Peter Fisk Associates

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Critical literature review of analytical methods applicable to environmental fate studies

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1. Abstract

The results from bioaccumulation and degradation simulation tests (e.g. OECD TG 305, 307, 308 and 309) will depend on the accuracy, precision and sensitivity of the analytical methods. This report describes the outcome of thorough, transparent and critical literature review of analytical methods (and their limitations) applicable to these environmental fate studies. Literature used for this report was obtained from peer-reviewed databases, governmental agency websites and textbooks.

Current methods used in environmental fate studies are reviewed and summarised in the report. These methods describe the analysis of parent substances that are radiolabelled, stable-isotope labelled or non-labelled, as well as the analyses of transformation/degradation products. Information regarding quality control such as recovery and detection limits is also summarized.

An overview of analytical methods applicable to environmental fate studies is given in this report based on the review of studies performed on relevant media (i.e. water, fish, soil and sediment). This includes information on techniques that can be used for the extraction, separation and detection of substances from water, fish, soil and sediment.

Certain behaviour of test substances due to their properties can result in various challenges when performing bioaccumulation or degradation studies. This report reviews some of these challenges for substances that are e.g. poorly soluble or highly volatile as well as challenges arising with the analysis of complex mixtures. Criteria for analytical method validation is also reviewed in this report.

2. Introduction

This document is the final report prepared on behalf of ECHA to produce a critical literature review of analytical methods applicable to environmental fate studies (reference ECHA/2019/236, under Framework Contract No ECHA/2015/50 Lot 1, Service Request No 17).

The study approach has been planned to include four discrete Work Packages (WPs), as identified in the Terms of Reference:

- WP1: Definition of the scope of the review and the methodology.
- WP2: Literature search and review.
- WP3: A transparent analysis of the data.
- WP4: Final report + Webex presentation to PBT Expert Group.

The mandatory outputs for this project as stated in the Terms of Reference (ToR) are summarised as follows:

- What are the requirements to achieve regulatory compliance under the REACH and Biocidal Products Regulations?
- What are the requirements of recommended technical guidelines (i.e. OECD Technical Guidance, EU Methods)?
- How can current limitations be overcome?
- What analytical chemistry methodologies are applicable to environmental fate studies (e.g. OECD TG 305, 307, 308 and 309)?
- What is the impact of the methodological set-up on the relevance and reliability of the information generated from environmental fate studies?
- What are the specific limitations regarding the type of test substance (i.e. mono-constituent vs. multi-constituents/UVCBs), structural functionality and test substance properties (e.g. physical state, solubility, volatility, sorptivity etc.)?

This report describes the findings in relation to the mandatory requirements for this project. The final report is based on the Interim Reports defining the scope of the review and methodology (IR1_Scoping_document), the literature search and review performed (IR2_Literature _sources) and the transparent analysis of the data (IR3_Analysis_of_information). The Final Report describes in a systematic and clear manner the findings in relation to the mandatory requirements for this tender. In addition, as required by the ECHA ToR, the final report should contain a table of contents, a list of abbreviations, a list of references, and an abstract of no more than 200 words (Deliverable 1). An executive summary, as a separate document, which provides information on the purpose, methodology, results and conclusions (Deliverable 2).

The outcome of the analysis of the sources of information was structured as follows:

- Requirements for regulatory compliance and test validity:
    - A summary is provided on guidelines for bioaccumulation and persistence testing under the Registration, Evaluation, Authorisation and Restriction of Chemicals Regulation (REACH), Plant Protection Products Regulation (PPPR), Biocidal Products Regulation (BPR) and European Medicines Agency (EMA) Regulation.
- Findings on current practice in guideline bioaccumulation and biodegradation studies:
    - An overview is given on analytical methods used in OECD guideline-based bioaccumulation or persistence testing published in the peer-reviewed literature, as well as on the use of analytical methods reported in EFSA Draft Assessment Reports.

- Key aspects to consider prior to conducting biodegradation and bioaccumulation studies:
    - This section provides information on characterisation of the test substance(s), physico-chemical properties of the substance, *in silico* prediction methods, calibration and internal standards and quality criteria for analytical method validation.
- Synthesis of radiolabelled material:
    - This section contains information on the synthesis of radiolabelled standards as well as important factors that should be considered in radiochemical labelling.
- General issues:
    - This section provides information on artefact formation during sampling, sample preparation and on mass spectrometry. It also reviews artefacts related to contamination of solvents and improper storage of samples.
- Sample handling and extraction:
    - This section provides information on sampling of soil, sediment, water and how to handle fish. It also reviews extraction techniques from solid matrices and water and concentration techniques.
- Isotopic labelling and quantification:
    - This section reviews analytical techniques used for quantifying radiolabelled substances and describes limit of quantitation determination.
- Chromatography and stable isotope detection:
    - This section reviews various separation (i.e. gas or liquid based chromatography or other techniques including thin layer chromatography, ion chromatography and size-exclusion chromatography) and detection techniques (mass spectrometry as the main detection technique, but other techniques are also reviewed).
- Summary of specific issues for difficult substance types:
    - This section reviews aspect that should be considered for the analyses of difficult to test substances such as hydrophobic substances, surfactants, ionic substances and volatile substances.

It should be noted that literature review conducted for this project was based solely on publicly available literature (i.e. literature from peer-reviewed academic journals or published texts, and reports, guidance and legislation published by regulatory and governmental organisations). The authors were not provided with access to any ‘grey’ literature (such as industry study reports). Consultation with Contract Research Organisations was similarly not in the scope of this project.

3. Requirements for regulatory compliance and test validity

Under the REACH Regulation (EC No. 1907/2006) a persistent, bioaccumulative and toxic (PBT) and very persistent and very bioaccumulative (vPvB) assessment is required for all substances manufactured or imported in amounts of 10 or more tonnes per year and containing an organic moiety for which a Chemical Safety Assessment (CSA) is required. The PBT/vPvB assessment must take into account constituents, impurities and additives present at a concentration of ≥ 0.1% (w/w) and relevant transformation/degradation products, unless a justification is provided by the registrant as to why they are not relevant for the PBT/vPvB assessment.

A PBT/vPvB assessment is also required under the Biocidal Products Regulation (EC No. 528/2012; BPR) and the Plant Protection Products Regulation (EC No. 1107/2009) (PPPR). Whilst the medicinal products regulation (EC No. 726/2004) does not have specific legal provisions relating to the assessment or authorisation of PBT/vPvB substances, the assessment is required, and relevant guidelines for the assessment are provided by the European Medicines Agency. Persistence is also a key parameter in identifying substance which meet criteria set out in the Stockholm Convention on Persistent Organic Pollutants (POPs) (UNEP, 2017) and the recent initiatives relating to identification of substances that are persistent and mobile (Crookes and Fisk, 2018; Neumann and Schliebner, 2017).

Under each of the above regulatory regimes, the criteria for the identification of PBT or vPvB substances set out under REACH Regulation (EC No. 1907/2006) have been adopted. The P/vP and B/vB criteria set out in REACH Regulation (EC No. 1907/2006) are summarised in Table 3.1 below:

Table 3.1: P/vP and B/vB criteria as set-out in Annex XIII of REACH Regulation (EC No. 1907/2006)

	PBT	vPvB
Persistence	The persistence criterion (P) is fulfilled when: a. the degradation half-life in marine water is > 60 days; OR b. the degradation half-life in fresh or estuarine water is > 40 days; OR c. the degradation half-life in marine sediment is >180 days; OR d. the degradation half-life in fresh or estuarine water sediment is >120 days; OR the degradation half-life in soil is > 120 days.	The "very persistent" criterion (vP) is fulfilled when: a. the degradation half-life in marine, fresh or estuarine water is > 60 days; OR b. the degradation half-life in marine, fresh or estuarine water sediment is > 180 days; OR the degradation half-life in soil is > 180 days
Bioaccumulation	The bioaccumulation criterion (B) is fulfilled when the bioconcentration factor in aquatic species is > 2000 l/kg	The "very bioaccumulative" criterion (vB) is fulfilled when the bioconcentration factor in aquatic species is > 5000 l/kg.

Whilst the standard information requirements for, and approach to conducting a PBT/vPvB assessment differs under each regulatory regime (Table 3.2), the parameters to be obtained are the same, i.e. degradation rates in surface water, soil and sediment under environmentally relevant conditions (e.g. those specified under OECD TG 307, 308, 309) and bioconcentration factors in aquatic species (e.g. OECD TG 305). Such studies may also be part of the standard information requirements to be submitted under the relevant regulation, for environmental fate assessment, environmental exposure assessment (degradation studies) or for human health exposure in the context of secondary poisoning. For PPPs, and most biocides and medicinal products, the standard information requirements for environmental risk assessment usually include sufficient information for PBT/vPvB identification. A PBT/vPvB assessment based on a direct comparison of standard study results to the threshold values in Table 3.1 is therefore possible in most cases. Under REACH, the standard information requirements vary depending on the quantity manufactured or imported each year; nonetheless, registrants at Annex VIII and above are required to provide information required for concluding the PBT assessment. However, the approach detailed in REACH guidance (ECHA, 2017) allows for a screening assessment and Weight-of-Evidence (WoE) approach to PBT/vPvB assessment, including non-standard studies, QSAR data etc.[1] The standard information requirements for registration under the various frameworks are set out in Table 3.2. The acceptable or recommended test guidelines for persistence or bioaccumulation assessments under the various regulatory frameworks are listed in Table 3.3.

Table 3.2: Standard information requirements for the different regulatory frameworks relevant to PBT assessment

	READY BIODEGRADABILITY	DEGRADATION SIMULATION STUDIES IN SOIL	DEGRADATION SIMULATION STUDIES IN SEDIMENT	DEGRADATION SIMULATION STUDIES IN SURFACE WATER	BIOACCUMULATION STUDY
REACH	Required for substances registered at >1 tpa.	If not readily biodegradable, study required in the relevant compartment(s) for substances registered at >100 tpa (or for substances registered at >10 tpa if triggered by PBT assessment).			Required for substances registered at >100 tpa, where the substance has high potential for bioaccumulation (e.g. Log K_{ow} > 3) and direct or indirect exposure of the aquatic compartment is likely. The

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[1] It is noted that further guidance is available on the principles to be considered to develop a systematic approach to Weight of Evidence in OECD, 2019.

	READY BIODEGRADABILITY	DEGRADATION SIMULATION STUDIES IN SOIL	DEGRADATION SIMULATION STUDIES IN SEDIMENT	DEGRADATION SIMULATION STUDIES IN SURFACE WATER	BIOACCUMULATION STUDY
					information may also be triggered at >10 tpa by the PBT assessment.
PPPR	Required.	Required in four soils if not readily biodegradable. Further field studies required if degradation half-life of >60 days obtained in simulation study.	Required in two sediments if not readily biodegradable	Required if not readily biodegradable.	Required.
BPR	Required.	Required in four soils if not readily biodegradable (dependent on emission pathway) Further field studies required if degradation half-life >60 days obtained in soil simulation study AND $PEC/PNEC_{soil}$ >1.	Required in two sediments if not readily biodegradable (dependent on emission pathway/substance properties)	Required if not readily biodegradable (dependent on emission pathway/substance properties)	Required if the substance has surface activity or structural features indicating bioaccumulation (or where there is a risk for secondary poisoning). Studies with invertebrates may be required in addition to fish studies.
EMA	Required.	Required in four soils if not readily biodegradable and $K_{oc} > 10\,000$ l/kg	Required in two sediments if triggered by PBT assessment or for PEC refinement for groundwater.	Only required if triggered by PBT assessment.	Required for substances where a risk for secondary poisoning is identified (log $K_{ow} \geq 3$)

Table 3.3: Summary of test guidelines for persistence or bioaccumulation assessments under the various regulatory frameworks

	REACH	PPPR	BPR	EMA
Bioaccumulation	OECD TG 305 (EC method C.13) Other test guidelines acceptable; ASTM E1022-94 OPPTS 850.1730	Test method not specified.	OECD TG 305/ EC method C.13 (Bioconcentration: Flow-Through Fish Test)	OECD TG 305
Degradation in soil systems	OECD TG 307/ EC method C.23 Other WoE studies acceptable: Lysimeter studies, field studies, mesocosm studies, where degradation rates have been obtained.	OECD TG 307/ EC method C.23 OCSPP 835.6100: Terrestrial field dissipation	OECD TG 307 / EC method C.23 OECD TG 304A NAFTA Regulatory Directive - DIR2006-01 Guidance Document for Conducting Terrestrial Field Dissipation Studies	OECD TG 307/ EC method C.23
Degradation in sediment systems	OECD TG 308 Other test guidelines acceptable: OPPTS 835.3180 Other WoE studies acceptable: Mesocosm/macrocosm studies, where degradation rates have been obtained.	OECD TG 308	OECD TG 308	OECD TG 308
Degradation in aquatic systems	OECD TG 309 Other test guidelines acceptable: ISO 14592-1 / ISO 14592-2 OPPTS 835.3170 Other WoE acceptable: Mesocosm/macrocosm studies, where degradation rates have been obtained.	OECD TG 309	OECD TG 309 ISO 14592 OPPTS 835.3100 (with non-adapted inoculum) #	OECD TG 309

Whilst this test method is listed in ECHA (2018) as appropriate for a water simulation study (for assessment of aerobic aquatic degradation), the requirement of the test method is solely to measure mineralisation by trapping and quantification of CO_2 and therefore is not considered further in the present work.

3.1 Existing regulatory guidance for the design and interpretation of bioaccumulation studies

For the regulatory regimes assessed, the ECHA Guidance on Information Requirements and Chemical Safety Assessment (also known as Guidance on IR&CSA and from now on referred to as ECHA R.x) R.7c and R.11 provide the most detailed information on the considerations necessary in design or evaluation of a bioaccumulation study (ECHA R.7c: R.7.10.3.1, R7.10.4.1, ECHA R.11: R.11.4.1.2). ECHA Guidance on BPR (Volume IV Part A, V1.2) predominantly establishes whether testing is necessary for the substance under evaluation, and cross-references ECHA R.7c for additional information. The PPP regulation and associated communications specify that assessment of bioaccumulation shall be based on measured data (Regulation (EC) No 1107/2009, Section 3.7.2.2) but provides no additional guidance on the conduct of the study. The draft guidance under preparation by the EMA (EMEA/CHMP/SWP/4447/00 Rev. 1) notes elements in study design which are considered to be good practice. Regulatory guidance on key elements of study design and interpretation in the various frameworks are further summarised below.

3.1.1 Acceptable test systems

The PPPR and associated communications (Commission communication 2013/C 95/01; OECD series on pesticides, number 25 (OECD, 2005)) specify that assessment of bioaccumulation shall be based on measured data on bioconcentration in freshwater or marine species. Under the BPR, bioaccumulation studies with marine invertebrates may be required for some product-types, such as antifouling products, or if a direct release to marine or brackish environments occurs (ECHA Guidance on the BPR, Section 2.1.1.7, Section 5 (ECHA, 2018)). Furthermore, both the REACH guidance (ECHA R.7c, Section R7.10.3.1) and the draft EMA guidance notes (EMEA/CHMP/SWP/4447/00 Rev. 1, Section 5.2.2.2) comment that other aquatic species than fish (e.g., mussels) may be tested, and the resulting BCF compared to the B criteria. Whilst, generally, this involves the generation of a fish bioconcentration factor using an aqueous exposure, the REACH guidance (ECHA R.11, Section R11.4.1.2.3) allows for the assessment of bioaccumulation using a dietary exposure study (preferably OECD TG 305-III: Dietary Exposure Bioaccumulation Fish) for substances "for which it is not possible to maintain and measure aqueous concentrations reliably and/or potential bioaccumulation may be predominantly expected from uptake via feed (e.g. for substances with extremely low water solubility and high K_{oc}, which will usually dissipate from water to organic matter)". For strongly hydrophobic substances (log K_{ow} > 5, water solubility below ~ 0.01-0.1 mg/l), testing via aqueous exposure may become increasingly difficult. It is further noted, however, that registrants should initially consider whether the use of an improved analytical technique, dosing method or of a radiolabelled substance before concluding that a dietary test is the only feasible option.

It should be noted that the REACH guidance (ECHA R.11, Section R.11.4.1.2.1) notes that bioaccumulation in air-breathing organisms may also be a concern. Where a substance meets the screening criteria (Log K_{ow} >2, log K_{oa} >5), it should first be considered whether aquatic bioaccumulation testing is expected to reflect a 'worst-case'. If aquatic bioaccumulation testing is carried out and indicates no bioaccumulation potential, it may still be necessary to generate further data in air-breathing organisms. No specific test guidelines or B/vB metrics for air-breathing organisms are available at present, however this area is being developed and it can be anticipated that such methods will introduce new study design and analytical methodology challenges.

3.1.2 Requirements for use of radiolabelled test substance

ECHA guidance (R.7c, Section R.7.10.4) recommends that radiolabelled test substances are used where possible, since they can be useful to detect organ specific enrichment or in cases where there are analytical difficulties. However, total radioactivity measurements alone can lead to an overestimation of the parent substance concentration, due to radiolabelled impurities present in the test material which may have a higher BCF than the test substance itself[2], or due to the presence of biodegradation/biotransformation products in tissues sampled (if one or more of the transformation products contains the radiolabel, the BCF based on total radioactivity will be overly conservative for the parent substance). A parent compound-specific chemical analytical technique or selective clean-up procedure should therefore be utilised throughout the exposure period.

The draft guidance under preparation by the EMA (EMEA/CHMP/SWP/4447/00 Rev. 1) notes that bioaccumulation studies should preferably be performed with radiolabelled compounds (EMEA/CHMP/SWP/4447/00 Rev. 1, Section 5.2.2.2).

3.1.3 Recommendations in relation to sampling/clean-up, extraction and analysis

The ECHA guidance R.7c, Section R.7.10.4 includes a number of specific recommendations which relate to the sampling, extraction and analysis requirements for aqueous and dietary bioaccumulation studies. These relate to the assessment of validity and reliability for existing studies and are therefore taken implicitly as recommendations for such studies. The guidance notes the following points:

Sampling

- In an aqueous exposure study, both fish and water are sampled at regular time-intervals and the concentration of test substance measured (test substance concentration in the test medium must be less than its solubility in the dilution water).

Analysis

- The study should utilise a parent substance-specific analytical method in both exposure medium (aqueous or dietary) and fish tissue.
- Concentration of the test substance in/on the whole fish, or in specified tissues of the fish may be measured[3]. Additionally, it is noted that in fish dietary studies (OECD TG 305-III), the removal of guts prior to analysis is recommended for certain substances (e.g. poorly assimilated or highly metabolised substances). The guts should be retained for further analysis if required.

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[2] The present authors consider that this would already be accounted for, since the purity of the radiolabelled test substance should be ascertained prior to the initiation of any study.

[3] R7.10.4 further notes that "in cases where BCFs are specified on tissue types other than whole body (e.g. liver), the results cannot be used unless tissue-specific BCF values can be normalised to lipid content and converted to a whole body BCF based on pharmacokinetic considerations."

- In a dietary study (OECD TG 305-III) the homogeneity of the test substance in the spiked food should be established (should homogenous distribution of the test substance in the spiked food not be achieved, the test organism may preferentially select food which does not contain or contains minimal test substance, leading to a falsely low BMF).

Extraction

The draft guidance under preparation by the EMA (EMEA/CHMP/SWP/4447/00 Rev. 1) notes that bioaccumulation studies should be completed using the best possible extraction methods (EMEA/CHMP/SWP/4447/00 Rev. 1, Section 5.2.2.2).

3.1.4 Requirement for the identification of metabolites

ECHA guidance (R.7c, Section R.7.10.4) also notes that, for substances which undergo biotransformation (metabolism), the bioaccumulation potential of the transformation products (metabolites) should be scrutinised in the PBT/vPvB assessment (specifically, when these metabolites are identified as persistent and could be potentially of concern). The assessment of bioaccumulation potential of metabolites has implications both on study design (number of fish, pooling of samples) and the analytical method (ability to distinguish between metabolites and parent substance, sensitivity and LOQ/LOD).

3.2 Comparison of test guidelines for bioaccumulation studies

In addition to the review of the regulatory guidance, a comparison of the test methods recommended within these guidance documents for bioaccumulation studies has been made (see Table 3.4), similarly focussing specifically on the aspects of the guidance relating to radiolabelling, sampling/clean-up, extraction and analysis. Three test guidelines are acceptable under the various regulatory frameworks, with the OECD TG 305 the most commonly preferred method[4].

Table 3.4: Comparison of methods for bioaccumulation studies

Test Guideline	OECD TG 305 (aqueous and dietary exposure methods)	OPPTS 850.1730 (aqueous exposure only)	ASTM E1022 (aqueous exposure only)
Applicability of test guideline	Aqueous exposure test is most appropriately applied to stable organic chemicals with log K_{OW} values between 1.5 and 6.0, though may be applied to strongly hydrophobic substances if a stable dissolved concentration of the test substance in water can be achieved. Dietary exposure is intended for poorly soluble non-polar organic substances; aqueous exposure should be preferred	No guidance provided on the applicability of the test guideline to substance types.	Test considered to be applicable to all chemicals that can be measured accurately at the necessary concentrations in water and in appropriate tissues. Guidelines developed for non-ionizable organic chemicals and might not apply to ionizable or inorganic chemicals.

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[4] It is noted that the current EU C.13 method is based on the OECD test guideline (TG) 305, 1996, which was updated in October 2012. The EU C.13 guideline does not provide any information or guidance which differs to or goes beyond that provided in the updated OECD TG 305, and is therefore not considered further here.

Test Guideline	OECD TG 305 (aqueous and dietary exposure methods)	OPPTS 850.1730 (aqueous exposure only)	ASTM E1022 (aqueous exposure only)
	unless adequate justification can be provided indicating that the study is not technically feasible.		
Use of radiolabelling.	Use of radiolabelled test substance is preferred. Radiolabelled test chemical purity should preferably be > 98%; the percentage of radioactivity associated with impurities should be known.	Use of radiolabelled test substance preferred in order to simplify analyses. When a radiolabelled substance is used, the precise position of the labelled atoms, the radiopurity, and the percentage of radioactivity associated with impurities should be identified. Radiolabelled test chemical purity should be greater than 95%, but greater than 98% is preferred.	Radiolabelled test materials are considered more useful for demonstrating the presence of metabolites than for measuring BCF. If radiolabelled material is used, total radioactivity should be measured on all samples (it is noted that combustion of samples and trapping of $^{14}CO_2$ is easier than Liquid Scintillation Counting). Use of chromatographic methods to verify that radioactivity is associated with the parent chemical is usually recommended (see below regarding acceptability of the test method).
Study validity criteria relating to analytical methodology	None.	None.	For the test to be acceptable, the percentage of radioactivity associated with impurities in both water and tissue must be determined using gas or liquid chromatography when a radiolabelled test material is used.
Requirements or recommendations for the analytical methodology	Analytical method of known accuracy, precision, and sensitivity in water and biological tissues	Pre-test method development should be conducted to minimise results reported as	The precision and bias of each analytical method used should be determined in

Test Guideline	OECD TG 305 (aqueous and dietary exposure methods)	OPPTS 850.1730 (aqueous exposure only)	ASTM E1022 (aqueous exposure only)
	(aqueous exposure) and in food and biological tissues (dietary exposure). Quantification limit of the test substance in both exposure medium (water/diet) and fish tissues should be known. The sensitivity of the analytical technique for quantifying test substance and metabolites in tissue and aqueous or food concentrations should also be known.	<LoD. Validation of the analytical method should be conducted prior to the test; relevant MDL and LoQ should be reported.	appropriate matrices.
Requirement to identify metabolites / degradation products	If the BCF is to be based on the parent substance, the major metabolites should be characterised, as a minimum at the end of the uptake phase.	If the BCF in terms of total radiolabelled residues is greater than or equal to 500, identification and quantification of test substance metabolic products or degradants representing greater than or equal to 10% of total residues in fish tissues at steady state should be attempted.	Analysis of tissue samples for likely reaction and degradation products of the test material is desirable, especially if a radiolabelled test material is used.
Fish sampling	For determination of the BCF analysis of the whole fish is required. Additional analyses of specific organs (e.g. muscle, liver) or of edible (fillet) and non-edible (viscera) fractions may be performed, depending on the purpose of the study. For determination of BMF, analysis of the whole fish is normally required. Removal and separate analysis of the gastrointestinal tract may be employed to determine the contribution to whole fish concentrations for sample points at the end of the uptake	For determination of the BCF, analysis of the whole fish is required. Analyses of the edible (fillet) and non-edible (viscera) should be performed whenever possible. Additional analyses of specific organs may be performed. It is noted that, if a sufficiently sensitive analytical method is not available, then pooling of the fish is necessary to constitute a sample for measurement. Pooling restricts the statistical procedures which can be applied to the data.	The whole body should be either analysed for test material. In tests with fish four samples of muscle (with or without skin) or adductor muscle, respectively, should be obtained at the end of the uptake phase from additional organisms for measurement of the test material in edible tissue.

Test Guideline	OECD TG 305 (aqueous and dietary exposure methods)	OPPTS 850.1730 (aqueous exposure only)	ASTM E1022 (aqueous exposure only)
	phase and near the beginning of the depuration phase, or as part of a mass balance approach. Pooling of fish samples is allowed if necessary, to ensure detectable substance concentrations and/or lipid content determination.		
Fish lipid determination	The lipid content of the fish should ideally be determined on the same extract as that produced for analysis for the test substance, since the lipids often have to be removed from the extract before it can be analysed chromatographically. However, it is noted that analysis of test substance often requires specific extraction procedures which might be in contradiction to the guidelines for lipid determination.	When possible, the analysis for total lipid should be made on the same extract as that produced for analysis for the test substance. Lipid material should not be present in samples used for chromatographic analysis	Fish lipid content should be determined in the same tissues in which test material is measured. "In bioconcentration tests on organic chemicals, "lipids" are sometimes measured by evaporating and weighing a portion of the extract obtained in the extraction of the test material from the tissue, using an organic solvent."
Water sampling and sample clean-up	In an aqueous exposure study, sampling of the water should be achieved by siphoning through inert tubing from a central point in the test chamber. Notes that neither filtration nor centrifuging appears always to separate the non-bioavailable fraction of the test substance from that which is bioavailable.	No guidance is provided on sampling or clean-up of the water sample.	Sampling of the water should be achieved by siphoning through inert tubing from a central point in the test chamber. A second sample should be taken and analysed after filtration or centrifugation to determine the percentage of test material associated with particulate matter.

Test Guideline	OECD TG 305 (aqueous and dietary exposure methods)	OPPTS 850.1730 (aqueous exposure only)	ASTM E1022 (aqueous exposure only)
	These techniques should not be used for highly absorbing substances (log K_{ow} >5). It is recommended that the tanks should be kept as clean as possible (e.g. by daily siphoning uneaten food and faeces from the test chamber) and the TOC content should be monitored during the uptake and depuration phases. Justification for any separation technique applied should be provided in the study report. Measurement of concentrations in water is not usually required in a dietary study.		

