO_2 production.*" (OECD, 2006)

Risk assessment: *"A process intended to calculate or estimate the risk to a given target organism, system, or (sub)population, including the identification of attendant uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system"* (WHO IPCS, 2004). The risk assessment process includes the four steps, i.e. (1) hazard identification) and (2) hazard characterisation (together: hazard assessment); (3) exposure assessment; (4) risk characterisation. (adapted from: WHO IPCS (2004))

Risk characterisation: *"The qualitative and, wherever possible, quantitative determination, including attendant uncertainties, of the probability of occurrence of known and potential adverse effects of an agent in a given organism, system, or (sub)population, under defined exposure conditions."* (WHO IPCS, 2004)

Risk characterisation ratio (RCR): Ratio of predicted environmental concentration (PEC) and predicted no-effect concentration (PNEC). (ECETOC, 2003)

Sorption: *"A general term used to encompass the processes of absorption, adsorption, ion exchange, and chemisorption."* (US EPA, 1997a)

Surface tension: "*The free surface enthalpy per unit of surface area*" (Council, 2008)

UVCB (substances of unknown or variable composition, complex reaction products or biological materials): "*A substance that cannot be sufficiently identified by its chemical composition, because (1) the number of constituents is relatively large and/or (2) the composition is, to a significant part, unknown and/or (3) the variability of composition is relatively large or poorly predictable."* (ECHA, 2012)

Water-accommodated fraction (WAF): "*The aqueous fraction containing the dissolved and/or suspended and/or emulsified fraction of a multicomponent substance.*" (OECD, 2019)

Water extractability / extractivity: The preferable extraction of some molecules in water leaving others remaining within the bulk substance (adapted from Environment Canada, 2009).

Weight-average molecular weight (M_w): $M_w = \frac{\sum_{i=1}^{n} H_i \times M_i}{\sum_{i=1}^{n} M_i}$ where H_i is the level of the detector signal from the baseline for the retention volume V_i, M_i is the molecular weight of the polymer fraction at the retention volume V_i, and n is the number of data points. The breadth of the MWD, which is a measure of the dispersity of the system, is given by the ratio M_w /M_n (see definition for number-average molecular weight (M_n)). (OECD TG 118)

ABBREVIATIONS

3D: Three-dimensional

AAS: Atomic absorption spectroscopy

AISE: International Association for Soaps, Detergents and Maintenance Products

ART: Advanced REACH Tool

ASTM: American Society for Testing and Materials

BAF: Bioaccumulation factor

BAT: Bioaccumulation Assessment Tool

BCF: Bioconcentration factor

BMF: Biomagnification factor

Cefic: European Chemical Industry Council

CF4Polymers: Conceptual Framework for Polymer Risk Assessment

CEM: Consumer Exposure Model (US EPA)

CEN: European Committee for Standardisation

CHESAR: Chemical safety assessment and reporting tool (ECHA)

Const.: Control of constituents (Tables 4A and 4C)

Da: Dalton (g/mol)

dART: Dermal Advanced REACH Tool

DIN: Deutsches Institut für Normung (German Standards Institute) (Table 1)

DM(T)A: Dynamic mechanical (thermal) analysis (Table 1)

DOC: Dissolved organic carbon

DREAM: Dose Related Risk and Effects (model)

DSC: Differential scanning calorimetry (Table 1)

DT_{50}: Disappearance time 50 (biodegradation)

ECB: European Chemicals Bureau

ECETOC: European Centre for Ecotoxicology and Toxicology of Chemicals

ECHA: European Chemicals Agency

ECPA: European Crop Protection Association

E-FAST: Exposure and Fate Assessment Screening Tool (US EPA)

EFSA: European Food Safety Authority

EMPA: Swiss Federal Laboratories for Material Testing and Research

EN: European Norm

EP: European Parliament

EPA: Environmental Protection Agency

EUSES: European Union System for the Evaluation of Substances

FET: Fish embryo acute toxicity (Figure 6)

FTIR: Fourier-transform infrared spectroscopy

GD: Guidance document

GES: Generic exposure scenario

GPC: Gel permeation chromatography

HDPE: High-density polyethylene (see Table 4A)