3.3 Existing regulatory guidance for design and interpretation of degradation simulation studies (surface water, sediment, and soil)

Degradation simulation studies may be used in environmental risk assessment, and in some cases are a standard information requirement under the regulatory assessment frameworks and according to the specified testing strategies. For example, under the medicinal products framework, substances for which a Phase II risk assessment has been performed inclusive of the soil compartment assessment, no additional testing for the PBT assessment will be required (i.e. degradation simulation studies in soil and/or sediment are performed as part of the risk assessment (EMEA/CHMP/SWP/4447/00 Rev. 1, Section 5)). Similarly, for PPPR and BPR products, the requirements of the environmental risk assessment usually generate the data required for PBT assessment. Under REACH, the standard information requirements depend on the tonnage of the substance manufactured/imported; degradation simulation studies are stipulated for substances where manufacture/import exceeds 100 tpa (though registrants at Annex VIII and above are required to provide all information required for PBT assessment). In the context of the PBT/vPvB assessment, in order to determine the persistence of the substance (and degradation products) within the compartment of interest, ECHA Guidance R.11 (Section R.11.4.1.1.3) states:

"*In principle, degradation simulation studies performed in appropriate environmental media and at environmentally realistic conditions are the only tests that can provide a definitive degradation half-life that can be compared directly to the persistence criteria as defined in REACH Annex XIII. The simulation tests as described in OECD TGs 307, 308 and 309 address the fate and behaviour of a substance as it may be expected in the environment including information about partitioning in the test system, primary or complete degradation, adsorption behaviour and route(s) of degradation (degradation* *products). The endpoints that need to be addressed are primary or ultimate degradation rate and degradation half-lives (DegT50) or dissipation half-lives (DT_{50}) for the compartments included in the test system as well as the route of degradation, metabolites and non-extractable residues."*

3.3.1 Requirements for use of radiolabelled test substance

ECHA Guidance R.7b (Section 7.9.4.1) notes that, whilst methods will normally be substance specific, radio-labelling and specific chemical analyses may allow measurement of metabolite formation and decay. It is recommended that specific-chemical analyses be used in parallel with radio-detection methods. The use of radiolabelled substance specific chemical analyses can also be used to identify and quantify transformation products. ECHA Guidance R.11 (Section R.11.4.1.1.3) notes that, wherever feasible, a simulation study should be performed using a radio-labelled molecule. A mass balance should be included in simulation tests so that possible losses from the test system during the test period can also be quantified.

The draft guidance under preparation by the EMA (EMEA/CHMP/SWP/4447/00 Rev. 1) recommends that degradation studies should be preferably performed with radiolabelled compounds and using the best possible extraction methods. The draft guidance further states that only in exceptional cases (where it can be shown to be technically not feasible to synthesise a radiolabelled test substance) may acceptable degradation data be produced using an unlabelled test substance since the mass balance requirement cannot be met.

3.3.2 Identification of transformation/degradation products

As stated in Section 3.1.4 above, should a transformation/degradation product meet the criteria for persistence, the bioaccumulation potential should also be assessed. ECHA Guidance R.7b (Section 7.9.4.1) notes that, whilst methods will normally be substance specific, radio-labelling and specific chemical analyses may allow measurement of metabolite formation and decay and states that "*where analytically possible, identification, stability, behaviour, molar quantity of metabolites relative to the parent substance should be evaluated*". The technical limitations associated with low test substance concentrations, and consequential low metabolite concentrations are acknowledged.

The ECHA BPR guidance (Section 2.2.6.1; Section 1.6) requires that identification of all major degradation products[5] must be included in the studies on degradation in soil, water and sediments. Any simulation test must at least fulfil the following criteria (Section 4.2.5):

- give measured rates for primary degradation and an indication of the mineralisation potential.
- allow for quantification and identification of metabolites formed during the test; and

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[5] Major degradation products are defined as those which account for ≥ 10% of the active substance at any time of the degradation studies under consideration, or where the degradant appears at two consecutive sampling points at amounts ≥ 5%, or where, at the end of the study, the maximum of formation is not yet reached but accounts for ≥ 5% of the active substance at the final time point;

- provide an indication of the degradation rates or persistence of the metabolites.

The guidance further indicates that, should the degradation rate for the metabolite not be determined (from the soil simulation) study on the active substance, there may be a need to perform separate studies for the metabolites.

Similarly, under the PPPR data requirements for assessment of degradation in soil, where a simulation study is required the kinetic formation fraction and degradation rates of potentially relevant metabolites should be established (FOCUS, 2014), and reliable degradation of 50% and 90% (DegT50 and 90) values for metabolites, breakdown and reaction products should be provided. In aquatic and sediment simulation studies, the PPPR guidance (Section 9.2.1) recommends that the pathways involved in the degradation are reported schematically, and a mass balance is provided which show the distribution of radio-label in water/sediment as a function of time, including radioactivity associated with the active substance; CO_2; volatile compounds other than CO_2; individual identified transformation products; extractable substances not identified; and non-extractable residues in sediment. It is noted that, in order to determine the degradation pattern of potentially relevant metabolites occurring within the soil and water/sediment studies, extension of the study for the active substance may be also required. Although this may not be recommended as the biogeochemical equilibrium of the test system may collapse due to extension of the test.

3.3.3 Simulation study test temperature

The reference temperature for simulation tests (against which the P/vP criteria are assessed) stated by ECHA Guidance R.11 (Section R.11.4.1.1.3) is 12°C for surface water environment and 9°C for a marine environment[6]. Ideally, new studies should be conducted at environmentally relevant temperature (9-12°C) to determine degradation rate constants. Where this is not possible, the degradation half-lives can be normalised to 12°C using the Arrhenius equation (further guidance on the temperature correction is provided in ECHA Guidance R.7b. (Section R.7.9.4.1), FOCUS Guidance (FOCUS, 2014) and EFSA (2007)). The use of a low test temperature has implications for the sensitivity required for the analytical method, since the formation of degradation products will be slower at low test temperatures, resulting in smaller quantities of degradation products formed during the study and consequently requiring more sensitive analytical methods. ECHA Guidance R7b (Section 7.9.4.1) states that "for the purpose of identifying degradation products, a higher test temperature (but within the frame provided by the study guideline) could be used to overcome potential analytical limitations for the identification and quantification of those degradation products.". In certain circumstances, it may be appropriate to conduct a parallel study with a higher test substance loading in order to analytically determine the transformation and degradation products.

3.3.4 Requirement to conduct further studies

Furthermore, under PPPR and in some circumstances under BPR, field studies are also be required for assessment of degradation in soil. Where possible DegT50 and DegT90 times of the test substance under field conditions should be obtained from such studies (in addition to the dissipation half-life; DT_{50}), and information on metabolites, degradation and reaction products must be reported.

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[6] It is noted that the test temperature required by the test guideline may differ from the ECHA requirements in the REACH guidelines, e.g. the OECD TG 309 requires testing at 'field temperature' or a standard tempterature of 20-25°C.

3.3.5 Analytical methodology

Under the PPPR, information on data requirements (Commission Regulation (EU) No. 284/2013) further guidance is provided regarding the submission of information on the analytical methods used for generation of pre-authorisation data and required for post-authorisation control and monitoring. Registrants are required to submit methods used for the determination of residues[7] (including full description), for the determination of non-isotope-labelled residues in soil, water, sediment, air and any additional matrices used in support of environmental fate studies (Section 5.2.1).

3.4 Comparison of test guidelines for degradation simulation studies

In addition to the review of the regulatory guidance, a comparison of the test methods recommended within these guidance documents for degradation simulation studies has been performed (Tables 3.5, 3.6 and 3.7, for water, sediment and soil, respectively), similarly focussing specifically on the aspects of the guidance relating to radiolabelling, sampling/clean-up, extraction and analysis. The OECD TG 309 is based on the ISO 14592-1; therefore, the requirements of both methods are very similar.

Table 3.5: Comparison of methods for degradation in surface water

	OECD TG 309	ISO 14592-1 [8]
Principle of the method	The principal objective of the test is to determine the mineralisation of the test substance. However, a secondary objective of the test is to obtain information on the primary degradation and the formation of major transformation products. Identification of transformation products, and if possible, quantification of their concentrations, are especially important for substances that are very slowly mineralised (e.g. with half-lives for total residual ^{14}C exceeding 60 days). It is recommended that transformation products detected at ≥ 10% of the applied radioactivity, at any sampling time should be identified unless reasonably justified otherwise. Transformation products	Measurement of the degradation of the test compound is carried out either by a radiotracer technique, normally using ^{14}C-labelling and liquid scintillation counting, or by specific chemical analysis, if a sufficiently sensitive analytical method is available. Using the ^{14}C technique and labelling the most persistent part of the molecule with ^{14}C, total mineralization or ultimate biodegradation can be assessed, while only primary biodegradation can be measured with specific analysis.

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[7] Where ‘residues’ means “*one or more substances present in or on plants or plant products, edible animal products, drinking water or elsewhere in the environment and resulting from the use of a plant protection product, including their metabolites, breakdown or reaction products*” [as defined in Regulation(EC) No 1107/2009].

[8] ISO 14592-2 is a continuous river flow simulation so has not been reviewed here.

	OECD TG 309	ISO 14592-1 [8]
	for which concentrations are continuously increasing during the study should also be considered for identification, even if their concentrations do not exceed the limit given above, as this may indicate persistence. Higher concentrations of the test substance (e.g. >100 µg/l) is recommended for identification and quantification of major transformation products due to analytical limitations (providing that the test substance concentration does not exceed the solubility of the substance in test media).	
Applicability of test guideline	Applicable to non-volatile or slightly volatile organic substances tested at low concentrations.	Applicable to organic test compounds present in lower concentration (normally below 100 µg/l) than those of natural carbon substrates present in the system. Method may not be well suited to studies on metabolite formation (since this requires higher test concentrations).
Radiolabelled substance recommendations	The test may be conducted using radiolabelled or non-radiolabelled test substance. For substances containing more than one aromatic ring, one or more carbons in each ring should preferably be ^{14}C-labelled. In addition, one or more carbons on both sides of easily degradable linkages should preferably be ^{14}C-labelled. The chemical and/or radiochemical purity of the test substance should be >95%; a specific activity of approx. 50 µCi/mg (1.85 MBq/mg) or more is preferred in order to facilitate ^{14}C measurements in tests conducted with low initial concentrations.	The test may be conducted using radiolabelled or non-radiolabelled test substance. Radiolabelled chemicals should be of high chemical purity.
Test temperature	Should be carried out at environmentally relevant temperature, either ‘field temperature’ (temperature of the sample at sampling time or average field temperature at the sampling site) or at a standard temperature of 20-25°C.	Either at field temperature or at a temperature of 20-25°C.
Recommendation for analytical methods	Analytical methods for quantification of the test substance and its transformation products should be available Concentrations of ^{14}C-labelled test substance and transformation products may be determined by use of radio-chromatography (e.g. thin layer chromatography, RAD-TLC) or HPLC with radiochemical detection. Information on the analytical	Not specified.

	OECD TG 309	ISO 14592-1 [8]
	techniques and the methods used for radiochemical measurements and for mass balance check and measurements of phase distribution, and the repeatability and sensitivity of the analytical methods used, including the limit of detection (LOD) and the limit of quantification (LOQ) must be reported.	
Sample clean-up requirements	If the analytical method involves either solvent extraction or solid phase extraction (SPE), the extraction should be performed immediately after sampling or after storing the sample refrigerated for a maximum of 24 hours.	Not specified.
Quality criteria	Initial test concentration should be verified by measurements of ^{14}C activity, or by chemical analyses in the case of non-labelled substances, in at least duplicate samples. The limit of detection (LOD) of the analytical method for the test substance and for the transformation products should be at least 1% of the initial amount applied to the test system if possible. The limit of quantification (LOQ) should be equal to or less than 10% of the applied concentration.	Not specified.
Validity criteria	The reference substance must be degraded within the expected time interval. The determination of the total recovery (mass balance) at the end of the experiment is mandatory. Radiolabelled mass balance should range from 90% to 110%. For non-labelled substances, the analytical accuracy should lead to an initial recovery of between 70% and 110%. (It is noted that these ranges should be considered to be targets and not used as acceptance criteria for the test)	Not specified.

Table 3.6: Comparison of methods for degradation in sediment

	OECD TG 308 (AEROBIC AND ANAEROBIC)	OPPTS 835.3180
Principle of the method	The method is intended to allow measurement of the dissipation or transformation rate of the test substance in the total water-sediment system, and in the sediment compartment alone. It further allows measurement of the mineralisation rate of the test substance and/or its transformation products (when ^{14}C-labelled test substance is used), and the identification and quantification of transformation products in water and sediment phases including mass balance.	A mass balance approach is used where the test substance's transport to or from and appearance in all applicable media of the microcosm, including sediment, overlying water, interstitial water, off-gases, and test equipment washings is determined, and formation of ^{14}C-labeled CO_2 is determined if radiolabelled parent compound is used. Primary and ultimate biodegradation rates may be obtained by analysis of the water, sediment and off-gases for disappearance of the parent compound and appearance of metabolites (if feasible).
Applicability of the test guideline	Applicable to slightly volatile, non-volatile, water-soluble or poorly water-soluble compounds. The test should not be applied to chemicals which are highly volatile from water (e.g. fumigants, organic solvents) and thus are not retained in water and/or sediment under the experimental conditions of this test.	Applicable to various classes of inorganic and organic compounds (the specific type of test compound used should be considered in selecting an appropriate microcosm design, testing protocol, and analytical technique.)
Radiolabelled substance recommendations	^{14}C-labelling is recommended, but the use of other isotopes, such as ^{13}C, ^{15}N, ^{3}H, ^{32}P, may also be useful. The radiolabel should be positioned on the most stable part of the molecule. Radiochemical purity should be at least 95%.	The use of ^{14}C-labeled test substances is recommended. The position of the radiolabel must be specified in the study report.
Test temperature	The test should be performed at constant temperature in the range of 10 to 30°C.	Temperature should simulate the environmental conditions at the sampling site.
Recommendations for analytical methods	Analytical methods (including extraction and clean-up methods) for identification and quantification of the test substance and its transformation products in water and in sediment should be available. Chemical analysis of test substance and transformation products is required (e.g. by gas liquid chromatography (GLC), high performance liquid chromatography (HPLC), thin-layer chromatography (TLC), mass spectroscopy (MS), gas chromatography-mass spectroscopy (GC-MS), liquid chromatography-mass spectrometry (LC-MS), nuclear magnetic resonance (NMR), etc.), including detection systems for radiolabelled or non-labelled chemicals as appropriate. When radiolabelled material is used a liquid scintillation counter and combustion oxidiser (for the combustion of sediment samples prior to analysis of	Compound-specific analytical methods are required. Gas chromatography (GC) and high performance liquid chromatography (HPLC) are suitable for the quantification of many test compounds. Use of appropriately radiolabelled test substances is recommended, especially when quantifying mineralization or identifying degradation products that need further characterization by conventional analysis. The identity of each metabolite with >10 % yield should be determined by TLC, HPLC or other analytical technique suitable for identifying metabolites.

	OECD TG 308 (AEROBIC AND ANAEROBIC)	OPPTS 835.3180
	radioactivity) will also be required. The analytical methods and detection limits used, and the methods for characterisation/identification of transformation products must be reported in the study report.	
Sample clean-up requirements	The extraction and characterisation of the test substance and transformation products should follow appropriate analytical procedures. Extraction methods and efficiencies must be reported in the study report.	Not specified.
Quality criteria	Initial test concentration should be verified by measurements of ^{14}C activity, or by chemical analyses in the case of non-labelled substances, in at least duplicate samples. Radiolabelled mass balance should range from 90% to 110%. For non-labelled substances, the analytical accuracy should lead to an initial recovery of between 70% and 110%. The repeatability of the analytical method for quantification of test substance and transformation products should be checked by duplicate analysis of the same extract of the water or the sediment samples which were incubated long enough for formation of transformation products. The limit of detection (LOD) of the analytical method for the test substance and for the transformation products should be at least 0.01 mg/kg (test substance) in water or sediment or 1% of the initial amount applied to the test system, whichever is lower. The limit of quantification (LOQ) should be specified.	It is noted that the sensitivity of the test depends upon the analytical methods used.

Table 3.7: Comparison of methods for degradation in soil

	OECD TG 307 (aerobic and anaerobic)	OPPTS 835.3300
Principle of the method	The method is intended to allow measurement of the transformation rate of the test substance in a soil system. It further allows measurement of the mineralisation rate of the test substance and/or its transformation products (when ^{14}C-labelled test substance is used), and the identification and quantification of transformation products in the soil, including mass balance.	Both a basic mineralisation test and additional tests describing the determination of volatilisation of parent/metabolites and determination of soil extractable and non-extractable residues are included.
Applicability of test guidelines	Applicable to slightly volatile, non-volatile, water-soluble or water-insoluble compounds. The test should not be applied to chemicals which are highly volatile from soil (e.g. fumigants, organic solvents) and thus cannot be kept in soil under the experimental conditions of this test.	The test is applicable to volatile or non-volatile, soluble or insoluble compounds which are not inhibitory to microorganisms.
Radiolabelled substance recommendations	^{14}C-labelling is recommended, but the use of other isotopes, such as ^{13}C, ^{15}N, ^{3}H, ^{32}P, may also be useful. The radiolabel should be positioned on the most stable part of the molecule. Radiochemical purity should be at least 95%.	^{14}C-labelled material is required (37–185 kBq (≈1–5 mCi)/100 µL) The mineralisation rate refers to the labelled carbonation only. Therefore, the location of the labelling within the structure and the specificity of the label need careful consideration.
Test temperature	The test temperature should be representative of the climatic conditions where use or release will occur (20±2°C recommended for test substances which may reach soil in temperate climates. For test substances likely to be released in colder climates, additional replicates should be prepared and incubated at a lower temperature, e.g. 10±2°C)	Test temperature of 22 ± 2°C.
Recommendations for analytical methods	Analytical methods (including extraction and clean-up methods) for quantification and identification of the test substance and its transformation products should be available. Repeatability of the analytical method (excluding the initial extraction efficiency) to quantify test substance and transformation products should be checked by duplicate analysis of the same extract of the soil, incubated long enough for formation of transformation products. The limit of detection (LOD) of the analytical method for the test substance and for the transformation products should be	Liquid scintillation counting is used. No chemical-specific analysis is required.

	OECD TG 307 (aerobic and anaerobic)	OPPTS 835.3300
	at least 0.01 mg/kg soil (as test substance) or 1% of applied dose whichever is lower. The limit of quantification (LOQ) should also be specified. The repeatability and sensitivity of the analytical methods used must be reported in the study report.	
Sample clean-up requirements	None specified.	To determine the soil-extractable residues, the soil is extracted with 100 mL acetone (5 min ultrasonic treatment) followed by an extraction with methanol in the same manner. Aliquots of the combined extracts are taken for liquid scintillation counting. Other extract portions may be used—if necessary—for further identification studies. To determine an estimation of evaporation, a PU plug is used to absorb volatile parent/metabolites. This plug should be extracted in a Soxhlet apparatus with n-hexane:methanol mixture (1:4). Aliquots are taken for scintillation counting.
Quality criteria	Initial test concentration should be verified by measurements of ^{14}C activity, or by chemical analyses in the case of non-labelled substances, in at least duplicate samples. Radiolabelled mass balance should range from 90% to 110%. For non-labelled substances, the analytical accuracy should lead to an initial recovery of between 70% and 110%. The limit of detection (LOD) of the analytical method for the test substance and for the transformation products should be at least 0.01 mg/kg (test substance) in soil or 1% of the initial amount applied to the test system, whichever is lower. The limit of quantification (LOQ) should be specified.	Sensitivity and specificity are considered to be very good, since the use of radiolabelled compounds is required.

3.5 Conclusions on the regulatory requirements for bioaccumulation and degradation simulation studies

The findings summarised above in Section 3 consolidate the requirements for the analytical methodology required for bioaccumulation and degradation simulation studies conducted for European chemical regulatory requirements. It is apparent that, whilst some detailed guidance is provided in relation to study design, good practice for sample clean-up, extraction and analysis is generally regarded as being substance specific and only general recommendations are provided. Recommendations for radiolabelling of test material are detailed, in terms of radiochemical purity and activity (where applicable). Some guidance is provided on the location of the radiolabel.

4. Methodology of the literature search

The literature search performed for this project was divided into four sections intended to determine:

1. The current state of the science: analytical methods currently used in guideline bioaccumulation and persistence studies and the challenges associated with these analyses (Section 4.1).
2. The challenges and uncertainties associated with analysis of chemicals in relevant media (water, fish, soil and sediment) and the approaches used for each stage of the analytical process. This search was not limited to bioaccumulation and persistence testing and includes insights from the field of environmental trace analysis[9] (Section 4.2).
3. Guidelines for validation of analytical methods in environmental fate studies (Section 4.3).
4. The use of *in silico* tools for the prediction of transformation and degradation products (Section 4.4)

4.1 Current state of the science for environmental fate studies

4.1.1 Keywords and information sources

A specific literature search was targeted at finding information on analytical methods currently used in guideline bioaccumulation and persistence studies and the challenges associated with these analyses. The literature search was limited to academic literature, and publicly available information sources (e.g. EFSA Draft Assessment Reports (see Section 4.1.4) and governmental agency resources (see Section 4.3)). Studies reported in the academic literature may not have been performed for any regulatory purpose, may not be performed according to GLP, and the data may not have undergone rigorous QA/QC procedures (this is further discussed in Section 5).

The keywords listed in Table 4.1 were used in Science Direct[10] and Wiley library[11] (SETAC journals, namely 'Environmental Toxicology and Chemistry', and 'Integrated Environmental Assessment and Management').

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[9] Environmental trace analysis is the detection (and quantification) of contaminants present at trace levels in the environment.

[10] https://www.sciencedirect.com/

[11] https://setac.onlinelibrary.wiley.com/

Table 4.1: Keywords for the literature search

KEYWORDS	NUMBER OF RESULTS	NUMBER OF ARTICLES SELECTED AFTER INITIAL SCREENING
OECD TG 305 or OECD 305	103	46
OECD TG 307 or OECD 307	30	20
OECD TG 308 or OECD 308	41	19
OECD TG 309 or OECD 309	19	10

It was clear that the set of keywords in Table 4.1 would not produce a comprehensive list of published bioaccumulation and biodegradation studies. However, the use of these key words was intended to target literature in which the specific OECD guidelines had been followed. This was so that the analytical methods used to support these studies could be understood. After screening of the obtained literature, the number of relevant papers for each study type was large enough to allow conclusions to be drawn but few enough that the review can be conducted in the available time.

4.1.2 Inclusion and exclusion criteria

The results obtained from the searches in Science Direct and Wiley Library contained studies that can be used for this project and studies that fall outside of the scope of the project. Therefore, all obtained literature has been through at least part of a multi-step inclusion/exclusion screening process.

An initial screening was performed based on the title and abstract of the obtained literature, to decide whether the study was relevant or not. Selected studies after the initial screening that were studies published after the year 2000, written in English and obtained from the peer-reviewed and grey literature. The studies were also laboratory-based, and the data were obtained experimentally (QSAR methods for prediction of bioaccumulation factor or degradation rate are not considered). Studies falling outside these criteria and studies on nanomaterials and methods were excluded.

The studies that were selected after the initial screening based on the title and abstract underwent a second screening whereby the method section of the study was reviewed. It was decided whether the study was relevant or not if they contained a detailed description of the extraction, clean-up and detection methods, as well as information on method validation (recovery, repeatability and precision) and detection and/or quantification limits. Studies reporting on regulatory interpretation, modelling of bioaccumulation or degradation, or studies not containing the above-mentioned information on the analytical methods used, were not considered relevant for this project.

4.1.3 Quality criteria

In order to be included in this review, the articles must be published in a peer-reviewed journal. Careful examination of the review methodology was carried out and evaluation of the review criteria against the inclusion/exclusion criteria listed above.

Experimental studies reflecting the current state of the science section after the initial screening of the title and abstract were assessed for reliability using the Klimisch scoring system used in REACH guidance[12]:

<u>1 = reliable without restrictions</u>: studies that were carried out according to generally valid

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[12] Guidance on information requirements and chemical safety assessment, Chapter R.4: Evaluation of available information. ECHA; 2011.

and/or internationally accepted testing guidelines or in which the test parameters documented are based on a specific (national) testing guideline or in which all parameters described are closely related/comparable to a guideline method.

2 = reliable with restriction: studies in which the test parameters documented do not totally comply with the specific testing guideline but are sufficient to accept the data or in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable.

3 = not reliable: studies in which there were interferences between the measuring system and the test substance or in which organisms/test systems were used which are not relevant in relation to the exposure or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for assessment and which is not convincing for an expert judgment.

4 = not assignable: studies which do not give sufficient experimental details, and which are only listed in short abstracts or secondary literature.

Studies with a scoring of 1-3 were included for further analyses of the analytical methods used for the environmental fate studies.

4.1.4 Insights from EFSA Draft Assessment Reports

Data produced under the Plant Protection Products regulations (as set out in the EU Commission (in accordance with EC Regulation No 1107/2009) and the associated Commission Communication 2013/C 95/01 which records the test methods and guidance) contain specific elements, particularly with regard to the identification of transformation and degradation products, which was considered to be useful for the purposes of this review. These are summarised as follows:

4.1.4.1 Plant Protection Products (PPP, 'Pesticides') Literature

Plant protection products (PPP), is the general term (which includes insecticides, acaricides, fungicides, herbicides and plant growth regulators) covering the main group of biologically active substances, which are deliberately released into the environment in any quantity during application of the PPP. In the European Union, authorisation of plant protection products has been administered by the European Food Safety Authority (ESFA) Pesticide Unit since 2003. Registration and authorisation of a novel active substance on the pesticide market requires the submission of dossiers for the approval of PPP active substances to EFSA. This dossier should contain a substantial amount of scientific data relating to pesticide behaviour and toxicity, amongst which is information on pesticide residues in the field and on crops, metabolic breakdown and behaviour in the environment as well as metabolism and toxicokinetics in target organisms. Consequently, because of the concern for the release of intentionally biologically active substances into the environment, the level of analysis of environmental fate is greater for PPPs than for substances covered by the REACH regulations (an overview of the comparison of REACH, PPP and BPR (Biocidal Products Regulations) regulations was summarised in WP1. Further comparison of the data required for these regulations will be discussed in WP3.) Hence, information submitted under EU Regulation No 284/2013 would be expected to yield more in-depth information on substance analysis, which would be potentially useful for this project.

The regulations required for PPP authorisation has also meant that the Plant Protection Industry has been at the forefront in developing analytical methods for generating these data. These methods include radiolabelling and analytical trace analysis, in which the limits of detection have become ever lower from year to year as a result of the development of new methods of separation and detection. Hence, it would be expected that useful information would be found in PPP dossiers relating to methods of carrying out environmental fate analysis, including information on the actual tests conducted and the methods of analysis and detection of the active substance and its breakdown products. Although some of the studies relating to data for PPP dossiers have been published in the open scientific literature, the bulk of the information is in the form of proprietary reports. These reports are not generally publicly available but are referenced in dossiers submitted to EFSA for authorisation of PPP active substances. Publicly accessible summaries of PPP dossiers are however available in the form of Draft Assessment Reports (DARs, see below) and it was the objective of searching this literature to see whether the DARs contained enough detail to extract useful information for this project.

4.1.4.2 European Food Safety Authority (EFSA) DARs for PPPs

EFSA DARs and additional reports, which are in the public domain are available from the EFSA web site: http://dar.efsa.europa.eu/dar-web/provision. DARs are available in the year range 2005 to 2015, but also can contain information from earlier years, sometimes much earlier. Newer reports are not accessible although a limited number of Renewal Assessment Reports (RARs) are. The reports cover about 420 active substances and are arranged in date order of filing. Some active substances have multiple entries, for example Fenazaquin has three, consequently, they are not searchable in the normal way that one would search a standard database, and reports must be downloaded individually.

4.1.4.2.1 Searching DARs

Information from the ESFA web site was copied and pasted into Excel and sorted alphabetically by active substance name. Substances for review were selected as outlined in 4.1.5.2.2 below. These documents were downloaded from the EFSA web site by completing the online request form for each selected PPP. An e-mail (from EFSA) providing a link to the relevant documentation was then provided. The report files were searched for relevant information separately as this was found preferable to using the tables of contents in most cases. Some reports did not have useful titles and the information required for this project was best searched using the Adobe Acrobat search facility across multiple files e.g. all the downloaded pdf files for Zetacypermethrin. The following search terms were used to find studies on fish bioaccumulation: "bioaccumulation", "bioconcentration", "bio-concentration", "BCF". Additionally, it was found useful to search some files individually using the term "fish", which finds all fish bioaccumulation and toxicity studies. For the soil/sediment studies it was found necessary to use the table of contents for the Annex B8 Environmental fate and behaviour volume as "soil" gave too many results.

4.1.4.2.2 Active substances assessed.

Table 4.2: List of Plant Protection Products (PPP) Draft Assessment Reports Reviewed for Environmental Fate and Behaviour Data

SUBSTANCE NAME	TYPE OF PPP	LOG K_{OW}
Abamectin	Acaricide/Anthelmintic	4.0
Bifenthrin	Insecticide (Pyrethroid)	6.0
Chlorsulfuron	Herbicide	0.74 (pH 5) -1.34 (pH 7)
Esfenvalerate	Insecticide (Pyrethroid)	6.2
Fenazaquin	Acaricide	5.5
Zetacypermethrin	Insecticide (Pyrethroid)	6.6

Because it would be impracticable to search the DARs of over 400 active substances, individual documents were selected that were expected to contain bioaccumulation studies. According to the Guidance Document on Risk Assessment for Birds and Mammals under Council Directive 91/414/EEC (SANCO/4145/2000) issued 25/09/2002 the bioaccumulation potential for pesticides should be evaluated for substances with a log K_{ow} value of more than 3.0. Therefore, only DARs of PPPs with a log K_{ow} value of > 3.0 would be expected to contain information on fish bioaccumulation, whereas all DARs have data on the fate and behaviour in soils, because this information is a requirement for their registration as PPPs. Six pesticides were chosen on a random basis, of which 5 have log K_{ow} values > 3.0 and one has a log K_{ow} value < 3.0. These are presented in Table 4.2 above). Note that most pesticides with a log K_{ow} > 3.0 (those with a potential to bioaccumulate) tend to be insecticides, whereas most of the more polar PPPs are herbicides or fungicides, though the division is by no means absolute.

The laboratory-based environmental fate and behaviour study requirements for PPP active substances, in accordance with Regulation (EC) No 1107/2009, which were used to assess analytical methods for this study are as follows:

Fate and Behaviour in the Environment

- Aerobic degradation of the active substance.
- Aerobic degradation of metabolites, breakdown and reaction products.
- Anaerobic degradation of the active substance.
- Anaerobic degradation of metabolites, breakdown and reaction products.
- Fate and behaviour in water and sediment

Route and rate of degradation in aquatic systems

- Route and rate of biological degradation in aquatic systems.

Ecotoxicological Studies

- Bioconcentration in fish.

Residue Analysis

- Several studies that measured residues in or on treated products, food and feed that are required for PPP authorisation have data on analytical methods useful for this study.

4.2 Insights from related areas: challenges and techniques

4.2.1 Introduction

The first part of the literature search (Section 4.1) was intended to specifically provide information about analytical methods currently used in guideline bioaccumulation and biodegradation studies.

The second part of the literature search, outlined in the current section, covered a broader range of literature. It aimed to:

- Understand the challenges and uncertainties associated with analysis of chemicals in relevant media (water, fish, soil and sediment).
- Identify the challenges and approaches for specific stages of the analytical process.
- Identify the challenges and approaches for specific ‘difficult substance’ types.

These goals are linked to addressing elements C to F of the mandatory outputs as listed in Section 1:

C. How can current limitations be overcome?
D. What analytical chemistry methodologies are applicable to environmental fate studies (e.g. OECD test guidelines 305, 307, 308 and 309)?
E. What is the impact of the methodological set-up on the relevance and reliability of the information generated from environmental fate studies?
F. What are the specific limitations with regard to the type of test substance (i.e. mono-constituent vs. multi-constituents/UVCBs), structural functionality and test substance properties (e.g. physical state, solubility, volatility, sorptivity etc.)?

4.2.2 Keywords for literature searching

Several different combinations of keywords were used and are listed in Tables 4.3 – 4.6. The keyword combinations were used in Science Direct.[13] Unless otherwise stated in Tables 4.3 - 4.6, these were conducted as ‘Title, abstract or author-specified keywords’ searches.

The searches were focused on:

1. Challenges and uncertainties associated with analysis of chemicals in relevant media (Table 4.3). The goal of these searches was to identify limitations in analysis of chemicals in relevant media (linked to mandatory output element C).
2. Difficult substances types (Table 4.4). The substances types[14] were:
    a. multi-constituent/UVCB,
    b. poorly soluble,
    c. highly sorptive,[15]
    d. volatile,[16]
    e. surface active,
    f. ionising.

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[13] https://www.sciencedirect.com/. Accessed December 2019.

[14] Transformation products with ‘difficult’ properties and substances where radiolabelling is not possible have also been identified as difficult substance types to consider. It has not been possible to identify specific search terms for these areas, but they should be addressed by searches relating to difficult properties and to radiolabelling, respectively.

[15] These searches were unsuccessful. Highly sorptive substances are generally poorly soluble so some information is available from these searches. We will review whether it is necessary to perform further searches on specific substances known to have this property.

[16] The search terms will not differentiate between volatile and semi-volatile substances and whether the parent or transformation products are volatile.

These searches link to element F of the mandatory outputs.

3. Specific techniques for the extraction and chromatography/detection stages (Table 4.5). The identification of the techniques covered by this search is discussed below. These searches (along with work outlined in Section 4) aim to find out what methods are applicable to environmental fates studies (element D) and to explore techniques that could be used to overcome challenges (element C), particularly for difficult substances (element F).
4. Challenges and approaches for specific stages of the analytical process not covered by point 3 above (Table 4.6). These searches have similar aims to the searches in point 3, but for different stages of the analytical process.

In considering information from all searches, the aim was to understand the impact of aspects of the analytical process on the relevance and reliability of the information generated from the studies (linked to element E).

The list of potentially relevant analytical techniques for the searches in point 3 above was compiled based on:

- Nießner and Schäffer (2017),
- Royal Society of Chemistry (2019),
- the initial review of the environmental fate studies (Section 5),
- the initial review of the studies found using the searches in points 1 and 2 above.

In order to obtain an overview of the current use of these techniques in laboratory simulation and environmental monitoring studies, a series of search terms were constructed. Further literature searches were then conducted in Science Direct during December 2019. These searches were conducted as ‘Title, abstract or author-specified keywords’ searches, and limited to review articles in the first instance. The accepted abbreviations for some extraction and chromatographic techniques were used, since these abbreviations were found to be consistently present in the abstract and/or keywords, and their use minimises alternative phrase or spellings in the search terms (a glossary is provided in Section 13).

4.2.3 Inclusion and exclusion criteria

The results obtained from the searches in Science Direct contained studies that could be used for this project and studies that were outside of the scope of the project. Therefore, a screening process was necessary.

In the first instance, only review articles were screened as these should provide an efficient way of obtaining an overview of relevant areas. If a relatively small number of results was obtained, then research articles were also screened (these searches are identified in Tables 4.3 - 4.6).

A “Title, Abstract and Keyword” search was performed, limited to studies published after the year 2000 and written in English. The titles and abstracts of the articles were screened for relevance, selecting articles which refer to analysis[17] of xenobiotic compounds in relevant media (water, fish, sediment, soil) in laboratory or field-based studies and contain information about the analytical methods used. Articles reporting the analysis of relevant compounds in other media (for example, plants, mammals, birds, or food) were excluded at this stage. The selected articles include data for organic or organometallic compounds; studies solely reporting data on metals, inorganics, nanomaterials or microplastics were excluded. For specific searches, it was necessary to apply further exclusion criteria, as set out in Table 4.3.

Table 4.3: Exclusion criteria applied to specific searches

SEARCH TERM	EXCLUSION CRITERIA	JUSTIFICATION
(water OR biota OR soil OR sediment) AND (analytical OR analytics OR "residue analysis" OR "trace analysis") AND (volatile OR volatility OR VOC)	Sources particularly relating to small polar substance	These types of substances are unlikely to screen as PBT
(water OR biota OR soil OR sediment) AND (analytical OR analytics OR "residue analysis" OR "trace analysis") AND (ionising OR ionisable)	Sources where the key word relates only to the method (e.g. electron-capture ionisation mass spectrometry)	Results where the key word was not related to the substance type (but rather to the method) were excluded.
surfactant AND (analytical OR analytics OR "residue analysis" OR "trace analysis") AND (water OR biota OR soil OR sediment)	Articles relating to the use of surfactants as aids in the analytical process	Does not relate to the analysis of surfactants themselves
challenges AND (analytics OR analytical OR extraction OR "residue analysis") AND (soil OR sediment OR water OR biota)	Effects directed analysis	This technique aims to identify chemicals exerting a specific toxicity in a complex mixture; it is not considered relevant for environmental fate studies
Searches including the term "soil"	Study relates to analysis of soil structure/composition rather than presence of contaminants	Does not relate to analysis of exogenous chemicals

4.2.4 Outcome of the literature search

Tables 4.4 – 4.7 list the keywords used for the literature searching and the number of

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[17] Analysis here refers to the entire analytical process including sampling, extraction, chromatography, detection and interpretation.

results obtained before and after the screening.

Most of the literature that has been found relates to analysis of trace contaminants in the environment rather than to laboratory studies. However, this appears to be a valuable source of information on analytical methods and procedures that could be relevant for environmental fate laboratory studies. The following keyword combinations were used in Science Direct.[18] Unless otherwise stated, these were conducted as 'Title, abstract or author-specified keywords' searches.

Table 4.4: Keywords for searches on challenges and uncertainties associated with analysis of chemicals in relevant media

SEARCH TERMS	NUMBER OF RESULTS (REVIEW ARTICLES ONLY UNLESS SPECIFIED)	NUMBER OF ARTICLES SELECTED AFTER INITIAL SCREENING[19]
challenges AND (analytics OR analytical OR extraction OR “residue analysis”) AND soil	52	7
challenges AND (analytics OR analytical OR extraction OR “residue analysis”) AND sediment	32	9
challenges AND (analytics OR analytical OR extraction OR “residue analysis”) AND water	182	21
challenges AND (analytics OR analytical OR extraction OR “residue analysis”) AND biota	13	5
uncertainty AND (analytical OR analytics OR "residue analysis" OR “trace analysis”) AND (water OR biota OR soil OR sediment)	28	9
uncertainty AND (analysis OR analytics OR "residue analysis" OR “trace analysis”) AND (biodegradation OR bioaccumulation)[20]	79	6

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[18] https://www.sciencedirect.com/. Accessed December 2019.

[19] Some articles are found by more than one search.

[20] Review and research articles considered.

Table 4.5: Keywords for searches on difficult substance types.

SEARCH TERMS	NUMBER OF RESULTS (REVIEW ARTICLES ONLY UNLESS SPECIFIED)	NUMBER OF ARTICLES SELECTED AFTER INITIAL SCREENING[21]
multi-residue AND (analytical OR analytics OR "residue analysis" OR “trace analysis”) AND (water OR biota OR soil OR sediment)	6	3
UVCB AND (analytical OR analytics OR "residue analysis" OR “trace analysis”) AND (water OR biota OR soil OR sediment)[22]	60	4
multi-constituent AND (analytical OR analytics OR "residue analysis" OR “trace analysis”) AND (water OR biota OR soil OR sediment)[23]	37	1
surfactant AND (analytical OR analytics OR "residue analysis" OR “trace analysis”) AND (water OR biota OR soil OR sediment)	20	7
"surface active" AND (analytical OR analytics OR "residue analysis" OR “trace analysis”) AND (water OR biota OR soil OR sediment)[23]	12	0
"poorly soluble" AND (water OR biota OR soil OR sediment) AND (analytical OR analytics OR "residue analysis" OR “trace analysis”)	2	0
HOC AND (water OR biota OR soil OR sediment) AND (analytical OR analytics OR "residue analysis" OR “trace analysis”)[23]	29	7
hydrophobic AND (water OR biota OR soil OR sediment) AND (analytical OR analytics OR "residue analysis" OR “trace analysis”)	19	6
poorly soluble AND (analytical OR analytics OR "residue analysis" OR “trace analysis”)	14	0
poorly soluble AND (bioaccumulation OR BCF OR biodegradation)	14	3
"sorptive" AND (water OR biota OR soil OR sediment) AND (analytical OR analytics OR "residue analysis" OR “trace analysis”)	5	0
(water OR biota OR soil OR sediment) AND (analytical OR analytics OR "residue analysis" OR “trace analysis”) AND volatile	31	11
(water OR biota OR soil OR sediment) AND (analytical OR analytics OR "residue analysis" OR “trace analysis”) AND volatility	31	11 (same as above)
(water OR biota OR soil OR sediment) AND (analytical OR analytics OR "residue analysis" OR “trace analysis”) AND VOC	4	0 (nothing not found by above searches)
(water OR biota OR soil OR sediment) AND (analytical OR analytics OR "residue analysis" OR “trace analysis”) AND ionisable	18	4
(water OR biota OR soil OR sediment) AND (analytical OR analytics OR "residue analysis" OR “trace analysis”) AND ionising	18	4 (same as above)

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[21] Some articles are found by more than one search.

[22] terms searched in the whole of the article, not just ‘Title, abstract or author-specified keywords’ as very few hits were found in ‘Title, abstract or author-specified keywords’. Review and research articles considered.

[23] Review and research articles considered.

Table 4.6: Keywords for searches on specific techniques for the extraction and chromatography/detection stages

SEARCH TERMS	NUMBER OF RESULTS (REVIEW ARTICLES ONLY UNLESS SPECIFIED)	NUMBER OF ARTICLES SELECTED AFTER INITIAL SCREENING[24]
extraction AND (ASE OR PLE OR PSE) AND (water OR aqueous OR soil OR sediment OR biota OR fish)	20	7
extraction AND (ASE OR PLE OR PSE) AND (contaminant OR pollutant OR environmental)	28	16
extraction AND SFE AND (water OR aqueous OR soil OR sediment OR biota OR fish)	12	3
extraction AND SFE AND (contaminant OR pollutant OR environmental)	17	4
extraction AND MAE AND (water OR aqueous OR soil OR sediment OR biota OR fish)	5	2
extraction AND MAE AND (contaminant OR pollutant OR environmental)	8	5
extraction AND UAE AND (water OR aqueous OR soil OR sediment OR biota OR fish)	8	2
extraction AND UAE AND (contaminant OR pollutant OR environmental)	14	4
extraction AND (SPE OR SPME) AND (water OR aqueous OR soil OR sediment OR biota OR fish) AND NOT metal	22	12
extraction AND (SPE OR SPME) AND (contaminant OR pollutant OR environmental) AND NOT metal	48	14
chromatography AND “ion-pairing” AND (water OR aqueous OR soil OR sediment OR biota OR fish)	7	2
chromatography AND “ion-pairing” AND (contaminant OR pollutant OR environmental)	6	1
chromatography AND HILIC AND (water OR aqueous OR soil OR sediment OR biota OR fish)	8	1
chromatography AND HILIC AND (contaminant OR pollutant OR environmental)	6	3
“capillary electrophoresis” AND (water OR aqueous OR soil OR sediment OR biota OR fish)	53	5
“capillary electrophoresis” AND (contaminant OR pollutant OR environmental)	62	9
“size exclusion chromatography” AND (water OR aqueous OR soil OR sediment OR biota OR fish)	9	1
“size exclusion chromatography” AND (contaminant OR pollutant OR environmental)	3	1
(derivatisation OR derivatization) AND (water OR aqueous OR soil OR sediment OR biota OR fish)	56	14
(derivatisation OR derivatization) AND (contaminant OR pollutant OR environmental)	48	13

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[24] Some articles are found by more than one search.

SEARCH TERMS	NUMBER OF RESULTS (REVIEW ARTICLES ONLY UNLESS SPECIFIED)	NUMBER OF ARTICLES SELECTED AFTER INITIAL SCREENING[24]
chromatography AND pyrolysis AND (water OR aqueous OR soil OR sediment OR biota OR fish)	7	0
chromatography AND pyrolysis AND (contaminant OR pollutant OR environmental)	3	0
QuECHERs	33	6

Table 4.7: Keywords for searches on challenges and approaches for specific stages of the analytical process

SEARCH TERMS	NUMBER OF RESULTS (REVIEW ARTICLES ONLY UNLESS SPECIFIED)	NUMBER OF ARTICLES SELECTED AFTER INITIAL SCREENING[25]
"sample handling" AND (water OR biota OR soil OR sediment) AND (analytical OR analytics OR "residue analysis" OR "trace analysis")[26]	56	18
"sample preparation" AND (water OR biota OR soil OR sediment) AND (analytical OR analytics OR "residue analysis" OR "trace analysis")	90	46
(water OR biota OR soil OR sediment) AND (analytical OR analytics OR "residue analysis" OR "trace analysis") AND "reference standards"[26]	43	9
artefact AND (analytical OR analytics OR "residue analysis" OR "trace analysis") AND (water OR biota OR soil OR sediment)	7	1
radiolabelling AND (water OR biota OR soil OR sediment)	1	0
radiolabelling AND challenges	6	0

4.2.5 Additional sources

In addition to the sources identified by keyword searching (see Section 4.2.4), the following sources were already known to the present authors and considered to be useful.

Additional sources which have been evaluated:

- Nießner, R., Schäffer, A., 2017. *Organic Trace Analysis*. De Gruyter. p2. https://doi.org/10.1515/9783110441154. This textbook was used a starting point for identifying methods for extraction, chromatography and detection that may be useful for environmental fate studies.
- RSC, 2019. *Challenges in Analysis of Complex Natural Mixtures*, Faraday Discussions. Volume 218, Royal Society of Chemistry. A collection of papers on the state-of-the-art determination of molecules contained within unresolved complex mixtures. The topics covered included:
    - Dealing with complexity: latest advances in mass spectrometry and chromatography

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[25] Some articles are found by more than one search.

[26] Review and research articles considered.

    - High-resolution techniques, from high-resolution mass spectrometry to NMR spectroscopy
    - Data mining and visualisation. Future challenges and new approaches.

- Green, John W., Springer, Timothy A., Holbech, Henrik, 2018. Statistical Analysis of Ecotoxicity Studies. Wiley. This is an up to date guide to the issues relevant to the design, analysis, and interpretation of toxicity studies that examine chemicals for use in the environment. The text contains myriad datasets (from laboratory and field research) that clearly illustrate the topics. The datasets reveal the techniques, pitfalls, and precautions derived from these studies. The authors also discuss the regulatory process indicating how test guidelines are developed and review the statistical methodology in current or pending OECD and USEPA ecotoxicity guidelines.
  - Validated methods from the US EPA's Environmental Sampling and Analytical Methods (ESAM) Program (https://www.epa.gov/esam/selected-analytical-methods-environmental-remediation-and-recovery-sam). These methods are intended to be used by laboratories performing analyses of environmental and building material samples following a contamination event. Appropriate methods for over 1000 analyte/sample type combinations are identified (Campisano *et al*. 2017). Although most industrial chemicals are not included, it may be possible to identify appropriate methods based on similarity in physicochemical properties to listed substances.
  - Some additional references, which had not been captured through the literature searches, were identified through consultation with ECHA.

4.2.6 Criteria for review of literature sources

Prioritisation of articles for review was based on the following aspects:

- Does the article include discussion of strengths/weaknesses, disadvantages/advantages of the techniques used? Is there discussion of challenges/problems encountered with the study?
- What level of detail of the analytical methods is presented?
- Is information on method validation (recovery, repeatability, precision) and detection and/or quantification limits presented?
- What methods are used for extraction, separation, detection?
- What is the matrix?
- What is the analyte? What are the physicochemical properties, and does it fit into one of the difficult substance types?

The intention was to identify:

- Literature that includes discussion of strengths/weaknesses, disadvantages/advantages and challenges/problems relating to the analytical process.
- A small number of examples covering:
  - each identified technique and/or stage of the analytical process, and each difficult substance type.

4.3 Guidelines for validation of analytical methods

4.3.1 Keywords and information sources

This search was intended to provide information about guidelines for validation of analytical methods. The search focussed not only on method validation of trace analyses of substances in environmental matrices but also on other matrices (for example food).

The search terms listed below were used on websites from governmental agencies such as the US EPA, European Commission and Australian Pesticides & Veterinary Medicines Authority.

Search terms:

- Validation of methods
- Analytical method validation
- Trace analysis validation

This set of search terms did not produce a comprehensive list of guidelines for validation of analytical methods; however, it will provide a solid basis for recommendations regarding the validation of analytical method used for environmental fate studies.

4.4 *In silico* tools for prediction of transformation and degradation products

This search was intended to locate information on *in silico* tools used for the identification of potential transformation and degradation products, with a focus on the use of such tools in environmental fate (bioaccumulation and degradation) studies.

4.4.1 Keywords and information sources

Searches of keyword combinations (see Table 4.8) were conducted in ScienceDirect from 2000 - 2020, in the 'title, abstract, keywords' field unless otherwise stated.
Table 4.8 contains information on the number of review articles obtained with each of the keyword combinations and the number of articles after screening. The obtained literature is reviewed in section 6.3 of this report.

Table 4.8: Search terms for *in silico* tools.

SEARCH TERMS	NUMBER OF REVIEW ARTICLES	NUMBER OF RESULTS AFTER SCREENING	REFERENCES OBTAINED AFTER SCREENING
("*in silico*" OR QSAR) AND (metabolism OR "degradation product") AND biodegradation	9	1	Dimitrov *et al.* 2010
("*in silico*" OR QSAR) AND (metabolism OR degradation) AND (environmental OR microbial)	25	1	Leonard 2019
("*in silico*" OR QSAR) AND metabolism AND xenobiotic	14	0	
("*in silico*" OR QSAR) AND metabolism	126	2	Kazmi *et al*. 2019 Piechota *et al*. 2013
("*in silico*" OR QSAR) AND "degradation pathway"	1	0	
(QSAR OR "*in silico*") AND metabolism AND microbial	10	0	

[1]*Search terms entered in 'find articles with these terms' field*

5. Findings on current practice in guideline bioaccumulation and persistence studies

This section describes the results from the literature search with the focus on the current state of the science on analytical methods used in bioaccumulation and persistence testing (i.e. OECD 305, 307, 308 and 309). It will include information on the extraction techniques and separation and detection techniques reported in the obtained literature. The following subsections will present information on the analytical methods obtained with the searches related to OECD 305, 307, 308 and 309 bioaccumulation or degradation testing.

5.1 Analytical methods used in fish bioaccumulation testing (OECD 305)

Searches of the peer-reviewed literature using the keywords "OECD TG 305" OR "OECD 305" resulted in 103 ‘hits’. After an initial screening of the title and abstract, 46 papers describing OECD 305 studies were found to be relevant based on the information provided in the abstract. After a second screening of the method section in the studies, 26 studies performing OECD 305 bioaccumulation testing were found to be relevant. Studies not found to be relevant after the second screening were studies that did not included information on analytical methods or were review papers or modelling studies.

A total of 26 studies identified as above focussed on substances that fall within the following chemical classes: hydrocarbons, organochlorine substances, flame retardants, veterinary medicines, pesticides, per- and polyfluoroalkyl substances (PFASs), pharmaceuticals, UV filters, siloxanes and nitrobenzenes (see Table 5.1 for individual substances included in the studies).

Studies conducted with radiolabelled substances:

From the 26 studies identified, three used ^{14}C-labelled substances and 23 studies used non-labelled substances. In the three studies using ^{14}C-labelled test substances, information on the location of the ^{14}C-label on the substance molecule and specific activity were provided. In these three studies, the organisms were exposed via water (flow-through/semi-static system) and/or diet.

Extraction & detection: Water samples were either directly analysed or were concentrated by using SPE technique followed by liquid scintillation counting (LSC) detection. The organisms (fish and amphipods) were analysed as whole-body samples and in some case individual tissues were separated. Samples either directly combusted and analysed by LSC or substances were extracted using solvents followed by LSC detection. Metabolites were monitored in two studies, using TLC and HPLC-RAD. Information on detection/quantification limits or other quality control were scarcely reported. For octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) LOQs in fish were 0.06-0.16 µg/g, quality control samples were within 20% of the prepared concentrations with precision having a relative standard deviation below 20% (Woodburn *et al*., 2013). Information on uptake/depuration kinetics were reported in all three studies.

Studies conducted with non-radiolabelled substances:

Extraction: In the studies using non-labelled substances, the aquatic organisms were exposed via the water or diet. Concentrations of the test substance was generally monitored by either direct analyses of the water or test-substances were isolated using solid-phase extraction (SPE), solid-phase micro-extraction (SPME) or liquid-liquid extraction (LLE) (see Table 5.1 for details per individual study). Whole body organisms (or in some cases individual tissues e.g. liver, GI tract) are generally homogenised followed by a solvent extraction in combination with sonication or by Pressurized Liquid Extraction (PLE). In some studies, an additional clean-up step was applied, i.e. SPE clean-up.

Detection: Separation and detection techniques for the test substances (and metabolites when included in the study) included LC-MS/MS, HPLC-FLD, HPLC-UV-Vis, GC-MS, GC-FID and GC-µECD. Analyses of metabolites was performed by several studies and using target-analyses. Detection and/or quantification limits were commonly reported in the obtained literature, as well as other QA/QC such as recoveries and precision. Information on uptake/depuration kinetics were reported in most of the 23 studies.

Transformation/degradation products: For studies where both the parent and transformation products were analysed, information on LOD/LOQ was limited. Wang *et al.* (2017) report on LOQs for organophosphate esters (OPEs) and their diester metabolites in water and fish tissue and found them to be comparable (e.g. 1.1-7 ng/g and 0.5-3.6 ng/g in fish for the OPEs and their metabolites, respectively). This study also reported on comparable recoveries for parents and metabolites and good reproducibility. Wang *et al.* (2017) was the only study that quantified metabolite concentrations.

An example of a study that reported detailed information on QA/QC is Nallani *et al*. (2011). Besides information on detection limits for Ibuprofen, this study provided details on recovery (91-104%), precision (RSD 0.6-12%) and accuracy (91-104%) of the analytical method for measurements in water and fish tissues. Other studies often provided information on LOD/LOQs and/or recoveries but no other QA/QC parameters.