HERA: Human and Environmental Risk Assessment (project; AISE and Cefic)

HMW: High molecular weight

HPLC: High-performance liquid chromatography

IAS: Intentionally added substances

ICP: Inductively coupled plasma

ICP-AES: Inductively coupled plasma atomic emission spectroscopy (Table 1)

ICP-OES: Inductively coupled plasma optical emission spectrometry (Table 1)

ISO: International Organisation for Standardisation

iTAP: Improved aquatic testing and assessment of cationic polymers (Cefic LRI ECO46)

IUPAC: International Union of Pure and Applied Chemistry

JRC: Joint Research Centre (European Commission)

K_d: Adsorption-desorption distribution coefficient

K_{oa}: n-Octanol/air partition coefficient

K_{oc}: Organic carbon/water partition coefficient

K_{ow}: n-Octanol/water partition coefficient

LET: Local Environment Tool (ECPA)

LMW: Low molecular weight

LRI: Long-range research initiative

MALS: Multi-angle light scattering (Table 1)

M_n: Number average molecular weight (for which acronym ‘NAMW’ was used in ECETOC TR. No. 133-1)

MPPD: Multi-Particle Pathway Distribution (model)

M_w: Weight-average molecular weight (by contrast, acronym ‘Mw’ used for ‘molecular weight’ in ECETOC TR. No. 133-1)

MWD: Molecular weight distribution

NIAS: Non-intentionally added substances

NICNAS: National Industrial Chemicals Notification and Assessment Scheme (Australia)

NMR: Nuclear magnetic resonance (Table 1)

OCSPP: Office of Chemical Safety and Pollution Prevention (US EPA)

OECD: Organisation for Economic Co-operation and Development

OPPTS: Office of Prevention, Pesticides and Toxic Substances (US EPA)

PBAT: Poly(butylene adipate-co-terephthalate) (Table 4C)

PBS: Polybutylene succinate (Table 4C)

PBT: Persistent, bioaccumulative, toxic

PCL: Polycaprolactone (Tables 4A and 4C)

PDMS: Polydimethylsiloxane

PEC: Predicted environmental concentration

PEG: Polyethylene glycol (Table 4A)

PFAS: Per- and polyfluoroalkyl substances

PHB: Polyhydroxybutyrate (Table 4A)

PHBV: Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (Table 4A)

pK_a: (Negative base-10 logarithm of) acid dissociation constant

PLA: Poly(lactic acid) (Tables 4A and 4C)

PLC: Polymer of low concern

PNEC: Predicted no-effect concentration

POP: Persistent organic pollutant

PVC: polyvinylchloride

QSAR: Quantitative structure-activity relationship

RCR: Risk characterisation ratio

REACH: Registration, evaluation, authorisation and restriction of chemicals

RIVM: National Institute for Public Health and the Environment (The Netherlands)

SEC: Size exclusion chromatography

SEM: Scanning electron microscopy (Table 1)

SHEDS: Stochastic human exposure and dose simulation

SMILES: Simplified molecular input line entry system

SPEC: Specification (Tables 4A-4C)

SpERC: Specific environmental release category (e.g. AISE, ECPA)

STD: Standard (Tables 4A-4C)

TEM: Transmission electron microscopy (Table 1)

TF: Task Force

TG: Test guideline

TGA: Thermogravimetry thermal analysis (Table 1)

THF: Tetrahydrofuran (Table 1)

$ThCO_2$: Theoretical CO_2 evolution

ThOD: Theoretical oxygen demand

TOC: Total organic carbon

TR: Technical Report

TRA: (ECETOC) Targeted Risk Assessment (tool)

UVCB: Substance of unknown or variable composition, complex reaction products and biological materials

vPvB: Very persistent and very bioaccumulative

WAF: Water-accommodated fraction

WHO IPCS: World Health Organisation - International Programme for Chemical Safety

WoE: Weight-of-evidence

WWTP: Waste water treatment plant

XRF: X-ray fluorescence (Table 1)

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