Overall, limited information on the QA/QC assurance of the analytical method was reported in studies investigating the bioaccumulation of substances in aquatic species. The majority of the studies provided information on LOD/LOQs and recoveries. Details on other QA/QC parameters were only provided in a few studies, and these methods appeared to be validated properly. Information on these QA/QC parameters are important in evaluating the quality and robustness of data generated by an analytical method.

Table 5.1: Summary of obtained studies reporting on analytical methods in bioconcentration/bioaccumulation fish testing (OECD 305).

Reference	Test compounds	Compound class	Use of ^{14}C-labelled substance	Sample extraction	Separation / detection technique	LOD/LOQ reported	Other QA/QC reported	Kinetics reported	Identity of degradation products reported
Pawlowski *et al*. (2019)	Octocrylene	UV filter	Yes - location of ^{14}C label, purity and activity provided. Also non-labelled	Water: direct; Whole fish and/or tissue: combustion	Parent: LSC	No	No	Yes	No
Raths *et al*. (2020)	Laurate		Yes - location of ^{14}C label, purity and activity provided.	Water: direct; Whole body: combustion or LLE	Parent: LSC, TLC; Metabolite: TLC; NER: combustion LSC	No	Recovery	Yes	Yes
Woodburn *et al*.	Octamethylcyclotetra siloxane (D4), decamethylcyclopent	Volatile cyclic siloxanes	Yes - location of ^{14}C	Fish: homogenised, liquid	Parent: LSC, GC-MS;	Yes	Recovery,	Yes	No

Reference	Test compounds	Compound class	Use of ^{14}C-labelled substance	Sample extraction	Separation / detection technique	LOD/LOQ reported	Other QA/QC reported	Kinetics reported	Identity of degradation products reported
(2013)	asiloxane (D5)	(VMS)	label, purity and activity provided.	extraction	Metabolite: HPLC-RAD		precision		
Adolfsson-Erici *et al*. (2012)	2,3,4-Trichoroanisole, p-diisopropylbenzene, musk xylene, Chlorpyrifos, Pentachlorobenzene 2,5-Dichlorobiphenyl, hexachlorobenzene, p,p-DDT	Various	No	Whole fish homogenate, liquid extraction; passive sampler: liquid extraction	Parent: GC-MS	Yes	Recovery, replicate, blanks	Yes	No
Adolfsson-Erici *et al*. (2012)	2,6-Diisopropyl-naphthalene, 2,3,4-trichoroanisole, musk xylene, 4-n-nonylphenol, Chlorpyrifos, pentachlorobenzene, 2,4,6-tributylphenol, pentachlorobenzene, 2,5-Dichlorobiphenyl, hexachlorobenzene, p,p-DDT	Various	No	Water: SPE; Whole fish homogenate, liquid extraction	Parent: GC-MS	No	No	Yes	No

Reference	Test compounds	Compound class	Use of ^{14}C-labelled substance	Sample extraction	Separation / detection technique	LOD/LOQ reported	Other QA/QC reported	Kinetics reported	Identity of degradation products reported
Bekele *et al*. (2018)	TBP, TCP, TCPP, TDCP, TEHP, TPP	Organophos-phate flame retardants	No	Tissues: PLE, SPE	Parent: GC-MS	Yes	Recovery, blanks	Yes	No
Böhm *et al*. (2017)	Hexachlorobenzene, o-terphenyl, PCB-153		No	Water: LLE or SPE; Whole fish homogenate, liquid extraction	Parent: GC-MS	No	No	Yes	No
Brooks *et al*. (2019)	Teflubenzuron, emamectin benzoate, deltamethrin, diflubenzuron	Veterinary medicinal products	No	Homogenized mussel: Liquid extraction, sonication, SPE	Parent: UPLC-MS/MS or GC-MS	Yes	Recovery	Yes	No
Camenzuli *et al*. (2019)	Isodecanol, isododecanol and isotridecanol. neodecanoic acid. cis-and trans-decalin, 2,6,10-trimethyldodecane, 2,2,4,6,6-pentamethylheptane	Hydrocarbons and alcohols	No	Water: direct analyses; Fish: PLE	Water: HS-GC-FID; SPME/HS-GC-MS. Fish: HS-GC-MS; GC-MS	Yes	No	Yes	No

Reference	Test compounds	Compound class	Use of ^{14}C-labelled substance	Sample extraction	Separation / detection technique	LOD/LOQ reported	Other QA/QC reported	Kinetics reported	Identity of degradation products reported
	, 2,2,4,4,6,8,8-heptamethylnonane, hexadecahydropyrene; 1,3,5-Triisopropylbenzene, Dicyclohexylbenzene, 1,2,3,4,5,6,7,8-octahydrophenanthrene, dodecahydrochrysene; Octahydrochrysene and hexahydroterphenyl; Chrysene, fluoranthene, m-Terphenyl, Hexahydrochrysene.								
El-Amrani *et al*. (2012)	Chlorpyrifos, atrazine, dicofol.	Pesticides	No	Water: LLE; Fish: freeze-dried, sonication extraction, C8 column clean up	GC-µECD	Yes	Recovery	Yes	No
El-Amrani *et al*. (2013)	Fluorene and anthracene	PAH	No	Water: LLE; Fish: Sonication, filtration	HPLC-FLD	Yes	Recovery	Yes	No
Garcia *et*	Carbamazepine	Anti-convulsant	No	Water: direct analysis; Fish:	LC-MS/MS	Yes	Recovery	Yes	No

Reference	Test compounds	Compound class	Use of ^{14}C-labelled substance	Sample extraction	Separation / detection technique	LOD/LOQ reported	Other QA/QC reported	Kinetics reported	Identity of degradation products reported
al. (2012)		drug		Tissue homogenisation, Liquid extraction					
Giraudo *et al*. (2017)	1,2-Bis(2,4,6-tribromophenoxy)ethane, 2-ethylhexyl 2,3,4,5-tetrabromobenzoate	Brominated Flame Retardants	No	Fish: homogenised, PLE, GPC, SPE	GC-MS	No	No	No	No
Hoke *et al*. (2015)	^{1}H,^{1}H,2H,2H-tridecafluoro-1-octanesulfonic acid (6:2-FTSA)	PFAS	No	Water: direct analysis; Fish: homogenised, liquid extraction, SPE	LC-MS/MS	Yes	Recovery	Yes	No
Hoke *et al*. (2016)	2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)-propanoate	PFAS	No	Not provided	LC-MS/MS	Yes	No	No	No
Lafontaine *et al*.	Chlordecone	Organochlo-	No	Tissue: PLE,	GC-ECD	Yes	Blank,	No (not for lab-based,	No

Reference	Test compounds	Compound class	Use of ^{14}C-labelled substance	Sample extraction	Separation / detection technique	LOD/LOQ reported	Other QA/QC reported	Kinetics reported	Identity of degradation products reported
(2017)		rine pesticide		acid clean up			recovery	yes for field-based	
Lo *et al*. (2015)	1,2,4,5-Tetrachlorobenzene, pentachlorobenzene, hexachlorobenzene, PCB 52, PCB 155, PCB 153, PCB 209, 1,2,3,4-tetramethyl benzene, b-HCH, trans-decalin, 9-methylanthracene, chrysene, hexylcyclohexane, 2,6-dimethyldecane, benzo[a]pyrene		No	Fish/liver: homogenisation, liquid extraction/sonication, florisil clean up	GC-MS	Yes	No	Yes	No
Lo *et al*. (2016)	Parent and alkylated aromatic hydrocarbons, cycloalkanes, and linear and branched aliphatic hydrocarbons, musk xylene, and methoxychlor		No	Fish: homogenised, PLE, SPE	Parent: GC-MS	No	Recovery	Yes	No
Miller *et al*. (2017)	Propranolol, warfarin, sulfamethazine, carbamazepine, Nimesulide,	Pharmaceuticals	No	Water: direct analysis; Fish: Homogenised, liquid extraction,	Parent and Metabolites: LC-MS/MS	No	Recovery	Yes	Yes

Reference	Test compounds	Compound class	Use of ^{14}C-labelled substance	Sample extraction	Separation / detection technique	LOD/LOQ reported	Other QA/QC reported	Kinetics reported	Identity of degradation products reported
	metoprolol tartrate salt, temazepam, Diazepam, nifedipine, oxazepam, nordiazepam, carbamazepine- 10,11-Epoxide, sulfamethazine, Trimethoprim,			SPE					
Nallani *et al*. (2011)	Ibuprofen	Pharmaceuticals	No	Water: LLE; Fish tissue: liquid extraction, SPE, derivatization	Parent: GC-MS; Metabolites: LC-MS	Yes	Recovery, precision, accuracy	Yes	Yes
Overturf *et al*. (2016)	Diazepam	Pharmaceuticals	No`	Tissue: liquid extraction, lipid removal by freezing	Parent and Metabolites: LC-MS/MS	Yes	Recovery	Yes	Yes

Reference	Test compounds	Compound class	Use of ^{14}C-labelled substance	Sample extraction	Separation / detection technique	LOD/LOQ reported	Other QA/QC reported	Kinetics reported	Identity of degradation products reported
Saunders *et al*. (2020)	2-Ethylhexyl-4-methoxycinnamate (EHMC), octocrylene (OCT)	Sunscreen agents	No	Liver, GI, Carcass: Liquid extraction (Quechers); dSPE	Parent: GC-MS	Yes	Recovery	Yes	No
Schlechtriem *et al*. (2017)	Hexachlorobenzene, o-Terphenyl, Dibenz[a,h]anthracene, PCB 153	Hydrophobic organic chemicals	No	Water: LLE; Fish: homogenisation, PLE, SPE	Parent: GC-MS	Yes	No	Yes	No
Shi *et al*. (2016)	Bisphenol AF	Fluorinated derivative of bisphenol A (BPA)	No	Water: not provided; Whole fish or tissue: homogenised, liquid extraction, sonication, centrifugation	Parent and metabolite: UPLC-MS/MS	No	No	No	Yes
Shi *et al*. (2017)	Hexabromocyclododecane	Brominated flame retardants	No	Water: SPE; whole organism: homogenised, liquid extraction, sonication	Parent: UPLC-MS/MS	No	Recovery	Yes	No
Wang *et al*. (2017)	TCEP, TPP, TNBP, TBOEP, TPHP, TDCIPP, TCP	Organophosphate esters	No	Fish tissues: liquid extraction; Water: SPE	Parent: GC-MS; metabolites: LC-	Yes	Recovery	Yes	Yes

Reference	Test compounds	Compound class	Use of ^{14}C-labelled substance	Sample extraction	Separation / detection technique	LOD/LOQ reported	Other QA/QC reported	Kinetics reported	Identity of degradation products reported
					MS/MS				
Yang *et al*. (2018)	2-Nitro-toluene, 4-nitro-toluene, 2-Nitro-phenol, 4-nitro-phenol and 2-nitro-aniline	Nitrobenze-nes	No	Water: direct; Fish: homogenised, liquid extraction, sonication	Parent: HPLC-UV/Vis	No	Recovery	Yes	No

5.2 Analytical methods used in persistence testing in soil (OECD 307)

Searches of the peer-reviewed literature using the keywords "OECD TG 307" OR "OECD 307" resulted in 30 ‘hits’. After an initial screening of the title and abstract, 20 papers describing OECD 307 studies were found to be relevant. After a second screening of the method section in the studies, 12 studies performing OECD 307 persistence testing were found to be relevant.

A total of 12 studies identified as above focussed on substances that fall within the following chemical classes: pharmaceuticals, artificial sweeteners, brominated flame retardants, antibiotics, antiparasitic agents, herbicides, insecticides and anti-microbial agents (see Table 5.2 for individual substances included in the studies).

From the 12 studies identified, seven used ^{14}C-labelled substances, one study used ^{13}C-labelled substances and four studies used non-labelled substances. In the seven studies using ^{14}C-labelled test substances, information on the location of the ^{14}C-label on the substance molecule, the purity and specific activity were provided.

Studies conducted with radiolabelled substances:

Extraction: Test substances were extracted from the soil using various solvents in a liquid extraction, Soxhlet extraction or PLE, followed in some studies by a SPE clean-up and centrifugation. Extraction efficiency was only reported by Claßen *et al.* (2019) who used Soxhlet extraction for 4-n-dodecylphenol, 4-n-dodecylbenzenesulfonic acid sodium salt and 4-n-dodecylbenzyltrimethylammonium chloride and ranged between 83 and 96%.

Detection: Test substances were detected using LSC, in some studies coupled to an HPLC or TLC. Three studies that monitored for the formation of metabolites used LC-MS/MS, LC-HRMS and RAD-TLC for metabolite identification.

LOD/LOQ: Information on LOD/LOQ and other QA/QC parameters reported in the studies were limited. Waria *et al*. (2011) reported an LOQ for triclosan and its transformation products of 0.16 Becquerel (Bq) (specific activity of the test substance was 1776 MBq/mmol), while other studies using ^{14}C-labelled substances reported LOQs determined with non-radio detectors and were 5 ng/g (Girardi *et al.* (2011) or 50 ng/ml for parent substances (LOQs for transformation products were not reported) (Liu *et al*. (2015).

NER quantification: With the exception of one study, the non-extractable residue fraction was determined by combustion-LSC in all studies.

Transformation/degradation products: Reporting of transformation products was on a qualitative basis, they were identified but no quantification was performed (Liu *et al*., 2015; Waria *et al.*, 2011).

One study used ^{13}C-labelled test substances, i.e. for ibuprofen and 2, 4-D (Girardi *et al*., 2013). For the extraction of the test substances from the soil PLE was used followed by an SPE clean-up. GC-MS and GC-C-IRMS were analytical techniques used for parent and metabolite measurements, while elemental analysis–isotope ratio monitoring mass spectrometry (EA-IRMS) was used for non-extractable residue (NER analyses).

Studies conducted with non-radiolabelled substances:

Extraction: Studies that were performed using non-labelled substances focussed on a range of pharmaceuticals and artificial sweeteners (Biel-Maeso *et al*., 2019), hexabromocyclododecane (HBCD) (Davis *et al*.,2005), abamectin (Dionisio *et al*., 2016) and pethoxamid (Rodriguez-Cruz *et al*., 2019) and used a liquid extraction with sonication or PLE for the extraction of the test substances from the soil, followed (in some studies) by an SPE clean-up. Where reported, extraction recoveries were satisfactory and fell within the range of 80 to 128% (Biel-Maeso *et al*., 2019; Davis *et al*.,2005; Rodriguez-Cruz *et al*., 2019).

Detection: In all four studies, parent substances were detected with an HPLC coupled to an MS or fluorescence detector. In the case for HBCD, metabolites were monitored using GC-MS. LOD/LOQs were in the range of 0.2 ng/g for pharmaceuticals and sweeteners (Biel-Maeso *et al.*, 2019) or 0.25 to 10 ng/ml for abamectin and pethoxamid (most likely instrumental LOQ were reported as they are on a volume concentration basis) (Biel-Maeso *et al.*, 2019; Dionisio *et al*., 2016).

Information of degradation kinetics were reported in all four studies, while none of them identified metabolites or reported on mass balance.

Overall, limited information on the quality control/quality assurance of the analytical method was reported in studies investigating the persistence of substances in soil. Half of the studies reported on LOD/LOQ information as well as information on the recovery of the analytical methods. LOD/LOQ were reported for parent substances, in studies where degradation products were identified, no information on LOD/LOQs for degradation products were provided. When reported, recoveries showed to be satisfactory when extraction techniques such as PLE or Soxhlet were used for various substances. Information on reproducibility, precision and accuracy was either sporadically reported or not at all. Dionisio *et al*. (2016) reported on these QA/QC parameters and demonstrated that their method was validated. Information on these QA/QC parameters are important in evaluating the quality and robustness of data generated by an analytical method.

Table 5.2: Summary of obtained studies reporting on analytical methods in soil persistence testing (OECD 307).

Reference	Test compounds	Compound class	Use of 14C-labelled substance	Sample extraction	Separation / detection technique	LOD/LOQ reported	Other QA/QC reported	Degradation kinetics reported	Identity of degradation products reported	Mass balance reported
Claßen *et al.* (2019)	4-n-Dodecylphenol, 4-n-dodecylbenzenesulfonic acid sodium salt, 4-n-dodecylbenzyltrimethylammonium chloride		Yes - location of ^{14}C label, purity and activity provided	Soxhlet	ER: LSC; NER Combustion-LSC	No	Recovery extraction	No	No	Yes
Girardi *et al.* (2011)	Ciprofloxacin	Antibiotics	Yes - location of ^{14}C label, purity and activity provided	PLE; SPE	Parent: TLC-LSC; Target: LC-MS/MS; Metabolites: LC-HRMS; NER - combustion-LSC	Yes	No	No	Yes	Yes
Hand *et al.* (2019)	Prometryn	Herbicide	Yes - location of ^{14}C label, purity and activity provided	Liquid extraction, centrifugation	Parent: HPLC-LSC; NER: combustion-LSC	No	No	Yes	No	Yes
Liu *et al.* (2015)	Cycloxaprid	Insecticide	Yes - location of ^{14}C label, purity and activity provided	Liquid extraction	Parent: HPLC-LSC; metabolites: LC-MS/MS	Yes	No	No	Yes	No
Liu *et al.* (2016)	Cycloxaprid	Insecticide	Yes - location of ^{14}C label, purity and activity	Liquid extraction	ER: LSC; NER: combustion-LSC	No	No	No	No	No

Reference	Test compounds	Compound class	Use of 14C-labelled substance	Sample extraction	Separation / detection technique	LOD/LOQ reported	Other QA/QC reported	Degradation kinetics reported	Identity of degradation products reported	Mass balance reported
			provided							
Wang *et al.* (2013)	Pyribambenz propyl	Herbicide	Yes - location of ^{14}C label, purity and activity provided	Liquid extraction, centrifugation	ER: LSC; NER: combustion-LSC	No	No	Yes	No	Yes
Waria *et al.* (2011)	Triclosan	Antimicrobial	Yes - location of ^{14}C label, purity and activity provided	Liquid extraction, sonication	ER: LSC; NER: combustion-LSC; parent and metabolite identity: RAD-TLC	Yes	No	Yes	No	Yes
Girardi *et al.* (2013)	Ibuprofen, 2,4-D	Pharmaceutical, herbicide	^{13}C-Ibu and ^{13}C-24 D	PLE; SPE	Parent: GC-MS; Parent & metabolite GC-C-IRMS; NER: EA-IRMS	No	No	Yes	Yes	Yes
Biel-Maeso *et al.* (2019)	Nadolol, sulfamethizole, sulfamethoxazole, sulfamethopyridazone, carbamazepine,	Pharmaceutical, artificial sweeteners	No	Freeze-dried, PLE; SPE clean-up	Parent: LC-MS/MS	Yes	Recovery IS	Yes	No	No

Reference	Test compounds	Compound class	Use of 14C-labelled substance	Sample extraction	Separation / detection technique	LOD/LOQ reported	Other QA/QC reported	Degradation kinetics reported	Identity of degradation products reported	Mass balance reported
	ibuprofen, diclofenac, hydrochlorothiazide, and gemfibrozil. acesulfame, saccharin, cyclamate, and sucralose									
Davis *et al.* (2005)	Hexabromocyclododecane	Brominated flame retardant	No	Liquid extraction	Parent: LC-APPI-MS/MS; Metabolites: GC-MS	No	Recovery extraction	Yes	No	No
Dionisio *et al.* (2016)	Abamectin	Antiparasitic agent	No	Liquid extraction, vortex; centrifugation	Parent: derivatization, HPLC-FLD.	Yes	Linear range, linearity, matrix effect, selectivity, intra-day and inter-day precision	Yes	No	No
Rodríguez-Cruz *et al.* (2019)	Pethoxamid	Herbicide	No	Liquid extraction, sonication, centrifugation	Parent: HPLC-MS	Yes	Recovery	Yes	No	No

5.3 Analytical methods used in persistence testing in sediment (OECD 308)

Searches of the peer-reviewed literature using the keywords "OECD TG 308" OR "OECD 308" resulted in 41 ‘hits’. After an initial screening of the title and abstract, 19 papers describing OECD 308 studies were found to be relevant. After a second screening of the method section in the studies, 11 studies performing OECD 308 persistence testing were found to be relevant.

A total of eleven studies identified as above focussed on substances that fall within the following chemical classes: surfactants, brominated flame retardants, antibiotics, pesticides, commonly-used tyre chemicals, herbicides, hydrocarbons and UV stabilizers (see Table 5.3 for individual substances included in the studies).

Studies conducted with radiolabelled substances:

Four of the studies used a ^{14}C-labelled test substance (see Table 5.3) (McAvoy *et al*. 2016; McDonough *et al*. 2016; Shrestha *et al*. 2020; Thomas *et al*. 2011). Information on the location of the ^{14}C-label on the substance molecule and/or specific activity were provided in these studies. Sediment samples were extracted using liquid extraction techniques (generally using acetonitrile) followed by sonication and centrifugation. The supernatant was analysed for test compounds by liquid scintillation counting (LSC). Information on the limit of detection or quantification was not provided. For further identification of parent and degradation production, separation techniques such as high-performance liquid chromatography (HPLC) coupled to a radioactivity monitoring detector (RAD) or thin-layer chromatography (TLC) coupled to LSC were applied. Although analyses for degradation products were performed, identification of all degradation products was not achieved. The four studies did report mass balances that included the test substance extracted from the water phase and the sediment phase, the fraction of the test substance remaining in the solids (NER) and the volatile radioactive fraction. Information on degradation kinetics of parent substances were reported in all the studies.

One study used a ^{13}C- and ^{15}N- stable isotope enriched test substance (Wang *et al*. 2016). The study that used a ^{13}C-^{15}N-labelled test substance did report on a mass balance and analysed the non-extractable residue fraction by Elemental Analyses-Combustion Isotope Ration Mass Spectrometry.

Studies conducted with non-radiolabelled substances:

Extraction: A total of six studies reported on analytical methods used in sediment degradation methods not using ^{14}C-labelled substances (see Table 5.3). Freeze-drying of samples was performed in several studies. Extraction of the test substances was done by either liquid extraction, followed by sonication and centrifugation, or by Pressurized Liquid Extraction (PLE). Three studies reported on further clean-up of the extract by either a silica clean-up or by solid phase extraction (SPE).

Detection: Analyses of the sediment extracts for parent test substances and metabolites was generally done by LC-MS/MS, although LC-HRMS and LC-TOF MS are also analytical techniques used for parent and metabolite analyses. For the studies using non-labelled test substances, no mass balance was performed.

QA/QC: From the seven studies mentioned above, six of them reported on detection and/or quantification limits for the test substances and/or degradation products. Quantification limits in water were reported to range between 0.2 and 9 ng/l for antibiotics (Liu *et al.*, 2019; Xu *et al.*, 2011) up until 0.5 to 25 µg/l for surfactants and herbicides (Corada-Fernandez *et al*., 2018; Wang *et al*. 2016). LOQs in sediment were generally in the sub ng/g dw concentrations range (0.05 - 2.9 ng/g dw) for antibiotics, herbicides and benzotriazole UV stabilizers (Liu *et al.* 2019; Wang *et al*. 2016; Wick *et al*. 2016; Xu *et al*., 2011), while LOQs for surfactants and commonly-used tyre chemicals were in the higher ng/g to µg/g concentration range Corada-Fernandez *et al*. 2018; Unice *et al*., 2015). When specifically reported, LOQs for parent and degradation products were in the same range, e.g. the LOQ for Sulfametoxazole in water and sediment were 1.3 ng/l and 0.8 ng/g, respectively, while LOQs for N4-acetyl-sulfamethoxazole were 9 ng/l and 0.8 ng/g, respectively (Wang *et al*. 2016; Xu *et al.*, 2011). Xu *et al*. (2011) reported that approximately 1% of the test substance Sulfametoxazole was degraded to N4-acetyl-sulfamethoxazole. Other studies that identified degradation products did not report on concentrations.

Other quality criteria that could be used to evaluate the analytical method reported in the above mentioned studies was limited. For antibiotics, commonly-used tyre chemicals, herbicides and benzotriazole UV stabilizers, recoveries were reported and were in the range of 56 – 127% (Liu *et al.*, 2019; Unice *et al*., 2015; Wang *et al*. 2016; Wick *et al*., 2016). Xu *et al*. (2011) reported on the reproducibility of the analysis of an antibiotic in water and sediment. The relative standard deviation (RSD) was reported to be comparable for the test substance and degradation product in water (0.6 – 2.3%) and sediment (0.5 – 5%).

Overall, limited information on the QA/QCof the analytical method was reported in studies investigating the persistence of substances in sediment. LOD/LOQ information was generally reported in studies using non ^{14}C-labelled substances, as well as information on the recovery of the analytical methods. Information on reproducibility, precision and accuracy was either sporadically reported or not at all. This information is important in evaluating the quality and robustness of data generated by an analytical method.

Table 5.3: Summary of obtained studies reporting on analytical methods in sediment persistence testing (OECD 308).

Reference	Test compounds	Compound class	Use of 14C-labelled substance	Sample extraction	Separation / detection technique	LOD/LOQ reported	Other QA/QC reported	Degradation kinetics reported	Identity of degradation products reported	Mass balance reported
McAvoy *et al*. (2016)	Tetrabromo-bisphenol A (TBBPA)	Brominated Flame Retardant	Yes - location of ^{14}C label, purity and activity provided	Liquid extraction; centrifugation.	Parent: LSC Parent/ degradation product identification: HPLC-RAD/TLC	No	No	Yes	Yes/No	Yes
McDonough *et al.* (2016)	Tetradecanol; C12 alcohol sulfate; Alcohol ethoxylate C14E9; Alcohol ethoxy sulfate C14E3S; C12 linear alkylbenzene sulfonate	Surfactants / fatty alcohols	Yes - location of ^{14}C label, purity and activity provided	Liquid extraction; centrifugation. Methylation fatty acids.	Parent: TLC-LSC Metabolites TLC-LSC NER: Combustion	No	No	Yes	No	Yes
Shrestha *et*	Benzo[a]pyrene,	Hydrocar-	Yes – purity	Liquid extraction	Parent/degradation product:	No	No	No	No	Yes

Reference	Test compounds	Compound class	Use of 14C-labelled substance	Sample extraction	Separation / detection technique	LOD/LOQ reported	Other QA/QC reported	Degradation kinetics reported	Identity of degradation products reported	Mass balance reported
al. (2020)	phenanthrene, biphenyl, tetralin, decane	bons	and activity provided	; sonication ; centrifugation	TLC-LSC; Radio-HPLC; GC-MS NER: Combustion					
Thomas *et al.* (2011)	Chlorotoluron; fludioxonil; Lufenuron; Pinoxaden metabolite; Prometryn; Propiconazole	Pesticides	Yes – location of ^{14}C-label and activity provided	Liquid extraction ; centrifugation.	Parent: HPLC, TLC, LSC	No	No	Yes	Yes/No	Yes
Wang *et al*. (2016)	Glyphosate	Herbicide	No, but ^{13}C ^{15}N label used	Liquid extraction ; derivatization	CO_2 by GC-IRMS Parent/metabolite: UPLC-MS/MS NER: EA-C-IRMS	Yes	Recovery	Yes	Yes	Yes
Corada-Fernandez *et al*. (2018)	Linear alkylbenzene sulfonates (LAS) C_{10-14}	Surfactants	No	Freeze-dried, PLE, SPE clean-up	Parent and metabolites: UPLC-TOF-MS	Yes	No	Yes	Yes	Yes
Davis *et al.* 2005	Hexabromocyclododecane (HBCD)	Brominated Flame Retardant	No	Liquid extraction	Parent: LC-APPI-MS/MS Metabolites:	No	Recovery	Yes	No	No

Reference	Test compounds	Compound class	Use of 14C-labelled substance	Sample extraction	Separation / detection technique	LOD/LOQ reported	Other QA/QC reported	Degradation kinetics reported	Identity of degradation products reported	Mass balance reported
					GC-MS					
Liu *et al.* (2019)	Sulfadiazine; Sulfamerazine; Sulfamethazine; Sulfadimethoxine; Sulfamethoxazole; Sulfathiazole; Ofloxacin; Norfloxacin; Ciprofloxacin; Oxytetracyc-line; Tetracycline; Erythromycin; Roxithromycin	Antibiotics	No	Freeze-dried, liquid extraction, sonication, centrifugation.	Parent: LC-MS/MS	Yes	Recovery	No	No	No
Unice *et al*. (2015)	(N-cyclohexylbenzothiazole-2-sulfenamide; N-(1,3-	Commonly used tyre chemicals	No	Liquid extraction; centrifuga	Parent/metabolites: LC-HRMS	Yes	Recovery	No	Yes	No

Reference	Test compounds	Compound class	Use of 14C-labelled substance	Sample extraction	Separation / detection technique	LOD/LOQ reported	Other QA/QC reported	Degradation kinetics reported	Identity of degradation products reported	Mass balance reported
	dimethylbutyl)-N′-phenyl-1,4-phenylenediamine; 1,3-diphenylguanidine			tion						
Wick *et al*. (2016)	UV-326; UV-320; UV-329; UV-350; UV-328; UV-327; UV-928; UV-234; UV-360	Benzotriazole, UV stabilizers	No	Freeze-dried, PLE, silica clean-up	Parent: LC-MS/MS	Yes	Recovery	No	No	No
Xu *et al*. (2011)	Sulfametoxazole	Antibiotic	No	Liquid extraction; sonication, SPE	Parent and metabolite: LC-MS/MS	Yes	Reproducibility	Yes	Yes	No

5.4 Analytical methods used in persistence testing in water (OECD 309)

Searches of the peer-reviewed literature using the keywords "OECD TG 309" OR "OECD 309" resulted in 19 ‘hits’. After an initial screening of the title and abstract, 10 papers describing OECD 307 studies were found to be relevant. After a second screening of the method section in the studies, seven studies performing OECD 309 persistence testing were found to be relevant.

A total of seven studies identified as above focussed on substances that fall within the following chemical classes: hydrocarbons, fungicide and various aniline based chemicals. (see Table 5.4 for individual substances included in the studies).

Studies conducted with radiolabelled substances:

Extraction & detection: From the five studies identified, three were performed with ^{14}C-labelled test substances (Hand *et al*., 2014; Toräng *et al*. 2002, 2005). Information on the location of the ^{14}C-label on the substance molecule, the purity and/or specific activity were provided in all studies. Water samples were analysed directly without sample treatment and quantified using LSC detection as well as HPLC-RAM in the isopyrazam study (Hand *et al*., 2014). In the aniline study, a non-labelled standard was also measured using HS-GC-FID (Toräng *et al*. 2002). Information on degradation kinetics were included in all three studies, while information on LOD/LOQ (with the exception of non-labelled aniline), other QA/QC parameters, identity of degradation products or mass balance was not included. The detection limit for analine using GC-FID was reported at 1 µg/l (Toräng *et al*., 2002).

Studies conducted with non-radiolabelled substances:

Extraction & detection: Four studies investigated the degradation of hydrocarbons in water using non-labelled test-substances (Birch *et al*. 2017a,b, 2018 and Hammershøj *et al*. 2019). In these studies samples were taken using SPME and analysed by GC-MS. No information was included on LOD/LOQ, while blanks were included. Degradation kinetics were reported, although no information on degradation products or mass balance were reported.

Overall, limited information on the quality control/quality assurance of the analytical method was reported in studies investigating the persistence of substances in water. LOD/LOQ information was only reported in one study, while several studies reported to have analysed blanks. Information on other QA/QC parameters such as reproducibility, precision and accuracy were not reported at all. This information is important in evaluating the quality and robustness of data generated by an analytical method.

Table 5.4: Summary of obtained studies reporting on analytical methods in water (OECD 309) persistence testing.

Reference	Test compounds	Compound class	Use of ^{14}C-labelled substance	Sample extraction	Separation / detection technique	LOD/LOQ reported	Other QA/QC reported	Degradation kinetics reported	Identity of degradation products reported	Mass balance reported
Hand *et al*. (2014)	Isopyrazam	Fungicide	Yes - location of ^{14}C label and activity provided	Direct analysis	LSC; HPLC-RAM	No	No	Yes	No	Yes
Toräng *et al*. (2002)	Aniline		Yes - location of ^{14}C label, purity and activity provided. Also non-labelled	Direct analysis	Non-label: HS-GC-FID; ^{14}C: LSC	Yes (non-label)	No	Yes	No	Yes
Toräng *et al*. (2005)	Aniline, 4-nitrophenol, 2,4-dichlorophenoxyacetic acid, 4-chloroaniline	Various	Yes - location of ^{14}C label, purity and activity provided	Direct analysis	LSC	No	No	Yes	No	No
Birch *et al*. (2017a)	n-Decane, tetralin, biphenyl, trans-decalin, bicyclohexyl, 1,2,4-trimethylbenzene (tmbenzene), naphthalene, 2,3-dimethylheptane (dmheptane), 1,3,5-trimethylcyclohexane (tmcyclohexane)	Hydrocarbons	No	HS-SPME	Parent: GC-MS	No	Blanks	Yes	No	No
Birch *et al*. (2017b)	n-Decane, tetralin, biphenyl, trans-decalin, bicyclohexyl, 1,2,4-	Hydrocarbons	No	HS-SPME	Parent: GC-MS	No	Blanks	Yes	No	No

Reference	Test compounds	Compound class	Use of ^{14}C-labelled substance	Sample extraction	Separation / detection technique	LOD/LOQ reported	Other QA/QC reported	Degradation kinetics reported	Identity of degradation products reported	Mass balance reported
	trimethylbenzene (tmbenzene), naphthalene, 2,3-dimethylheptane (dmheptane), 1,3,5-trimethylcyclohexane (tmcyclohexane)									
Birch *et al.* (2018)	53 hydrocarbons (C8-C20)	Hydrocarbons	No	SPME	Parent: GC-MS	No	Blanks	Yes	No	No
Hammershøj *et al.* (2019)	1,2,4-Trimethylbenzene, trans-decahydronaphthalene, n-decane, naphthalene, tetrahydronaphthalene, bicyclohexyl, p-xylene, biphenyl, 2-methylnonane, cis^{-1},2-dimethylcyclohexane, n-dodecane, 2,3-dimethylheptane, n-octylcyclohexane, and 1,2,3,4,5,6,7,8-octahydrophenanthrene, perhydrofluorene and dimethylisopropyl-perhydrophenanthrene	Hydrocarbons	No	HS-SPME	Parent: GC-MS	No	Blanks	Yes	No	No

5.5 Isotopic Labelling Methods Recommended in OECD 305, 307, 308 and 309

The regulatory guidelines for the use of isotopic labelling in OECD 305 (Fish Bioaccumulation), OECD 307 (Aerobic and Anaerobic Transformation in Soil), OECD 308 (Aerobic and Anaerobic Transformation in Aquatic Sediment Systems) and OECD 309 (Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test) are uneven in their requirements, with OECD 309 being the most stringent. These were discussed in the IR1 Scoping document (WP1) of this report, and are reproduced in Table 5.5 below:

Table 5.5: Isotopic Labelling Methods Recommended in OECD 305, 307, 308 and 309.

OECD TEST	RECOMMENDATIONS/REQUIREMENTS
305	Use of radiolabelled test substance is preferred. Radiolabelled test chemical purity should preferably be >98%; the percentage of radioactivity associated with impurities should be known.
307 and 308	^{14}C-labelling is recommended, but the use of other isotopes, such as ^{13}C, ^{15}N [*stable isotopes*], ^{3}H, ^{32}P, may also be useful. The radiolabel should be positioned on the most stable part of the molecule. Radiochemical purity should be at least 95%. When radiolabelled material is used a liquid scintillation counter and combustion oxidiser (for the combustion of sediment samples prior to analysis of radioactivity) will also be required. Radiolabelled mass balance should range from 90% to 110%. For non-labelled substances, the analytical accuracy should lead to an initial recovery of between 70% and 110%.
309	The test may be conducted using radiolabelled or non-radiolabelled test substance. For substances containing more than one aromatic ring, one or more carbons in each ring should preferably be ^{14}C-labelled. In addition, one or more carbons on both sides of easily degradable linkages should preferably be ^{14}C-labelled. The chemical and/or radiochemical purity of the test substance should be >95%; a specific activity of approx. 50 µCi/mg (1.85 MBq/mg) or more is preferred in order to facilitate ^{14}C measurements in tests conducted with low initial concentrations For substances containing more than one aromatic ring, one or more carbons in each ring should preferably be ^{14}C-labelled. In addition, one or more carbons on both sides of easily degradable linkages should preferably be ^{14}C-labelled. The chemical and/or radiochemical purity of the test substance should be >95%, a specific activity of approx. 50 µCi/mg (1.85 MBq/mg) or more is preferred in order to facilitate ^{14}C measurements in tests conducted with low initial concentrations. Concentrations of ^{14}C-labelled test substance and major transformation products may be determined by use of radiochromatography (e.g. thin layer chromatography, RAD-TLC) or HPLC with radiochemical detection. Information on the analytical techniques and the methods used for radiochemical measurements and for mass balance check and measurements of phase distribution, and the repeatability and sensitivity of the analytical methods used, including the limit of detection (LOD) and the limit of quantification (LOQ) must be reported. The determination of the total recovery (mass balance) at the end of the experiment is mandatory. Radiolabelled mass balance should range from 90% to 110%.

Additionally, specifically in the case of OECD 305, the ECHA guidance (R.7c, Section R.7.10.4) makes a number of specific recommendations in relation to radiolabelling, sampling/clean-up, extraction and analysis and as a minimum requirement it could be suggested that recommendations for OECD 305, 307, 308 and 309 guidelines are harmonised at the level of OECD 309 guidelines, which corresponds to methodology approaching what is considered to be best practice.

5.6 Isotopic Labelling Analysis Methods Used in European Food Safety Authority (EFSA) Draft Assessment Reports (DARs) for PPPs

The six substances for review: Abamectin, Bifenthrin, Chlorsulfuron, Esfenvalerate, Fenazaquin and Zetacypermethrin were selected as outlined in IR2. The relevant documents were downloaded from the EFSA web site. The summary data for fish bioaccumulation studies were not suitable for critical assessment, but those pertaining to aerobic and anaerobic transformation in soils, aerobic and anaerobic transformation in sediments and aerobic mineralisation in surface water were suitable for critical review. It was found necessary to use the table of contents for the Annex B8 Environmental fate and behaviour volume in order to find the relevant studies. These studies were conducted under the requirements of OECD TG 307, 308 and 309 respectively, although they also complied with the additional stipulations for PPPR authorisation (see Section 3 above), for example the identification of transformation/degradation products and the construction of metabolic pathways.

All studies used radiolabelled pesticides. Where there was a labile bond in the structure (e.g. for pyrethroids), radiolabelled analogues were used where each half of the molecule was labelled. Therefore, metabolites arising from the cyclopropyl acid and 3-phenoxybenzyl alcohol moieties could be identified. Most studies used ^{14}C labelled material, although ^{3}H was also used in one study. There were often multiple studies performed using the same test, for example aerobic breakdown in soil, using different soil samples (according to the OECD 307 guideline requirement to test four soils). Study guidelines and whether GLP or not, were always reported.

It must be appreciated that the EFSA documents reviewed are summaries, so that detailed descriptions of the analytical methods are not routinely documented. Most, but not all studies stated the radiochemical purity and other details of the radiolabelled compound (such as position of the radiolabel(s)). Sample extractions from soil, sediment or water were usually ambient temperature solvent extraction followed by a series of solvents with differing polarities. Hot solvent (Soxhlet) extraction was also used. Just one study of those examined used a solid phase extraction (Empore disc) method. The methods used to separate metabolites were HPLC and/or TLC. Identification of metabolites was usually by co-elution on HPLC or TLC (1 D and 2 D) with authenticated cold standards, although NMR and MS were also used. The LOD/LOQ was stated in very few studies, although the text often indicated it had been measured, just not stated. Most studies used un-labelled reference metabolites produced in-house and characterised by ^{1}H, MS and HPLC, although specific details were often not available in the DAR summaries. All studies measured unextractable residues by combustion analysis and volatiles (including CO_2) were trapped and quantified. Consequently, mass balance, which was always stated, was usually in the range 95-115%. Degradation kinetics were usually reported, together with the derived DT_{50} values. Major metabolites were always identified, but not minor ones <5%. These studies are summarised in Table 5.6 below.

Table 5.6: Summary of obtained studies reporting on analytical methods in EFSA DARs for PPPs

PPP TESTED	PPP CLASS	REFERENCE	TEST	TEST SUBSTANCE	RADIOCHEMICAL PURITY %	SAMPLE EXTRACTION	SEPARATION TECHNIQUE	QUANTIFICATION METHOD	LOD/LOQ REPORTED	REFERENCE METABOLITES	IDENTIFICATION OF METABOLITES METHOD(S)	BOUND RESIDUES	VOLATILES MEASURED	DEGRADATION KINETICS	IDENTITY OF DEGRADATION	MASS BALANCE REPORTED
Abamectin [1]	Insecticide/ anthelmintic	Abamectin_DAR_06_Vol_3_B8_public.pdf	Transformation in Soil (I)	[23-^{14}**C**]-avermectin B1a	99.3	Solvent (ambient temp. and reflux)	HPLC and TLC	LSC and 2 D-TLC, HPLC-UV (220 nm)	No	Yes (5)	NMR and LC/MS	Yes	Yes	Yes	Yes	Yes
Abamectin [1]	Insecticide/ anthelmintic	Abamectin_DAR_06_Vol_3_B8_public.pdf	Transformation in Soil (II)	[23-^{14}**C**]-avermectin B1a	97.0	Solvent (ambient temp. and reflux)	HPLC and TLC	LSC and 2 D-TLC, HPLC-UV (220 nm)	Yes	Yes (5)	NMR and LC/MS	Yes	Yes	Yes	Yes	Yes
Abamectin	Insecticide/ anthelmintic	Abamectin_DAR_06_Vol_3_B8_public.pdf	Transformation in sediment I	[23-^{14}**C**]-avermectin B1a	99.4	Solvent extraction (ambient temperature)	HPLC and TLC	2 D-TLC and/or HPLC-UV (243 nm)	No	Yes (5)	NMR and LC/MS	Yes	Yes	Yes	Yes	Yes
Abamectin	Insecticide/ anthelmintic	Abamectin_DAR_06_Vol_3_B8_public.pdf	Transformation in sediment II	[5-^{3}**H**]-avermectin B1 [25-^{14}**C**]-avermectin B1a	>99.0 (both)	Solvent (ambient temp.)	LC	LSC	No	Yes (5)	Not stated	Yes	Yes	Yes	Yes	Yes
Bifenthrin	Insecticide	Bifenthrin_DAR_06_Vol_3_B8_public.pdf	Transformation in soil (I)	cyclopropenyl $^{1\text{-}14}$C- and (UL)-^{14}C-bifenthrin	Not stated	Solvent (ambient temp.)	HPLC and TLC	LSC	No	Yes (3)	HPLC/TLC co-elution	Yes	Yes	Yes	Yes	Yes

PPP TESTED	PPP CLASS	REFERENCE	TEST	TEST SUBSTANCE	RADIOCHEMICAL PURITY %	SAMPLE EXTRACTION	SEPARATION TECHNIQUE	QUANTIFICATION METHOD	LOD/LOQ REPORTED	REFERENCE METABOLITES	IDENTIFICATION OF METABOLITES METHOD(S)	BOUND RESIDUES	VOLATILES MEASURED	DEGRADATION KINETICS	IDENTITY OF DEGRADATION	MASS BALANCE REPORTED
Bifenthrin	Insecticide	Bifenthrin_DAR_06_Vol_3_B8_public.pdf	Transformation in soil (II)	(phenyl)-^{14}C-bifenthrin	Not stated	Solvent (ambient temp.)	HPLC	LSC	No	Yes (1)	Not stated	Yes	Yes	Yes	Yes but not minor	Yes
Bifenthrin	Insecticide	Bifenthrin_DAR_06_Vol_3_B8_public.pdf	Transformation in soil (III)	(phenyl)-^{14}C-bifenthrin	Not stated	Solvent (ambient temp.)/ Soxhlet	TLC and LC	LSC	No	Yes (4)	Co-elution HPLC	Yes	No	No	Yes	Yes
Bifenthrin	Insecticide	Bifenthrin_DAR_06_Vol_3_B8_public.pdf	Transformation in soil (IV)	cyclopropenyl $^{1-14}$C-bifenthrin	Not stated	Solvent (ambient temp.)	TLC	LSC	No	Yes	Co-elution TLC	Yes	Yes	No	Yes but not minor	Yes
Bifenthrin	Insecticide	Bifenthrin_DAR_06_Vol_3_B8_public.pdf	Transformation in soil (anaerobic) (V)	cyclopropenyl $^{1-14}$C-bifenthrin	Not stated	Solvent (ambient temp.)	HPLC and TLC	LSC	No	Yes	Co-elution TLC	Yes	No	No	Yes but not minor	Yes

PPP TESTED	PPP CLASS	REFERENCE	TEST	TEST SUBSTANCE	RADIOCHEMICAL PURITY %	SAMPLE EXTRACTION	SEPARATION TECHNIQUE	QUANTIFICATION METHOD	LOD/LOQ REPORTED	REFERENCE METABOLITES	IDENTIFICATION OF METABOLITES METHOD(S)	BOUND RESIDUES	VOLATILES MEASURED	DEGRADATION KINETICS	IDENTITY OF DEGRADATION	MASS BALANCE REPORTED
Bifenthrin	Insecticide	Bifenthrin_DAR_06_Vol_3_B8_public.pdf	Transformation in water/ sediment (aerobic) I	^{14}C-cyclopropyl bifenthrin and^{14}C-phenyl bifenthrin	Not stated	Ambient temp. solvent and Soxhlet	TLC	LSC	Not stated	Yes	Co-elution	Yes	Yes	Yes	No	Yes
Bifenthrin	Insecticide	Bifenthrin_DAR_06_Vol_3_B8_public.pdf	Transformation in water/ sediment (aerobic) II	^{14}C-cyclopropyl bifenthrin and^{14}C-phenyl bifenthrin	Not stated	SPE (Empore) and ambient temp. solvent	HPLC and TLC	LSC	Not stated	Yes	Co-elution, LC-MS and GC-MS	Yes	Yes	Yes	Yes	Yes
Chlorsulfu-ron	Herbicide	Chlorsulfuron_AddendumB_post-approbation_January_2012.pdf	Transformation in soil (aerobic and anaerobic) I	phenyl(U) ^{14}C]chlorsulfuron and [triazine-2-^{14}C]chlorsulfuron	97.8 and 98.7 respectively	Ambient temp. solvent	HPLC	LSC	Yes	Yes	C0-elution	Yes	Yes	Yes	Yes	Yes
Chlorsulfu-ron	Herbicide	Chlorsulfuron_AddendumB_post-approbation_January_2012.pdf	Transformation in soil (aerobic) II	phenyl(U) ^{14}C]chlorsulfuron and [triazine-2-^{14}C]chlorsulfuron	97.8 and 98.7 respectively	Ambient temp. solvent	None	Combustion analysis	N/A	No	N/A	Yes	Yes	Yes	Yes	Yes

PPP TESTED	PPP CLASS	REFERENCE	TEST	TEST SUBSTANCE	RADIOCHEMICAL PURITY %	SAMPLE EXTRACTION	SEPARATION TECHNIQUE	QUANTIFICATION METHOD	LOD/LOQ REPORTED	REFERENCE METABOLITES	IDENTIFICATION OF METABOLITES METHOD(S)	BOUND RESIDUES	VOLATILES MEASURED	DEGRADATION KINETICS	IDENTITY OF DEGRADATION	MASS BALANCE REPORTED
Chlorsulfu-ron	Herbicide	Chlorsulfuron_AddendumB_post-approbation_January_2012.pdf	Transformation in soil (aerobic) III	phenyl(U) ^{14}C]chlorsulfuron and [triazine-2-^{14}C]chlorsulfuron	97.8 and 98.7 respectively	Ambient temp. solvent	None	Combustion analysis	N/A	No	N/A	Yes	Yes	Yes	No	Yes
Chlorsulfu-ron	Herbicide	Chlorsulfuron_AddendumB_post-approbation_January_2012.pdf	Transformation in water/sediment (aerobic)	phenyl(U) ^{14}C]chlorsulfuron and [triazine-2-^{14}C]chlorsulfuron	97.8 and 98.7 respectively	Ambient temp. solvent	HPLC	LSC	No	Yes	Co-elution	Yes	Yes	Yes	Yes	Yes

PPP TESTED	PPP CLASS	REFERENCE	TEST	TEST SUBSTANCE	RADIOCHEMICAL PURITY %	SAMPLE EXTRACTION	SEPARATION TECHNIQUE	QUANTIFICATION METHOD	LOD/LOQ REPORTED	REFERENCE METABOLITES	IDENTIFICATION OF METABOLITES METHOD(S)	BOUND RESIDUES	VOLATILES MEASURED	DEGRADATION KINETICS	IDENTITY OF DEGRADATION	MASS BALANCE REPORTED
Esfenvale-rate	Insecticide	Esfenvalerate_RAR_11_Volume_3_B-8_2013-07-31.pdf	Route and rate of degradation in soil I	^{14}C--phenoxyphenyl esfenvalerate and ^{14}C-phenoxyphenyl fenvalerate	99.0 (each compound)	Not stated	TLC	LSC	No	Yes	Co-elution	Yes	Yes	Yes	Yes	Yes
Esfenvalerate	Insecticide	Esfenvalerate_RAR_11_Volume_3_B-8_2013-07-31.pdf	Route and rate of degradation in soil II	^{14}C-benzylmethyne esfenvalerat	98.6	Not stated	TLC	LSC	No	Yes	Not stated	Yes	Yes	Yes	Yes	Yes
Esfenvalerate	Insecticide	Esfenvalerate_RAR_11_Volume_3_B-8_2013-07-31.pdf	Transformation in water/sediment	^{14}C-phenoxyphenyl and ^{14}C-chlorophenyl esfenvalerate	97.2 and 98.0 respectively	Not stated	TLC	LSC	No	Yes	Not stated	Yes	Yes	Yes	Yes	Yes
Fenazaquin	Acaricide	Fenazaquin_DAR_08_Vol3_B8_public.pdf	Transformation in soil I	^{14}C-phenyl Fenazaquin	Not stated	Ambient temp. solvent	TLC	LSC	No	Yes	Co-elution	Yes	Yes	Yes	Yes	Yes

PPP TESTED	PPP CLASS	REFERENCE	TEST	TEST SUBSTANCE	RADIOCHEMICAL PURITY %	SAMPLE EXTRACTION	SEPARATION TECHNIQUE	QUANTIFICATION METHOD	LOD/LOQ REPORTED	REFERENCE METABOLITES	IDENTIFICATION OF METABOLITES METHOD(S)	BOUND RESIDUES	VOLATILES MEASURED	DEGRADATION KINETICS	IDENTITY OF DEGRADATION	MASS BALANCE REPORTED
Fenazaquin	Acaricide	Fenazaquin_DAR_08_Vol3_B8_public.pdf	Transformation in soil II	^{14}C-phenyl Fenazaquin	Not stated	Ambient temp. solvent	TLC	LSC	No	Yes	Co-elution	Yes	No	Yes	Yes	Yes
Fenazaquin	Acaricide	Fenazaquin_DAR_08_Vol3_B8_public.pdf	Transformation in water/ sediment	^{14}C-quinazoline and ^{14}C-phenyl quinazoline	Not stated	Ambient temp. solvent	HPLC and TLC	LSC	No	Yes	Not stated	Yes	Yes	Yes	Yes	Yes
Zeta-cypermethrin	Insecticide	Zeta-cypermethrin_DAR_09_Vol3_B8_public.pdf	Transformation in soil	^{14}C-Cypermethrin (benzyl label), cyclopropyl-1-^{14}C-cypermethrin	97.3, 96.3 respectively	Ambient temp. solvent and Soxhlet	TLC and HPLC	LSC	No	Yes	TLC, HPLC-UV and HPLC–MS	Yes	Yes	Yes	Yes	Yes
Zeta-cypermethrin	Insecticide	Zeta-cypermethrin_DAR_09_Vol3_B8_public.pdf	Transformation in soil II	cis- and trans-Cypermethrin, [^{14}C-labelled benzyl ring]	Not stated	Soxhlet	TLC	LSC	Not stated	Yes	Co-elution	Yes	Yes	Yes	Yes	Yes

PPP TESTED	PPP CLASS	REFERENCE	TEST	TEST SUBSTANCE	RADIOCHEMICAL PURITY %	SAMPLE EXTRACTION	SEPARATION TECHNIQUE	QUANTIFICATION METHOD	LOD/LOQ REPORTED	REFERENCE METABOLITES	IDENTIFICATION OF METABOLITES METHOD(S)	BOUND RESIDUES	VOLATILES MEASURED	DEGRADATION KINETICS	IDENTITY OF DEGRADATION	MASS BALANCE REPORTED
Zeta-cypermethrin	Insecticide	Zeta-cypermethrin_DAR_09_Vol3_B8_public.pdf	Transformation in water/sediment	Zeta-Cypermethrin[^{14}C-cyclopropyl-labelled and Zeta-Cypermethrin[^{14}C-benzyl-labelled]	99.0 (both compounds)	Ambient temp. solvent	TLC and HPLC	LSC and radio-scanning TLC plates	Not stated	Yes	Co-elution	Yes	Yes	Yes	Yes	Yes

6. Key aspects to consider prior to conducting biodegradation and bioaccumulation studies

This section describes the key aspects and areas that will impact the analytical method development and the validity of the results of analysis. Discussion on these topics is limited to the key elements which could affect decisions on study design and subsequent interpretation of the study; this is not the focus of the literature review.

6.1 Characterisation of the substance

Test substances for environmental fate studies, as well as any synthesised metabolites, should be fully characterised:

- It is important to identify impurities (including any unreacted starting material) as these may be mistaken for metabolites.
- Characterisation should be carried out using different chromatographic and detection methods against a certified reference standard.

6.2 Key physico-chemical properties of the test substance: Water solubility

Prior to conducting any biodegradation or bioaccumulation study, an understanding of the key physico-chemical properties of the test substance is critical. These key properties are listed in the relevant OECD test guidelines, and include properties such as vapour pressure, octanol-water partition coefficient (K_{ow}), biotic or abiotic degradation in water (including hydrolysis rate), surface tension, dissociation constant (acid and alkali). Certain properties (or combinations of properties) may lead to a substance being considered 'difficult-to-test'; in some cases, it may not be feasible to conduct a particular study for these substances. Further discussion of these 'difficult-to-test' substances is provided in Section 10.

The availability of reliable data for these key physico-chemical properties should be assessed. In particular, water solubility is a fundamental property that affects the hazard and exposure assessment of chemicals. The availability of a reliable water solubility value is vital, both for the determination of BCF (Arnot and Gobas, 2006) and for degradation studies as well as for evaluating the feasibility of the methods used. The most recent OECD guideline (OECD TG 105) for measuring solubility, dates from 1995 and states that it is applicable "essentially pure substances which are stable in water and not volatile." The OECD TG 105 guideline is not suited for several groups of difficult-to-test substances, such as highly hydrophobic chemicals, volatile chemicals, surfactants, multi-constituent and UVCBs. Birch *et al*. (2019) reviewed solubility measurement methods for highly hydrophobic and volatile chemicals, and methods to rapidly saturate water with fast degrading chemicals are also reviewed. They present a useful decision tree outlining the preferred choice of method for each chemical group. This study also reviewed measurement methods for critical micelle concentrations that set the upper concentration limit for freely dissolved surfactants. Finally, they described strategies to measure solubility parameters for multi-constituent substances and UVCBs.

The applicability domains of the OECD 305 and 309 test guidelines are partially defined in terms of the test substance solubility in the test media (it should be noted that solubility in test media may differ from the water solubility measured by an OECD TG 305 study). In the OECD TGs 305 (aqueous exposure) and 309, the exposure concentration must be below the limit of solubility of the test substance in the test media. When stable aqueous concentrations cannot be demonstrated, for example with highly volatile substances, an OECD TG 305 dietary study should be considered. The OECD TG 307 is applicable soluble or water-insoluble compounds, whilst the OECD TG 308 is applicable to water-soluble or poorly water-soluble compounds.

According to OECD TGs 305, 308 and 309, the water solubility according to OECD TG 105 must be known prior to environmental fate testing and should be assessed in natural water (influence of salt content and pH should be investigated). It is the experience of the authors of this report that registrants sometimes rely on QSAR predictions of water solubility and that QSAR predictions may not be sufficiently accurate. If it is technically not feasible to conduct a guideline water solubility study, then this is a challenge for those environmental fate studies where study design is determined based on solubility in water . In some cases, the environmental fate test guidelines allow amended study designs with the aid of solvents. The OECD TG 305 allows for the use of a solvent (or dispersing agent) in order to produce a suitably concentrated stock solution for aqueous exposure studies, though it is recommended that the use of solvent should be minimised as it may impact the bioavailability of the substance. Similarly, whilst a solvent may be used in a OECD TG 309 study, the guideline notes that care are must be taken to limit the amount of solvent transferred to what is absolutely necessary and to ensure that the amount of test substance can dissolve in the final volume of test water. In the OECD TG 307 and 308, the test substance may be dissolved in minimum solvent for application to the soil/sediment, however it must be demonstrated that the solvent selected has no adverse effects on the test system. The use of a volatile solvent such as acetone is recommended as it will be removed from the test system fairly quickly. The use of readily biodegradable solvents should be considered with caution (e.g. in aqueous bioaccumulation studies, these can cause problems with bacterial growth). Generally, the applicability of the method for the substance must be considered carefully; e.g. it is unlikely to be appropriate to conduct an OECD TG 309 with a substance for which a water solubility study is not feasible; where it is not possible to prepare a stock solution for an aqueous bioaccumulation study without the use of a solvent, a dietary exposure study should be considered.

Knowledge of the water solubility of chemicals is of prime importance for a BCF study or a degradation study, to allow homogeneous solutions to be produced. It may be necessary to consider the use of passive dosing techniques in order to achieve homogenous solutions (see Section 10.3.1). Arnot and Gobas (2006) state that it is good practice for the exposure concentration in an aqueous bioaccumulation study to be ≤ 20% of the aqueous solubility of the chemical, to ensure that a homogeneous solution is maintained. It should be noted that OECD TG 305 states that, in general, the aqueous concentrations in the uptake phase of a bioaccumulation study should be at least an order of magnitude above the limit of quantification (LOQ) of the analytical method so that more than one half-life of body burden can be measured in the depuration phase of the study.

The solubility of a substance in test media (in an aqueous bioaccumulation study) or in natural water may not be the same as the solubility determined by an OECD TG 105 study (or similar), as test media and natural waters may contain undissolved organic matter, salts, etc., and is therefore more complex than distilled water (OECD 23, 2019).

Water and solvent solubility are also very important when considering the methods of extraction and chromatography as these are often based upon partition between water and solvent or solid phase.

6.3 In silico methods

In silico methods may be used in several areas when preparing to conduct environmental fate studies:

- Prediction of key physicochemical properties when generating experimental data is not feasible, which are required prior to conduct of the test (for example log K_{ow}, water solubility and vapour pressure).
- Prediction of metabolites that may be formed during degradation studies.
- Use of environmental fate modelling to identify compartments of concern.

In all cases, the validity of the method, its applicability to the substance of interest and the adequacy of the prediction for the purpose must be assessed (ECHA Guidance R.6). For physicochemical properties, there will be cases where a prediction is not adequate for the purpose of the study design. For example, where the proposed concentration in water is close to the predicted solubility or where a log K_{ow} value is a factor in determining the type of bioaccumulation testing to be conducted (BCF or BMF), the uncertainty in a predicted value may be too high; in this case a measured value would be needed.

A number of *in silico* tools are available for prediction of metabolites, including EAWAG-BBD Pathway Prediction System,[27] Catalogic,[28] PathPred & KEGG databases,[29] OECD QSAR Toolbox (microbial metabolism profiler),[30] and Biotransformer.[31] REACH guidance documents R.7 and R.11 refer to the use of some of these methods for prediction of metabolites. The majority of published literature in the area relates to tools for drug discovery. *In silico* approaches and tools for the prediction of drug metabolism have been the subject of various reviews, including Kazmi *et al.* (2019) and Piechota *et al.* (2013).

Some of the available tools predict only sites of metabolism, others also predict metabolites generated through any one of the many known metabolic pathways, especially from cytochrome P450-mediated processes. Some tools can estimate the likelihood of formation of metabolites generated, however, formation and dissipation kinetics are not predicted. A common downside of these tools is that all possible known reaction schemes are included in a library and so any hypothetical metabolite that may be generated from that particular scheme will be predicted (Leonard, 2019). Another limitation in prediction of metabolites in a biodegradation simulation study is that library reaction schemes may be based on non-microbial metabolic mechanisms (e.g., rodent or human metabolism). Metabolic information available to build these models is also incomplete for some substance types, leading to low predictability (Dimitrov *et al.* 2010). As with all *in silico* methods, these tools

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[27] http://eawag-bbd.ethz.ch/predict/

[28] http://oasis-lmc.org/products/software/catalogic.aspx

[29] http://www.genome.jp/tools/pathpred/

[30] https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm

[31] http://biotransformer.ca/

will perform better when similar molecules[32] are included in the model training set, and when the degradation pathways have been thoroughly documented.

Therefore, these tools may be helpful in indicating potential metabolites for the purpose of designing studies to identify metabolites or indicating whether metabolites of concern could form. However, the low accuracy of the predictions, the lack of quantitative information and the other limitations discussed above mean that predictive methods cannot replace the need for experimental identification of metabolites. Experimental methods for identification of observed metabolites could include the soft ionisation, tandem MS/MS and high-resolution MS methods discussed in Section 9.5.

6.4 Artefact formation[33]

Consideration of the possibility of artefact formation is central to assessing the relevance and reliability of data provided by analytical methods. It should be noted that this would not be significant in radiolabel studies compared to non-radiolabelled studies. Artefacts are caused by contamination, within the mass spectrometer or from the matrix. They become increasingly significant as lower concentration levels are required. The most serious contamination occurs in the sampling, extraction and concentration stages.

Middleditch and Zlatkis (1987) stated that measurement artefacts are very common, rarely reported and have many different origins. One of the few books dealing with this subject is by Middleditch (1989) entitled *Analytical Artifacts.* In this compendium of problems encountered when using various commonly used analytical techniques.

The sections below summarise the very sparse literature on sources of artefact formation, particularly Middleditch (1989), Keller *et al*. (2008) and Nießner and Schäffer (2017). The examples given below are from environmental trace analysis, however similar issues would arise in environmental fate studies conducted in the laboratory.

6.4.1 Artefacts in Mass Spectrometry

Fischer Scientific (2019), in their poster entitled 'Interferences and contaminants encountered in modern mass spectrometry', states that the common background contamination ions encountered in mass spectrometers are polyethylene glycol, polypropylene glycol, phthalates, organic solvent clusters, solvent modifiers, fatty acids, metal ions, Triton-X, Tween® and siloxanes. Ende and Spiteller (1982) reviewed the contaminants in mass spectrometry. More recently, Millipore-Sigma (2017) produced a useful guide on LC-MS contaminants and how to avoid them.

6.4.2 Artefact formation and contamination from sampling and sample preparation

There are a number of sources of artefact formation and contamination from sampling and sample preparation:

- The act of separation and enrichment can increase the probability of reaction with other reactive chemicals in the same matrix. These processes include:
    - Hydrolysis.
    - Solvolysis.

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[32] It is noted that some types of substances are generally excluded from *in silico* predictors, including polymeric substances, organometallic, and inorganic substance. As these substances are outside of the scope of the present report, they are not considered here.

[33] Note the US spelling of Artifact with an "i" and the British English as Artefact.

  - Auto-radiolysis.
  - Aggregation.
  - Sorptivity for surfaces, such as cyclic siloxanes (Knoerr *et al.*, 2017) and PAHs for surfaces (Qian *et al.*, 2011).
  - Oxidation.
  - Thermal instability.
  - Visible light and pH changes can trigger isomerism.
  - Photo-oxidation.
  - Photo-dimerization of unsaturated compounds.
- Pipette tips should not be recycled, and sampling devices should be carefully cleaned before use.
- The analyst can be source of contamination.
  - Smokers should not perform a PAH analysis (Nießner and Schäffer, 2017).
  - The skin can be source for many organic compounds. For example, siloxanes present in skin care products have been detected in blanks (Horrii and Kannan, 2008).
  - Squalene and cholesterol are present in high concentrations in skin and are ubiquitous components of the dust that accumulates in most laboratories. These would show up with m/z 69 and 81. Clark and Shirley (1973) stated that simply touching the outside of the syringe needle with the fingers would give measurable amounts of squalene in the chromatogram.
- Filter membranes may contain melamine resins as binder so should be rinsed with solvent before use.

6.4.3 Radiolysis

One practical problem when using radioactive tracers is the radiolysis of samples. Radiolysis can be defined as chemical decomposition by the action of radiation and is thought to proceed mainly by a free radical mechanism in the case of β-emitters. The rate of chemical deterioration by radiolysis is dependent on the radioactive flux; consequently, it much more of a problem with tritiated compounds than for ^{14}C. It is of considerable importance when storing samples which have high radiochemical and chemical concentrations that will reduce the radiochemical purity of the test substance over time. The acceptable storage time depends on a number of factors, including radiochemical specific activity, chemical concentration, chemical stability, physical state (solid, liquid or solution) and temperature. These parameters are well-understood by suppliers of radiochemicals, who will recommend storage conditions such as: "Do not store as a solid. If possible, solubilise in toluene and ethanol (95:5) as this can quench any stray energy. This solvent can also be removed from quickly under a stream of nitrogen when the test substance is required. Store at -80°C where possible." Typical acceptable storage times for high specific activity tritiated substances are a few months and much longer for ^{14}C-labelled substances. Radiochemical purity should be re-assessed by HPLC or TLC whenever used. If the radiochemical purity is deemed too low for use, then it must be re-purified. Ideally all radiolabelled substances should be assessed for radiochemical purity upon receipt, re-assessed during storage and prior to use.

6.4.4 Contamination of the solvent

Solvents used in the extraction or chromatography states may also be a source of contamination:

- It is a false economy to not use the highest purity solvents available (Middleditch and Zlatkis, 1987). Recycling of used solvents should always be avoided.
- Ultrapure water must be checked for organic impurities. Usually, only conductance is monitored.
- Some ultrapure solvents may have contaminants leading to interferences especially in the case of fluorescence analysis.
- Un-stabilised chloroform may be oxidized to phosgene that could react with nitrogen containing compounds.
    - For example, Cone *et al.* (1982) contaminated chloroform in the extraction of nitrogen containing drugs (such as amphetamine, norcodeine, normeperidine, and nornicotine) from aqueous solution and this led to the formation of carbamoyl chlorides.
- Contamination from solvent stabiliser.
    - Ethanol is often used as a stabiliser for chloroform to inhibit the production of phosgene, but traces of phosgene may react with ethanol to afford ethyl chloroformate that then reacts with the nitrogen containing compounds to yield ethyl carbamates (Cone *et al*., 1982).
    - Tetrahydrofuran (THF) and peroxide formation.
    - The pH of volatile acids or alkalis used to amend extraction solvents and mobile phases can change with storage so should be prepared freshly.
- Chlorinated water samples are subject to further chlorination reactions within the sample.

6.4.5 Improper storage of samples

Storage of samples may also result in artefact formation:

- The analyte can adsorb strongly and instantly to the surface of containers such as synthetic pyrethroids (Albaseer *et al.*, 2011).
- Improper physical conditions (temperature, sunlight, etc.) may alter the constituents (bond breaking or isomerization).
- Plastics should be avoided for storing samples because the presence of stabilisers, plasticisers, and other additives that may leach out.
    - Most analysts realise that they should use glass rather than plastic containers, but few would anticipate the possibility of plasticizer residues on glassware washed using detergent from a plastic bottle (Middleditch (1989).
    - Phthalates (PAEs) are ubiquitous in water, organic solvents, ambient air, glassware and plastics (Net *et al.*, 2019). For example, poly (vinyl chloride), may comprise up to 45% of phthalate plasticisers usually di(2-ethylhexyl) phthalate, diisononyl phthalate or diisodecyl phthalate (Carlos *et al.*, 2018).
    - Other polymers that use plasticisers include acrylic polymers, polyamides, polyolefins, polyurethanes, certain fluoroplastics, and elastomers (Wei *et al*., 2019).
    - Verge and Agnes (2002) discussed plasticizer contamination in vacuum system O-rings in a quadrupole ion trap mass spectrometer.
    - Sometimes, the catalysts used for polymer production are still active at inner wall surfaces of plastic bottles or seals of screw caps leading to degradation of the analyte.
- Rubbers should also be avoided because they contain additives. Ball *et al.* (2012) published a recent survey of contamination from rubber and plastics; although it is stated that most of the work was done in the 1970s. Contamination from plastic additives includes, among others, 2,5-di-tert-pentylhydroquinone (Middleditch *et*

al. 1980), N-ethylaniline (Ulsaker and Teien 1979), 2,2'-methylenebis(4-ethyl-6-tert-butylphenol) (Shang-Qiang and Evenson 1983).
- Cross-contamination from leaking sample containers.
- Aqueous samples should not be frozen without prior analysis, where avoidable. A degree of de-mixing, precipitation can occur during freezing and thawing stages. Freezing solid samples is permissible.
- Microbes present (bacteria and protozoa) could metabolize organic chemicals over time.
- The stability of the samples in storage where solvent exchange has not occurred is always a great concern. Therefore, it is good practice to do the extractions at the time of sampling and store the extracts.

6.4.6 Contamination from glassware, external media or instrumentation

Other sources of contamination are:

- Contamination by reaction vessels, media and instrumentation. Carryover of substances has to be minimized by separation of working areas, as described in the rules for good laboratory practices (GLP guidelines).
- Glassware should be rigorously cleaned by strong oxidizing agents (e.g., a mixture of sulfuric acid and hydrogen peroxide) as needed.
- Gas impurities are generally underestimated. Despite the purity of a bottled gas being at 99.999%, there are still plenty of unwanted contaminants due to the amount of carrier gas used in gas chromatography.
- Carryover from high concentration standards can give ghost peaks.
- Late eluting peaks where the run-time is not long enough, or a high enough temperature maintained, can appear in the next run.
- GC columns operated at too high a temperature can cause degradation of the solid phase, which in turn can change retention times.
- Septum materials are known to be a source of contamination.

6.5 Calibration and Internal Standards

Radiolabelling is an absolute tracer method; all the other detection methods are relative, and quantification is performed by comparison to a standard, normally using a calibration curve. The availability of standards is an important consideration when undertaking stable isotope or non-labelled studies. Standards for transformation products may not be available. It may be problematic to assume that transformation products have the same response factor as the parent substance; in particular, oxygenated metabolites can have very different response factors from non-polar parent substances.

If using a substance with similar physical chemical properties to the analyte as an internal standard, selection of appropriate standard is crucial. The assumption is that the internal standard has the same extraction efficiency as the analyte. This is not always the case and can lead to significant systematic errors.

6.6 Quality criteria and method validation

6.6.1 Current guidelines on quality criteria for analytical methods in environmental fate studies

Precision, trueness, repeatability and sensitivity are key parameters that must be known of an analytical method used for bioaccumulation or degradation testing in order to generate reliable and robust data.

Current OECD guidelines on quality criteria for the analytical methods used for bioaccumulation and persistence testing (i.e. OECD 305, 307, 308 and 309) are summarized in Table 6.1. The OECD guideline for bioaccumulation in fish (OECD 305) does not state specific criteria for parameters such as recovery, repeatability and sensitivity, whereas the guidelines for persistence studies (OECD 307, 308, 309) do include specific criteria for recovery, repeatability and sensitivity. It is noted that quality criteria relating to other aspects of the studies are listed in the OECD TGs 305, 307, 308, 309. Where these criteria are not directly related to the analytical methodology, they are not discussed here.

Table 6.1: Quality criteria stated in OECD guidelines

OECD GUIDELINE	RECOVERY	REPEATABILITY	SENSITIVITY
OECD 305	Analytical method of known recovery in water and biological tissues (aqueous exposure) and in food and biological tissues (dietary exposure).	Analytical method of known repeatability in water and biological tissues (aqueous exposure) and in food and biological tissues (dietary exposure).	Quantification limit of the test substance in both exposure medium (water/diet) and fish tissues should be known.
OECD 307	Extraction and analysis of, at least, duplicate soil samples immediately after the addition of the test substance gives a first indication of the repeatability of the analytical method and of the uniformity of the application procedure for the test substance. Recoveries for later stages of the experiments are given by the respective mass balances. Recoveries should range from 90% to 110% for labelled chemicals and from 70% to 110% for non-labelled chemicals.	Repeatability of the analytical method (excluding the initial extraction efficiency) to quantify test substance and transformation products can be checked by duplicate analysis of the same extract of the soil, incubated long enough for formation of transformation products.	The limit of detection (LOD) of the analytical method for the test substance and for the transformation products should be at least 0.01 mg·kg^{-1} soil (as test substance) or 1% of applied dose whichever is lower. The limit of quantification (LOQ) should also be specified.
OECD 308	Extraction and analysis of, at least, duplicate water and sediment samples immediately after the addition of the test substance give a first indication of the repeatability of the analytical method and of the uniformity of the application procedure for the test substance. Recoveries for later stages of the experiments are given by the respective mass balances	Repeatability of the analytical method (excluding the initial extraction efficiency) to quantify test substance and transformation products can be checked by duplicate analysis of the same extract of the water or the sediment samples which were incubated long enough for formation of	The limit of detection (LOD) of the analytical method for the test substance and for the transformation products should be at least 0.01 mg·kg^{-1} in water or sediment (as test substance) or 1% of the initial amount applied to a test system whichever is

OECD GUIDELINE	RECOVERY	REPEATABILITY	SENSITIVITY
	(when labelled material is used). Recoveries should range from 90% to 110% for labelled chemicals and from 70% to 110% for non-labelled chemicals.	transformation products.	lower. The limit of quantification (LOQ) should also be specified.
OECD 309	For ^{14}C-labelled test substance, the level of recovery at the end of the experiment is given by mass balance. Ideally, the radiolabelled mass balance should range from 90% to 110%, whereas the analytical accuracy should lead to an initial recovery of between 70% and 110% for non-labelled test substances. These ranges should be interpreted as targets and should not be used as criteria for acceptance of the test. Optionally, the analytical accuracy may be determined for the test substance at a lower concentration than the initial concentration and for major transformation products.	Repeatability of the analytical method (including the efficiency of the initial extraction) to quantify the test substance, and transformation products, if appropriate, should be checked by five replicate analyses of the individual extracts of the surface water.	The limit of detection (LOD) of the analytical method for the test substance and for the transformation products should be at least 1% of the initial amount applied to the test system if possible. The limit of quantification (LOQ) should be equal to or less than 10% of the applied concentration. The chemical analyses of many organic substances and their transformation products frequently require that the test substance is applied at a relatively high concentration, i.e. >100 g/l.

6.6.2 Guidelines for validation of analytical methods used in other fields

OECD guidelines on bioaccumulation and persistence testing state quality criteria regarding recovery, repeatability and sensitivity as described above. There are, however, other quality parameters of an analytical method that should be known before applying the analytical method. These include the linearity of the analytical instrument used, accuracy, precision and stability of the analytes in the studied matrix.

A literature search was performed on guidelines for analytical method validation used on other related fields. Below several parameters commonly described in analytical method validation procedures from organisations such as the European Commission, Food and Drug Administration (FDA), European Medicines Agency (EMA) and the Australian Pesticide and Veterinary Medicines Authority (Table 6.2). These guidelines focus on the validation of analytical methods used for various pesticides and drugs measurements in biological matrices, food stuff, soil and water.

Commonly reported validation parameters are on the calibration curve, accuracy, precision, recovery, sensitivity and stability. More details on each of these parameters, including criteria set by the various guidelines are listed below and in Table 6.2.

Calibration curve

The response of the instrument in relation to the concentration of analyte should be known and evaluated over a specified concentration range. Some guidelines recommend that the calibration standards should be prepared in the same matrix as the matrix of the intended study samples by spiking the blank matrix with known concentrations of the analyte. The solvent composition (percent water and organic solvent) of the calibration standards and samples should be comparable. There should be one calibration curve for each analyte studied in the method validation and for each analytical run.

Based on the guidelines, a calibration curve should include blanks (no analyte, no internal standard), a zero-calibration standard (blank plus internal standard) and at least six or more calibration standards. The concentration range should span 80 to 120% of the expected concentration.

Criteria for the calibration curve include that non-zero calibration standards should be ± 15% of nominal (theoretical) concentrations, except for lower limit of quantitation (LLOQ) where the calibration standard should be ± 20% of the nominal concentrations in each validation run. 75% and a minimum of six non-zero calibration standards should meet the above criteria in each validation run. The calibration curve should also have a correlation coefficient (r) greater than 0.99 over the range (see Table 6.2).

Accuracy

The accuracy of an analytical method describes the closeness of the determined value obtained by the method to the nominal concentration of the analyte (expressed in percentage). Accuracy should be assessed on samples spiked with known amounts of the analyte; the quality control samples (QC samples). It is recommended by the guidelines that the accuracy is tested at different concentrations, for example at the lower limit of quantitation (LLOQ) and low, medium and high quality control samples.

The accuracy can be determined in several ways:

- by analysing a sample of known concentration and comparing the measured value to the 'true' value. However, a well characterised sample (e.g., reference standard) must be used;
- by spiking a known amount of a test substance to a blank sample (a sample that contains all other ingredients except the test substance). The results obtained are then compared to the expected results;
- by adding a known amount of the test substance to a sample previously analysed and analysing it again. The difference between both results are then compared to the expected result.

Accepted criteria for the accuracy vary among guidelines. For drug analyses the measured concentration should be ± 15% of nominal concentrations, except ± 20% at LLOQ. For pesticides, the criteria for accuracy is dependent on the concentration of the substance in the sample and range from 98 – 102% to 75 – 125% (see Table 6.2).

Table 6.2: Guidelines on validation criteria

VALIDATION PARAMETER	FOOD AND DRUG ADMINISTRATION (FDA)	EUROPEAN MEDICINES AGENCY	AUSTRALIAN PESTICIDE AND VETERINARY MEDICINES AUTHORITY	EUROPEAN COMMISSION
Calibration curve	A blank (no analyte, no IS), a zero calibrator (blank plus IS), and at least six, non-zero calibrator levels covering the quantitation range, including LLOQ in every run. **Criteria**: Non-zero calibrators should be ± 15% of nominal (theoretical) concentrations, except at LLOQ where the calibrator should be ± 20% of the nominal concentrations in each validation run. 75% and a minimum of six non-zero calibrator levels should meet the above criteria in each validation run.	A minimum of six calibration concentration levels should be used, in addition to the blank sample (processed matrix sample without analyte and without IS) and a zero sample (processed matrix with IS). **Criteria**: The back calculated concentrations of the calibration standards should be within ±15% of the nominal value, except for the LLOQ for which it should be within ±20%. At least 75% of the calibration Guideline on bioanalytical method validation standards, with a minimum of six calibration standard levels, must fulfil this criterion.	Linearity should be determined by using duplicate determinations at three or more concentrations, or a single determination at six or more concentrations that span 80 to 120 per cent of the expected nominal concentration. **Criteria**: The test results should not show a significant deviation from calculated results by the calibration equation—indicated by the correlation coefficient, r—greater than 0.99 over the range (80 to 120 per cent).	The calibration of the detection system shall be adequately demonstrated at a minimum of 3 concentration levels in duplicate or (preferably) 5 concentration levels with single determination. Calibration should be generated using standards prepared in blank matrix extracts (matrix matched standards) for all sample materials included in the corresponding validation study. Only, if experiments clearly demonstrate that matrix effects are not significant (i.e. < 20 %), calibration with standards in solvent may be used
Accuracy	Accuracy should be established with at least three independent runs, four QC levels per run (LLOQ, L, M, H QC), and ≥ five replicates per QC level.	Within-run accuracy Within-run accuracy should be determined by analysing in a single run a minimum of 5 samples per level at a	The accuracy should cover at least three concentrations (80, 100 and 120 per cent of the nominal concentration) in the expected range.	Not reported

VALIDATION PARAMETER	FOOD AND DRUG ADMINISTRATION (FDA)	EUROPEAN MEDICINES AGENCY	AUSTRALIAN PESTICIDE AND VETERINARY MEDICINES AUTHORITY	EUROPEAN COMMISSION
	Criteria: ± 15% of nominal concentrations; except ± 20% at LLOQ	minimum of 4 concentration levels which are covering the calibration curve range: the LLOQ, within three times the LLOQ (low QC), around 30 - 50% of the calibration curve range (medium QC), and at least at 75% of the upper calibration curve range (high QC). **Criteria:** The mean concentration should be within 15% of the nominal values for the QC samples, except for the LLOQ which should be within 20% of the nominal value. <u>Between –run accuracy</u> For the validation of the between-run accuracy, LLOQ, low, medium and high QC samples from at least three runs analysed on at least two different days should be evaluated. **Criteria:** The mean concentration should be within 15% of the nominal values for the QC samples, except for the LLOQ which should be within 20% of the nominal value.	**Criteria**: The acceptance criteria for the accuracy of the method are based on expected recovery. The mean percentage recovery of each of the three concentrations should be within the following ranges: Active constituent → Acceptable recovery >10% → 98 – 102% 1.0 – 10% → 90 – 110% 0.1 – 1.0% → 80 – 120% <0.1% → 75 – 125%	
Precision	Precision should be established with at least three independent runs, four QC levels per run (LLOQ, L, M, H	<u>Within-run precision</u> For the validation of the within-run precision, there should be	The precision of an analytical method expresses the closeness of agreement	The precision of a method shall be reported as the relative standard deviation (RSD) of

VALIDATION PARAMETER	FOOD AND DRUG ADMINISTRATION (FDA)	EUROPEAN MEDICINES AGENCY	AUSTRALIAN PESTICIDE AND VETERINARY MEDICINES AUTHORITY	EUROPEAN COMMISSION
	QC), and ≥ five replicates per QC level. Criteria: 15% CV, except ± 20% CV at LLOQ	a minimum of five samples per concentration level at LLOQ, low, medium and high QC samples in a single run. **Criteria:** The within-run CV value should not exceed 15% for the QC samples, except for the LLOQ which should not exceed 20%. <u>Between –run precision</u> For the validation of the between-run precision, LLOQ, low, medium and high QC samples from at least three runs analysed on at least two different days should be evaluated. **Criteria:** The between-run CV value should not exceed 15% for the QC samples, except for the LLOQ which should not exceed 20%.	(degree of scatter) between a series of measurements obtained from multiple sampling of the same sample under the same prescribed conditions. **Criteria**: The precision of an analytical procedure is usually expressed as the per cent relative standard deviation of a series of measurements and should fall within the following range: Substance measured in sample → Precision (%RSD) >10% → ≤2% 1.0 – 10% → ≤5% 0.1 – 1.0% → ≤10% <0.1% → ≤20%	recovery at each fortification level. **Criteria:** Concentration → %RSD >1 µg/kg ≤ 0.01 mg/kg → 30% >0.01 mg/kg ≤ 0.1 mg/kg → 20% >0.1 mg/kg ≤ 1.0 mg/kg → 15% >1 mg/kg → 10%

VALIDATION PARAMETER	FOOD AND DRUG ADMINISTRATION (FDA)	EUROPEAN MEDICINES AGENCY	AUSTRALIAN PESTICIDE AND VETERINARY MEDICINES AUTHORITY	EUROPEAN COMMISSION
Recovery	Extracted samples at L, M, and H QC concentrations versus extracts of blanks spiked with the analyte post extraction (at L, M, and H).	Not reported	Not reported	The recovery must be reported at several fortification levels. **Criteria:** Concentration → % recovery >1 µg/kg ≤ 0.01 mg/kg → 60 – 120% >0.01 mg/kg ≤ 0.1 mg/kg → 70 - 120% >0.1 mg/kg ≤ 1.0 mg/kg → 70 - 110% >1 mg/kg → 70 - 110%
Sensitivity	The lowest nonzero standard on the calibration curve defines the sensitivity (LLOQ). **Criteria**: The accuracy should be ± 20% of nominal concentration (from ≥ five replicates in at least three runs). The precision should be ± 20% CV (from ≥ five replicates in at least three runs).	The lower limit of quantification (LLOQ) is the lowest concentration of analyte in a sample which can be quantified reliably, with an acceptable accuracy and precision. **Criteria**: the analyte signal of the LLOQ sample should be at least 5 times the signal of a blank sample	The limit of detection (LOD) of an analytical method is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated as an exact value. The lowest concentration that produces a detectable peak response corresponding to the analyte should be normally measured with between 6 and 10 replicates. The LOD is the average response + 3 × SD. The limit of quantitation (LOQ) is the lowest amount of the analyte in the sample that can be quantitatively determined with defined precision under the stated experimental conditions. A reference standard should be measured with between 6 and 10 replicates. The LOQ is the average response + 10 × SD.	LOQs are set at maximum residue levels (MRLs) for pesticides.

VALIDATION PARAMETER	FOOD AND DRUG ADMINISTRATION (FDA)	EUROPEAN MEDICINES AGENCY	AUSTRALIAN PESTICIDE AND VETERINARY MEDICINES AUTHORITY	EUROPEAN COMMISSION
Stability	For auto-sampler, bench-top, extract, freeze-thaw, stock solution and long-term stability, perform at least three replicates at L and HQC concentrations. **Criteria**: The accuracy (% nominal) at each level should be ± 15%.	Stability of the analyte in the studied matrix is evaluated using low and high QC samples (blank matrix spiked with analyte at a concentration of a maximum of 3 times the LLOQ and close to the ULOQ) which are analysed immediately after preparation and after the applied storage conditions that are to be evaluated. **Criteria**: The mean concentration at each level should be within ±15% of the nominal concentration	Not reported.	Not reported

Precision

The precision of the analytical method describes the closeness of repeated individual measures of analyte. Precision is expressed as the coefficient of variation (CV) or as the relative standard deviation (RSD). Precision should be demonstrated at different concentrations, for example for the LLOQ, low, medium and high QC samples, within a single run and between different runs.

Accepted criteria for the precision vary among guidelines. For drug analyses the VC value measured should not exceed 15% for the QC samples, except for the LLOQ which should not exceed 20%. For pesticide analyses, the criteria for precision is indicated as %RSD and the criteria varies dependent on the concentration of the test substance in the samples. The RSD varies from 2 to 20% for substance concentrations ranging between <0.1% and >10% in the sample according to one guideline and varies between 10 and 30% for substance concentrations ranging between 1 µg/kg and >1 mg/kg (see Table 6.2).

Recovery

Recovery refers to the extraction efficiency of an analytical process, reported as a percentage of the known amount of an analyte carried through from the sample extraction and processing steps of the method. The recovery of the analyte should be optimized to ensure that the extraction is efficient and reproducible. Recovery need not be 100%, but the extent of the recovery of an analyte and of the internal standards should be consistent and reproducible.

The validation guidelines for medicines did not report on specific criteria for the recovery, while for pesticides the criteria for recovery were concentration dependent. It should range between 60 -120% and 70 – 110% for substance concentrations ranging between >1 µg/kg ≤ 0.01 mg/kg and >1 mg/kg (see Table 6.2).

Sensitivity

Sensitivity is defined as the lowest analyte concentration in the matrix that can be measured with acceptable accuracy and precision.

In drug analyses, the lowest non-zero standard on the calibration curve defines the sensitivity (i.e. lower limit of quantitation; LLOQ). The following criteria are set: the accuracy of the lowest non-zero standard should be ± 20% of nominal concentration (from ≥ five replicates in at least three runs), and the precision should be ± 20% coefficient of variation (CV) (from ≥ five replicates in at least three runs).

For pesticide analyses, the limit of quantification (LOQ) is the lowest amount of the analyte in the sample that can be quantitatively determined with defined precision under the stated experimental conditions. A reference standard should be measured with between 6 and 10 replicates. The LOQ is the average response + 10 × SD.

Stability

Stability is a measure of the intactness an analyte (lack of degradation) in a given matrix under specific storage and use conditions relative to the starting material for given time intervals.

Tests to determine the stability of the test substance is included in the validation methods for drugs but is not mentioned in the validation guidelines for pesticides. It should be performed at different concentrations and for different stages of the analytical method such as autosampler stability, extract (or processed sample) stability, freeze-thaw stability and long-term stability.

Criteria for the stability of the test substance is that concentrations in the sample after the applied storage conditions at each concentration level should be within ±15% of the nominal concentration (see Table 5.2).

7. Sample handling and extraction

The direct analysis of analytes in complex matrices such as water, soil, sediment and fish tissues is difficult because they may be present at low concentrations or be associated with the sample matrix. In order to analyse such samples, preparation, clean up and concentration steps may be required. This can include filtration, pH adjustment, extraction, clean-up and pre-concentration procedures to optimise the identification and quantification of analytes.

Extraction from the matrix is a crucial step in the analytical process and often more challenging than the subsequent chromatography and detection step. A sample preparation and clean up method should be selective enough to isolate the target compounds from matrix components. The test substance and its degradation products/metabolites can have very different properties, and therefore may require different extraction methods, increasing the complexity of the task. Issues such as efficiency and selectivity of the method for different components, potential for artefact formation, selection of appropriate internal standards and method validity again require careful consideration. The properties of expected degradation products/metabolites can be predicted, and this can therefore assist in selecting appropriate extraction methods.

The following section discusses the application of different sample preparation techniques such as solid phase extraction, solid phase microextraction, microwave assisted extraction, liquid-liquid extraction (LLE), liquid phase microextraction, pressurised liquid extraction (PLE) and stir bar sorptive extraction (SBME) method which, among others, have been used for preconcentration and extraction in different matrices.

7.1 Sampling

Poor sampling and analysis of non-representative samples is generally a dominant source of error leading to erroneous results and wrong conclusions. Therefore, collection of representative samples using proper sampling strategy that fits the goal of the study is essential (Petrovic, (2014).

7.1.1 Soils and Sediments

The dosing and adequate mixing of soils remains a major challenge due to their heterogeneity and polydisperse particle distribution. Only in the gas or liquid phase is homogeneous mixing easily achieved. Depending on the selected analytical determination, there might be differences from one subsample to the next due to heterogeneity of the sample. Therefore, it is recommended to analyse multiple subsamples (Nießner and Schäffer, 2017 p19). This is also very important consideration when selecting the method of spiking of soil/sediment in environmental fate studies.

The first few centimetres of the soil layer constitute the most active layer. The highest residues are normally found in the organo-mineral fraction of soil (Nießner and Schäffer, 2017 p55). The binding of the chemicals soils and sediments protect against microbial degradation.

7.1.2 Sampling of the aqueous phase

The difficulties in generating and maintaining a homogenous test solution with poorly soluble substances (see Section 10.3.1) may be compounded by the challenges in obtaining representative samples of the solution. Poorly soluble liquids may form heterogeneous aqueous solutions characterised by spatial gradients (stratification) or the formation of emulsions (droplets of undissolved test substance) leading to variation in measured concentrations. This variation in concentrations can be investigated by taking triplicate samples (or more) at the top, middle and bottom of the solution. Whereas in a degradation simulation study, variation in the dispersion of test substance may provide useful information on the behaviour of the test substance in the environment, in an aqueous bioaccumulation study the results of sampling of the exposure media should ideally be tightly distributed without any outliers and be devoid of evidence of stratification from the scatter of results from the above heterogeneity test.

Homogeneity of the sample should be determined by triplicate analysis by the chosen analytical method. Poorly water soluble substances can be quickly sorbed to solids and (if present) particulate matter. Many substances are also associated with temporally formed gel phases, either via incorporation or sorption. Aeration should be minimised in OECD 308 as this induces gel phase formation in the inoculated flasks and may also disturb the equilibrated layers of the upper portion of the sediment. It should however be sufficient to maintain aerobic conditions (if relevant) and allow formation of an oxic layer in the sediment.

7.1.3 Passive Sampling

Passive sampling techniques (also referred to as 'equilibrium sampling') are frequently deployed in environmental monitoring campaigns, providing time-weighted-average, equilibrium concentrations across the deployment period.

Two types of *in situ* passive integrative samplers used for ultra-trace (i.e. <1 ppt), and trace-level analysis (< 1ppb). They typically measure the freely dissolved concentrations of pollutants as a time-weighted average over deployment periods ranging from weeks to months (Alvarez, 2010) so produce information on their bioconcentration potential. Both devices consist of a receiving phase (sorbent or lipid) enclosed in a diffusion membrane. The accumulated analytes are extracted from these samplers in the laboratory and measured using suitable techniques. Mayer *et al*. (2014) and Ghosh *et al*. (2014) describe more recent developments with other sampling phases such as polydimethylsioxanes (PDMS), low density polyethylene (LDPE) and polyoxymethylene (POM) and sampling devices (sheets) or micro-extraction fibres (also see 7.3.2). Sampling kinetics and equilibration times differ for each sampler and depends on diffusion coefficients and size and volume of the sampler (Mayer *et al*. 2014). A protocol for the selection and application of passive samplers was recently published (Jonker *et al*. 2020).

Whilst the use of passive sampling in environmental fate laboratory studies is not commonplace, OECD 23 makes reference to the potential use of passive sampling in aqueous ecotoxicology studies, for example in measuring the freely dissolved concentrations of components of a UVCB prepared as a WAF and there is the potential for similar applications in environmental fate studies. However, OECD 23 notes that the registrant should consult the regulatory agency as to the acceptance of this approach initially, and a similar approach to assessing the acceptability of passive sampling in environmental fate studies may also be necessary prior to attempting a study.

Adolfsson-Erici *et al.* (2012) report the use of passive samplers in aqueous bioconcentration studies similar to the OECD 305 guideline. They investigated the potential for simultaneously measuring BCF_s of multiple chemicals in one experiment and deployed passive sampling to monitor the concentrations of each chemical in water. Passive samplers were deployed on four occasions during the uptake period of the study (on each occasion, the sampler was submerged in the outflow region of the aquaria for 3 hours, however the authors report that in preliminary experiments equilibrium concentrations in the passive samplers were not achieved after 20 hours). Confounding factors in the study make it difficult to draw conclusions on the suitability of passive sampling of the water phase in aqueous bioconcentration studies.

The use of passive sampling of hydrophobic organic compounds in homogenised fish tissue

(Jahnke *et al.* 2009, 2011), and in soil and sediment samples (Maenpää *et al.* 2011, Parkerton *et al.* 2014, Tcaciuc *et al.* 2018) are also reported.

However, the time taken to achieve equilibrium concentrations using this approach is frequently reported to be detrimental (Tcaciuc *et al.,* 2018), particularly with regard to fish tissue where degradation of the tissue may occur before equilibrium concentrations are reached. The study by Tcaciuc *et al.* (2018) further concluded that there may be potential for use of passive samplers in investigation of *in situ* degradation rates and pathways in sediment systems. Further work in this area could be beneficial.

Table 7.1: Types of passive sampling devices. (Alvarez, 2010).

PASSIVE SAMPLE	APPLICABILITY	EXAMPLE ANALYTES
SemiPermeable Membrane Device (SPDM)	A wide range of hydrophobic (non-polar) organic compounds with log K_{ow}) >3.	• Chlorinated pesticides. • Dioxins. • Furans. • Phthalates. (Net *et al*., 2015) • Polybrominated diphenyl ethers (PBDEs). • Polychlorinated biphenyls (PCBs). • Polycyclic aromatic hydrocarbons (PAHs).
Polar Organic Chemical Integrative Sampler (POCIS)	Hydrophilic (polar) organic compounds with log K_{ow} <3.	• Degradation products. • Illicit drugs. • Metabolites. • Most pharmaceuticals. • Phosphate flame retardants. • Polar pesticides. • Surfactants.

The limitations apply to all types of passive samplers (Albaseer *et al*., 2011).

- Passive samplers are designed to be long-term (weeks to months) integrative samplers.
- No benefit if the sample period is less than one week. Solid-phase microextraction device (SPME) are better suited for short-term samplings.
- Inexperience of analytical laboratories using passive samplers. Passive sampler extracts are often not as difficult to work with as samples of other environmental matrices and are compatible with common methods the laboratory may have in place.
- Passive sampling for environmental monitoring has only recently been accepted as a suitable method by the regulatory community.

7.2 Sample handling

Albaseer *et al.* (2011) showed that the sample procedures had a large influence upon the stability of synthetic pyrethroids with photodegradation being the dominant process. Hladik *et al.* (2009) also made specific recommendations on sampling, storage and sample preparation of synthetic pyrethroids from environmental samples for analysis. Table 7.2 summarises the recommended preventative measures from these two reviews. These would not be suitable for all substances but are a good guide.

Table 7.2: Processes affecting stability of organic analytes and recommended preventative measures Based on Albaseer *et al*. (2011), Hladik *et al*. (2009).

STAGE	PROCESSES AFFECTING STABILITY	PREVENTIVE MEASURES
Sampling	Adsorption onto sample containers	• Use Pyrex®/borosilicate or PTFE bottles. • Collect samples in smallest viable containers (volume-to-contact-area ratio should minimised) • Samples should be agitated vigorously for at least 1 minute immediately before being transferred to another container.
	Biodegradation	• Extract the sample as soon as possible. • Keep the sample refrigerated in the dark until analysis.
	Photodegradation	• Keep sample in the dark after sampling. Possibly use brown glass and / or cover in aluminium foil.
Sample storage	Adsorption onto storage container	• Use Pyrex®/borosilicate or PTFE bottles.
	Biodegradation	• Extract the samples as soon as possible. Sediment and soil samples can be frozen for a few months (prior to extraction), with insignificant or no changes in analytical integrity. Water samples should be analysed within 3 days of collection.
	Photodegradation	• Keep sample in the dark.
	Hydrolysis	• Assess whether a change of pH to an aqueous solution, or LLE to an appropriate solvent will prevent this happening (also prevents isomerisation in some cases)
	Racemisation	• Acidify to pH 2-4.
Extraction	Adsorption onto extraction devices	• Use PTFE containers • If filtration is required, then use those that have been assessed.
	Contamination	• Use only clean devices

Biological activity in water samples is normally reduced by storing samples at low temperatures (below 4°C) and keeping them in the dark until they are processed, acidifying to pH 2-4 or by adding a preservative (e.g., copper sulphate, formaldehyde) (Petrovich, 2014). These storage conditions should be assessed for individual substances as they could impact the substance.

Filtration can have a significant impact on the outcome of fate and behaviour studies. For example, Petrovich (2014) stated that a high proportion of pharmaceuticals are bound to filterable particulates and a clear distinction should be made between whole-water sample and dissolved fraction when reporting the results of analysis.

Redrup *et al*. (2016) made the following recommendations for the entire life span of the sample:

- Sampling procedures should be described in the protocol or within the laboratory manual. This information should include the volume of the sample to be collected, the required anticoagulant, light sensitivity, collection and storage containers, and labelling with a unique identifier.

- The correct procedures for processing and then storing the samples after collection at the clinical/non-clinical testing site and during shipment are also very important to ensure the analyte(s) stability and should be documented.
- Chain of custody for the samples must be maintained throughout the complete life span of each sample. This is typically maintained via paper and electronic data systems, including Laboratory Information Management Systems (LIMS) where available.
- Pre- and post-analysis storage location and conditions must also be clearly defined at the analytical laboratory. The storage temperature of the samples must be traceable and controlled by monitoring and warning alerts. The team suggests moving away from using temperatures and to adopt standard terminology of "room temperature," "refrigerator," "freezer," and "ultra-freezer" that have defined and industry-wide accepted temperature ranges.
- At the end of the study, documentation of sample disposal is required.

7.2.1 Handling of fish tissue samples

For an aqueous or dietary bioaccumulation study, the OECD TG 305 states that after sampling and euthanasia, fish should be rinsed with water and blotted dry; no further processes should be performed. Further sample preparation may be performed, e.g. where specified tissues or organs (muscle, liver, fat etc) are intended for analysis if the fish is large enough, or if the fish is to be divided into edible/non-edible fractions.

7.3 Extraction from water

Traditional extraction methods such as liquid-liquid extraction have been largely replaced by solid phase extraction. Over the last decade there has been more emphasis in the literature on microextraction methods. These drastically reduce or eliminate the use of solvents, especially chloroalkanes, that are toxic and hence expensive to dispose of. These techniques are included in a review by Petrovic and Barcelo (2004), Petrovich (2014), Dimpe and Nomngongo (2016).

The extraction techniques in Figure 7.1 are listed hierarchically with the orange boxes indicating the present author's preferred methods, based on the review presented in Section 7.3.

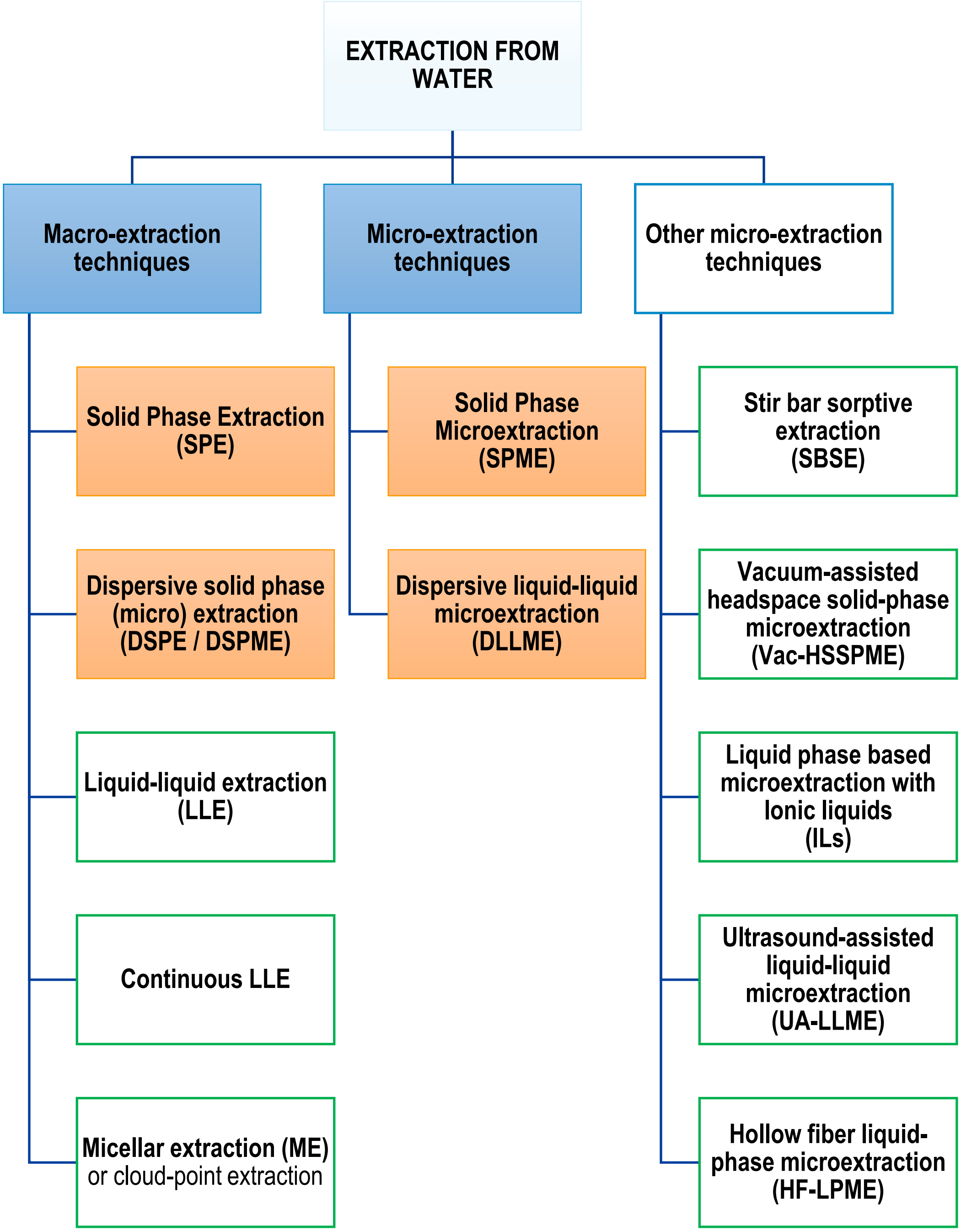

Figure 7.1: Choosing a technique for extraction from water.

7.3.1 Macro-extraction methods from water

A comparison of sample preparation techniques for the conventional macro-extraction and clean-up of aqueous samples is presented in Table 7.3.

Table 7.3: Overview of macro-extraction methods from water.

METHOD	COMMENTS	ADVANTAGE	DISADVANTAGES
Liquid-liquid extraction (LLE)	Uses the immiscibility properties of organic solvents to partition target analytes from raw extract to the extractant. The most common solvents are chloroform, dichloromethane, ethyl acetate, hexane, isooctane, toluene, and methylcyclohexane. Addition of salt (sodium chloride) can increase the partition and increase the efficiency of extraction. **Standard EPA Methods** US EPA (1996) *Method* 3510C: Separatory Funnel Liquid-Liquid Extraction	• Non-selective. • Largely replaced by SPE. • Use of an extractant immiscible with the sample. • Diluted extracts.	• Non selective. • Cannot be automated. • Increasingly expensive in solvent and disposal. • Slow. • Large solvent volume. • Suited for hydrophobic substances, therefore unsuitable for metabolites that are increasingly polar or ionic.
Continuous liquid-liquid extraction (Continuous LLE)	The extracting solvent is vaporised and condensed in a cooling column and the droplets are continuously passing through the aqueous phase. Requires the analyte to have low volatility. **Standard EPA Methods** US EPA (1994) *Method 3520C* for the Continuous Liquid-Liquid Extraction of water-insoluble and slightly soluble organic compounds.	• Higher efficiency. • Smaller extraction volumes used. • Similar in concept to Soxhlet Extraction of solids.	• Cannot be used for volatile and semi-volatile analytes unless fraction traps are employed
Solid phase extraction (SPE)	Solid phase extraction was first developed in the 1980s and since then, it has proven to be the most powerful tool for the isolation and purification of	• Wide selection of solid phases. • Simplicity. • Flexibility.	• Moderate amount of organic solvents but much lower than LLE.

METHOD	COMMENTS	ADVANTAGE	DISADVANTAGES
	target analysis. **Standard EPA Methods** US EPA (2007) Test *3535A: Solid-Phase Extraction (SPE)* **Examples** Lesueur *et al.* (2008) **Quick, Easy, Cheap, Effective, Rugged and Safe** (QuEChERS) is a standardised extraction procedure has been widely adopted for pesticide residues and increasingly for pharmaceuticals and their metabolites in the environment. **Review** Bruzzoniti *et al.*(2014) reviewed the use of QuEChERS in the determination of pesticides and other organic residues in environmental matrices. **Examples of QuEChERS** • Willkowska and Biziuk (2011) determined pesticide residues in food matrices using the QuEChERS methodology. • Rashid *et al.*(2010) used QuEChERS to determine organochlorine pesticides in soil using gas chromatography-tandem mass spectrometry. • Garcia Pinto *et al.*(2010) used QuEChERS to extract chlorinated compounds from soil samples. • Antonio Padilla·Sanchez *et al*. (2010) to simultaneously extract chlorophenols, alkylphenols, nitrophenols and cresols from soils.	• High selectivity by use of different sorbents. • Can be automated. • Rapidity. • High enrichment factors (concentration). • Absence of emulsion. • Better selectivity and sensitivity for environmentally relevant residue concentrations. • Combines extraction, clean-up, solvent exchange and concentration of analytes prior to analysis. • Normally at least a 10-fold concentration factor can be obtained. • SPE cartridges with different phases can be combined in series to maximise loading.	• Disposable cartridges, and discs. • One sorbent may not provide sufficient recovery of all relevant compounds (for example very polar, conjugated metabolites and/or transformation products). This results in some compounds would not be detected. [Miller *et al*., 2018: 132] • Continuous method development required for retention of all transformation products
Dispersive solid phase (micro)	Single extraction of the sample using a solid adsorbent and followed by	• Full interaction of the sorbent and the sorbent particles.	• Unable to change solvent between the extraction and

METHOD	COMMENTS	ADVANTAGE	DISADVANTAGES
extraction (DSPE / DSPME)	a clean-up process. Sorbents such as bonded silica, activated carbon, and primary secondary amine are directly dispersed into the sample solution instead of being packed in SPE columns. Then filtration or centrifugation. **Standard EPA Methods** None	• A great capacity per amount of sorbent. • Avoids the blockage of cartridges in traditional SPE. • Rapidity (no conditioning step). • Simplicity. • High enrichment factors. • Cost effective due to the reduction in sample size, sorbent amount, solvents, and waste.	preconcentration steps.
Micellar extraction (ME) / cloud-point extraction	This is extraction without solvents where less than 1% w/w Triton X-100 or Genapol 80 is used. The hydrophobic (low K_{ow}) analytes separate into micellular phase (MP). To undergo GC, it must be back extracted for GC and this has been performed for pesticides. **Standard EPA Methods** None **Review** Hinze and Pramauro (1995). **Examples** • Determination of or polychlorinated biphenyls (PCBs) in water. • Direct measurement within MP reported by synchronous luminescence of PAHs. • Back extraction before the determination of the pesticide, naporpamide in water.	• Of limited use.	• Requires to be back extracted for GC.

7.3.1.1 Sorbents for Solid Phase Extraction

The selectivity of solid phase extraction depends upon the type of support that is used. A wide range of SPE sorbents are available, these are summarised in Table 7.4.

Table 7.4: SPE sorbents.

SORBENT TYPE	SORBENT	CHARACTERISTICS
Polar sorbent	• Normal phase silica. • Florisil^{TM} • Cyano-, amino-, diol-modified silica or alumina;	• Polar compounds
Moderately polar sorbent	Reversed-phase hydrophobic-hydrophilic-balanced (HLB) polymeric sorbents. • Polyamide, • Poly-vinylpyrrolidone-divinylbenzene (DVB)], • Methacrylate DVB • Hydroxylated polystyrene DVB	• Stable in wide pH ranges, • No silanol interactions • No effect on sorbent drying. • Able to retain acidic, basic, and neutral analytes
Moderately polar sorbent	• Phenyl-, ethyl-, cyclohexyl-modified functionalised silica.	• Moderately polar substances.
Non-polar sorbent of alkyl-modified silica (reverse phase)	• C-18 non-polar phase • C-8 non-polar phase	• Non-polar substances.
Ion exchange phases		Ionic compounds.
Molecular imprinted sorbents (MIP)	Based on the principle of polymerisation of monomers in the presence of a template molecule such as a pesticide or pharmaceutical.	**Reviewed** Kubo *et al*. (2014) Maser and Hage (2010)
Immunoaffinity columns	The stationary phase consists of an antibody that is selective towards the analyte (antigen). Kubo *et al.* (2014)	• Selective to a specific analyte.
Polyoxymethylene solid phase extraction (POM-SPE)		Used in the determination of bioavailable concentrations
Novel Sorbents	These include carbon-based sorbents (graphitic, nanotubes, graphene), metal nanoparticles, metal-organic frameworks, and mesoporous hybrid materials. • Expensive and most are still experimental. • Magnetic nanoparticles have low stability in acidic media, low dispersibility in different sample matrices and its low sensitivity towards analytes.	• Increasing the detectability, enrichment efficiency, and selectivity. • Large surface area and permanent nanoscale porosity.

7.3.2 Microextraction

The advantages of microextraction-techniques are that they are considered to be efficient, environmentally friendly, faster than classical sample preparation techniques, use low solvent and sample volumes and permit automated or semi-automated procedures.

Carasek *et al*. (2018) provided an overview of the basic principles of the following microextraction techniques.

- Solid-phase microextraction (SPME),
- Stir bar sorptive extraction (SBSE),
- Bar adsorptive microextraction (BAμE),
- Rotating disk sorptive extraction (RDSE),
- Micro solid-phase extraction (μ-SPE)
- Liquid-phase microextraction (LPME).

Souza-Silva *et al*. (2015) emphasised the development of new microextraction devices, such as cold fibre and thin films to address quantitation in complex environmental matrices and calibration strategies for SPME methods.

Demeestere *et al*. (2007) reviewed the sample extraction methods for analysis of volatile organic compounds (VOC), from air and water matrices. These included solid-phase microextraction (SPME), single-drop microextraction (SDME), and liquid phase microextraction (LPME),

Holopainen *et al*. (2012) reviewed the current literature on the partition-based methods sample-extraction methods most feasible for aqueous samples prior to ion-mobility analysis. These included solid-phase microextraction (SPME), stir-bar sorptive extraction (SBSE), single-drop microextraction (SDME), hollow-fibre liquid-phase microextraction (HF-LPME).

7.3.2.1 Solid-phase microextraction (SPME)

Solid-phase microextraction (SPME) is a simple and effective adsorption and desorption technique that eliminates the need for solvents or complicated apparatus, for concentrating volatile or non-volatile compounds in liquid samples or headspace (see Table 7.5 for advantages and disadvantages). It is a solvent-free technique particularly useful for extraction in dilute systems, in which a polymer coated fibre is exposed to the analyte of interest in gas or liquid form. In their review, de Fátima Alpendurada (2000) stated that an analyst can ensure highly consistent, quantifiable results for low concentration analytes, providing that the polarity and thickness of the coating on the fibre is controlled, the sampling time is consistent, and the extraction parameters are appropriate. SPME is by far the most common of the microextraction techniques. At present, 27 variations of fibre coating and size are available. Among the newest are a fibre assembly with a dual coating of divinylbenzene and Carboxen suspended in poly(dimethylsiloxane), and a series of 23-gauge fibres intended for specific septum-less injection system.

Böhm *et al*. (2017) used automated solid-phase microextraction (SPME) as an alternative approach to conventional liquid-liquid extraction (LLE) for the water analysis in fish bioconcentration studies. The analytes were three hydrophobic organic chemicals (HOCs) with log K_{ow} 5.5–7.8) from flow-through studies with rainbow trout (*Oncorhynchus mykiss*). They found that the total concentrations extracted by SPME combined with internal standards and LLE were the same. They also verified that simultaneous extraction of total and freely dissolved HOC concentrations by SPME was possible.

The freely dissolved concentrations may allow the assessment of sorption and bioavailability of HOCs in bioconcentration studies and therefore their potential impact on resulting bioconcentration factors (BCF$_s$). Reduction in freely dissolved water concentrations may result in an underestimation of BCF$_s$ if they are calculated based on total water concentrations. For polychlorinated biphenyl (PCB), a significant increase in BCF value was observed when freely dissolved concentrations were taken into account. However, log BCF values calculated based on freely dissolved concentrations did not correlate linearly with log K_{ow} values above 5 to 6. Therefore, sorption to organic matter had occurred.

Whilst methods for the extraction of test compound are not prescribed in the guidance, OECD TG 305 mentions the use of SPME to obtain information on the proportions of bound and freely dissolved test materials, specifically when testing highly hydrophobic compounds. The use of SPME as an alternative analytical method for the determination of aqueous test substance concentrations within aqueous bioconcentration studies is further discussed in the Guidance Document on Aspects of OECD TG 305 (OECD, 2017).

Table 7.5: Solid phase microextraction (SPME)

METHOD	COMMENTS	ADVANTAGE	DISADVANTAGES
Solid phase microextraction (SPME)	Reliable and effective alternative extraction method to SPE. The extraction and pre-concentration of the analyte occur by a thin layer of a suitable polymer at the surface of a fused silica fibre. Quantification depends upon the exposure time, partition coefficient of the analyte, temperature and length of fibre. Three modes of extraction: • Headspace extraction (HS-SPME). • Direct-immersion extraction (DI-SPME) • Membrane protected SPME **Standard EPA Methods** US EPA, (2014) *Method 3511: Organic Compounds such as PAHs in Water by Microextraction* **Reviews** • Torres Padron *et al.*(2014) reviewed determination or organic micropollutants in environmental water samples. • Vas and Vekey (2004) reviewed the use of SPME as a clean-up before MS. • de Fátima Alpendurada (2000) reviewed the use of SPME in environmental analysis.	• Solvent free extraction method. • Wide choice of fibres. • Applicable to volatile compounds. • Small sample volumes. • Analyte concentration from liquid, gaseous and solid samples. • Easily automated to allow high-throughput analysis.	The main limitation is related to polymeric extractant phase and the desorption process. • Quantification can only be reliably performed with isotopically labelled samples and MS analysis.

METHOD	COMMENTS	ADVANTAGE	DISADVANTAGES
	• Aulakh (2005) reviewed solid phase micro extraction-high performance liquid chromatography (SPME·HPLC) for the analysis of pesticides • Moreda·Pineiro and Moreda·Pineiro (2015) looked at the recent advances in combining microextraction techniques for sample pre-treatment.		

7.3.2.2 *Other Microextraction techniques*

Whilst solid-phase microextraction is the more commonly used microextraction technique, the literature review identified a number of other microextraction techniques used in extraction from aqueous matrices. These are summarised in Table 7.6. The EPA do not currently have Standard Methods for these techniques.

Table 7.6: Other microextraction techniques.

METHOD	COMMENTS	ADVANTAGE	DISADVANTAGES
Dispersive liquid-liquid microextraction (DLLME)	Uses a tertiary solvent system in which small volumes of extracting solvent is dispersed by the action of a second solvent. **Standard EPA Methods** None **Reviews** Zgoła-Grześkowiak, and Grześkowiak (2011) **Examples** • Rezaee *et al*. (2006) determined organic compounds in water. • Berijani *et al*. (2006) extraction of organophosphorus pesticides in water and then GC-NPD.	• Very low solvent use. • Simple operation • Low cost • Low solvent consumption • Speed • The potential for automation • High enrichment factors. • Simple method development. • Can be used with simultaneous derivatisation of analytes • Can be connected to other sample-preparation techniques. • Use of low toxic hydrocarbons, alcohols and ionic	Extraction solvents limited to solvents of higher density than water

METHOD	COMMENTS	ADVANTAGE	DISADVANTAGES
		liquids instead of toxic chlorinated solvents. • Applications including pesticides, pharmaceuticals, phenols and other compounds.	
Ultrasound-assisted liquid-liquid microextraction (UA-LLME)	Uses ultrasound radiation for the acceleration of mass transfer process. **Standard EPA Methods** None **Example** Hou *et al.* (2014) used 1-dodecanol (extraction) and ethanol (dispersant) based upon the solidification of floating organic droplets in determining pyrethroid pesticides in tea. 100-fold enrichment and 92-100% recoveries.	• Very low solvent use. • Simple operation • Low cost • Low solvent consumption • Speed • The potential for automation • High enrichment factors.	• Extraction solvents limited to solvents of higher density than water. • Chlorinated solvents.
Hollow fibre liquid-phase microextraction (HF-LPME),	Similar to SPME but the solvent is placed inside the channel of the hollow fibre (HF) and the pores of its walls and an aqueous solution (acceptor) is then injected into the channel. Analytes are usually extracted through the organic phase inside the pores of the hollow fibre and straight into an acceptor solution. **Standard EPA Methods** None	• Low cost. • Great reduction in the volumetric ratio of the acceptor and the sample phases • Sample clean-up. • High enrichment (concentration) factor.	• Slow • Lacks commercially available equipment • The membranes in LPME acts as a barrier between the sample phase and the acceptor phase which increases the extraction time and reduces the extraction rate. • Air bubbles that are created on the surface of the HF decreases the reproducibility of the extraction and also reduces the transport rate.
Liquid phase based microextraction with Ionic liquids (ILs)	Ionic liquids (ILs) are ionic compounds made up of discrete cations and anions often liquid at or below 100°C. **Standard EPA Methods** None	• Miscible with water. • High solvation interaction. • Negligible vapour pressure. • High thermal stability. • Good conductivity. • Tunable viscosity	• Complex synthesis and hence costly. • Difficult to purify. • pyridinium or imidazolium based ionic liquids are toxic hence not “Green.”
Stir bar sorptive extraction (SBSE)	A solvent-free extraction that is usually performed on a 1.5 cm long glass magnetic stirrer coated with polydimethylsiloxane (PDMS) immersed in the	• Solvent free. • used for the extraction of polar and non-polar organic compounds from water, slurry,	• The PDMS coating poorly extracts extract polar compounds.

METHOD	COMMENTS	ADVANTAGE	DISADVANTAGES
	liquid phase and continuously stirred for a period of time. **Standard EPA Methods** None **Review** López-López (2019)	food, and biological samples.	
Vacuum-assisted headspace solid-phase microextraction (Vac-HSSPME)	Vacuum-assisted headspace solid-phase microextraction **Review** Zhakupbekova, *et al.* (2019)	• Shorter extraction times than headspace solid-phase microextraction (HSSPME) • Decreases the cross-contamination of samples from the laboratory air. • Simple equipment for the technique. • For volatile compounds analysed by GC.	• Wider application of the technique is limited by the lack of automation. • The analytes need to be volatile organic compounds (VOCs) for gas chromatography.

7.3.3 Extraction of ionic species from water by electro-membrane extraction

Electro-membrane extraction is an important method of extracting ionic species from water that does not involve ion pairing liquid-liquid extraction, chromatography or electrophoresis. The method is described by Pedersen-Bjergaard *et al*. (2017), summarised in Table 7.7.

Table 7.7: Extraction techniques of ionic species from water.

	COMMENTS	ADVANTAGE	DISADVANTAGES
Electromembrane extraction	The charged analytes (such as drug substances) are extracted from an aqueous sample (such as a biological fluid), through a supported liquid membrane (SLM) comprising a water immiscible organic solvent, and into an aqueous acceptor solution. The driving force for the extraction is an electrical potential (dc) applied across the SLM. **Review** Pedersen-Bjergaard *et al.*(2017)	• Cationic and anionic analytes.	

7.4 Extraction from Solid matrices

The extraction from fish, sediment and soils utilise similar techniques and similar issues require consideration; these will therefore be covered together. The extraction techniques in Figure 7.2 are listed hierarchically with the orange boxes indicating the present author's preferred methods, based on the review presented in Section 7.4.

The traditional methods for extraction from solid matrices are Soxhlet extraction and liquid-liquid extraction by agitation (e.g. sonication). Some modern studies have used pressurised liquid extraction. Based on a review of Nießner and Schäffer (2017), Faraday Discussions (2019) on *Challenges in Analysis of Complex Natural Mixtures* and the published literature, several more modern methods have been identified that can be applied in laboratory environmental fate studies. Some of these methods are already being applied, others are less common. An overview of these methods is given in Section 7.4.2.

The US EPA (2007) *SW-846 Test Method 3500C* provides guidance on selecting methods used in quantitative extraction of samples for analysis of semi-volatile or non-volatile organics by GC-MS methods. Most of these rely on a head-space heating prior to separation and must have suitable internal standards.

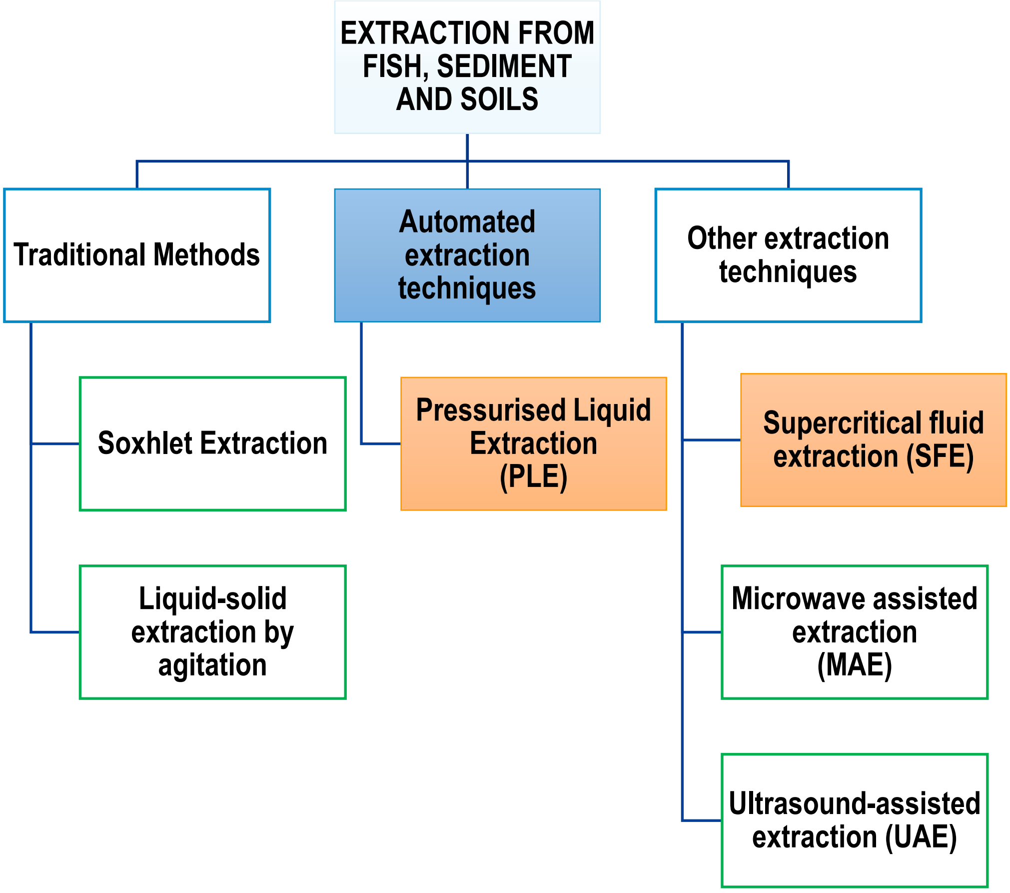

Figure 7.2: Choosing a technique for extraction from solid matrices such as fish, soil and sediment.

7.4.1 Overview of traditional extraction methods

Extraction from solids such as fish (i.e. animal tissue), soils and sediments has been traditionally performed by Soxhlet extraction or Liquid-solid extraction by agitation. A summary of these methods is presented in Table 7.8.

Table 7.8: Traditional method of extraction from solids.

METHOD	COMMENTS	ADVANTAGE	DISADVANTAGES
Soxhlet Extraction	The solid sample is loaded into a Soxhlet thimble of thick filter paper. The solvent is distilled from a reservoir and condensed with a water condenser. **Standard EPA Methods** US EPA (1996) *Method 3540C* for Soxhlet Extraction of non-volatile and semi-volatile organic compounds from solids such as soils, sludges, and wastes. US EPA (1994) *Method 3541* for Automated Soxhlet Extraction of organic analytes from soil, sediment, sludges, and waste solids using a commercially available three stage extraction system.	• Over 150 years, this has been the standard method of extraction from solid samples. • Easy to handle. • Inexpensive equipment. • No filtration required. • High matrix capacity.	• Slow and time consuming (6-48 hours) • Uses a large amount of high purity solvents (e.g. acetone, acetonitrile:water cyclohexane, heptane) up to 200 ml. • High cost of disposal of solvents. • Generates dirty extracts • Cannot be automated. • Due to heating, could cause degradation of the analyte. • Not suitable for semi-volatile and volatile analytes. • Thermal degradation can occur during the long extraction time.
Liquid-solid extraction by agitation	Agitation for 1-6 hours in an orbital or horizontal shaker with suitable solvents. Water:solvent combinations are recommended to keep the pores of soils open. This method is still used to estimate the bioavailable fraction. **Standard EPA Methods** None	• Different solvents or mixed solvents of different polarity can be used to understand the bioavailable fraction.	• Slow and time consuming (6-48 hours) • Uses a large amount of purified solvents up to 200 ml. • High cost of disposal of solvents. • Cannot be automated.

7.4.2 Overview of modern extraction methods

Over the last few decades, a series of other methods have been developed and through suitable modification can be selective and so reduce the background effects. An overview of these methods is presented in Table 7.9. Selection of extraction methods are dependent upon the matrix and the nature of the chemicals to be extracted.

Table 7.9: Overview of modern extraction methods of extraction from solids.

METHOD	COMMENTS	ADVANTAGE	DISADVANTAGES
Pressurised Liquid Extraction (PLE)	Pressure of 7-20 MPa and heating of extraction solvent to 50-200°C above their boiling point. This promotes the analyte solubility, reduces the surface tension and viscosity of the solvent so assisting it to reach areas of matrices easily and enhancing the extraction rate. The pressure increase retains the solvents in the liquid state even at higher temperatures. The main parameters which influence its extraction efficiency are namely the temperature, extraction time, flow rates and addition of modifiers/additives. Also known as Accelerated Solvent Extraction (ASE) – Trademark of Thermo Fischer as Dionex invented the method. Enhanced solvent extraction (ESE) High-Pressure High-Temperature Solvent Extraction (HPHTSE), High-Pressure Solvent extraction (HSE / HPSE). Pressurized Fluid Extraction (PFE), Pressurized Hot Solvent Extraction (PHSE), Pressurized Solvent Extraction (PSE), Subcritical Solvent Extraction (SSE). **EPA standard method** US EPA (2007) *Method 3545A* for the elevated temperature and pressure extraction of water insoluble or slightly water-soluble organic compounds from soils, clays, sediments, sludges, and waste solids. **Reviews** Bjorklund E *et al.* (2006) Dean and Ziong (2000) Ramos *et al.* (2002) Teo *et al*. (2010)	• Simple optimisation procedure so can adapt an existing Soxhlet or sonication procedure. • Fast extraction method (10-20 min). • Low consumption of organic solvents (20-30 ml). • Very selective. • High yields. • No filtration required. • Operating temperatures (50–200°C). • Automated, permitting high throughput and repeatability and ease of use. • Offers a wide range of polarities by changing the temperature and can easily provide class-selective extraction by temperature programming and/or the addition of modifier(s).	• Sample clean-up is still required after extraction. • Expensive specialised equipment. • High extraction temperature might cause decomposition of thermally unstable analytes of interest. • Thermal stability of the analytes needs to be considered. • Continuous method development may be required.
Supercritical fluid extraction (SFE)	This uses supercritical carbon dioxide as the solvent. **EPA standard method** US EPA (1996) *Method 3560* for supercritical fluid extraction (SFE) of the total recoverable	• Fast. • No need for organic solvent and hence extract is very pure.	• Expensive and complex equipment operating at elevated pressures and low temperatures.

METHOD	COMMENTS	ADVANTAGE	DISADVANTAGES
	petroleum hydrocarbons (TRPHs) from soils, sediments, fly ash, solid-phase extraction media, and other solid materials. US EPA (1996) *Method 3561* supercritical fluid extraction (SFE) of PAHs from soils, sediments, fly ash, solid-phase extraction media, and other solid materials US EPA (2007) *Method 3562* for the supercritical fluids for the extraction of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) from soils, sediments, fly ash, solid-phase extraction media, and other solid materials OECD 307 suggests the use of SFE for the analysis of non-extractable residues. **Reviews** Garcia·Rodriguez (2008) Souza Machado (2013) Anitescu (2006) Dean and Ziong (2000)	• Free of heavy metals and inorganic salts. • No chance of polar substances forming polymers. • High yield. • Low operating temperatures (40-80°C).	• CO_2 is highly selective and suitable for non-polar substances, although a small amount of solvent such as methanol can be used to increase its polarity for extraction of aromatic substances.
Microwave assisted extraction (MAE)	MAE uses microwave energy to warm the solvent in contact with the solid matrix to extract the contents from the sample. The influence of the microwave energy is dependent on the nature of the matrix and the solvent used. **EPA Standard Method** US EPA (2007) *Method 3546* for the microwave assisted extraction of water insoluble or slightly water-soluble organic compounds from soils, clays, sediments, sludges, and solid wastes.	• Several extractions can be performed at the same time (up to 12). • High level of automation. • Moderate investment. • Decreased extraction time (10-30 min). • Decreased solvent volume for the extraction of organic analytes from solid samples (10-70 ml). • Higher penetration of chosen solvent into cellular	• High power consumption. • Required further filtration of the extracts. • Solvents must absorb microwaves unless water is present in the matrix. • Non-homogeneous field inside the cavity. • Heating affects only in polar solvents. • Difficult to scale up. • Limited amount of sample (0.5 g) can be processed. • Volatile solvents should be avoided.

METHOD	COMMENTS	ADVANTAGE	DISADVANTAGES
		material, soils and sediments. - Higher extraction rates and lower temperatures.	- Does not permit the addition of solvents or reagents during operation - Lack of selectivity. - Long cooling times after pre-treatment.
Ultrasound-assisted extraction (UAE)	This uses ultrasound to penetrate the solvents in contact with the solid matrix to extract the content from the sample solution. **EPA Standard Method** US EPA (2007) *Method 3550C* for the ultrasound assisted extraction of non-volatile and semi-volatile organic compounds from solids such as soils, sludges, and wastes. **Reviews** Capelo and Mota (2005) Harrison *et al.* (2013) Szreniawa-Sztajnert *et al.* (2013)	- Decreased extraction time and solvent consumption. - Higher penetration of chosen solvent into cellular material, soils and sediments.	- High power consumption. - Difficult to scale up. - Required further filtration of the extracts.

7.5 Concentration techniques

Concentration methods are often required after traditional methods of extraction from water or solid matrices as large volumes of solvent are used. Techniques for concentration are summarised in Table 7.10. The stability of the sample during concentration is an important consideration. Concentration techniques have not received attention in recent literature; concentration technique may be specified in the methods but is not discussed further. Newer extraction methods, such as solid phase extraction, use lower volumes of solvent and do not require a separate concentration step.

Table 7.10: Concentration techniques.

CONCENTRATION TECHNIQUE	COMMENTS
Rotary evaporation	Subsequent purification is normally essential unless performed prior.
Centrifugal evaporation	Even solvents such as DMSO (bp 189°C) can be evaporated at 50°C. Solvents are condensed in a cold trap.
Freeze drying / lyophilization / cryodesiccation	Removal of solvent (normally water) in the frozen state under reduced pressure. Can lead to increased bound substance that cannot be re-solubilised.
Vacuum concentration	This is without freezing the organic solvent
Blow-down evaporation	Evaporation is achieved by directing a stream of inert gas (commonly nitrogen) onto the sample.

8. Isotopic Labelling and Quantification

Isotopic labelling can greatly facilitate the detection and quantification of compounds in environmental fate studies. The choice is between radiolabelling and stable isotope enrichment. The ease of experimentation in these studies is much enhanced by isotope-labelling, and particularly by the use of radioisotope-labelling, however the use of isotopically labelled materials greatly increases the cost of the study and may not be feasible in all instances. Advantages of using radioisotope-labelling include the quantification of non-extractable residues, thus enabling the calculation of a mass balance, and the identification and quantification of metabolites when used in conjunction with suitable chromatographic separation techniques. Additionally, the very low LOQs of radiolabelled substances means that, particularly in the case of bioaccumulation studies, they may be assessed at low environmentally relevant concentrations. This last point is particularly important with substances of very low water solubility, as they may be tested at concentrations of < 20% solubility without problems of quantification.

8.1 Synthesis of isotopically labelled materials

8.1.1 Stable Isotopes

The usual stable isotopes used in environmental studies are ^{2}H (deuterium, isotopic natural abundance 0.02%), ^{13}C (isotopic natural abundance 1.1%) and ^{15}N (isotopic natural abundance ca. 0.4%). The main advantages in using stable isotope enrichment as a label is that it can be used in situations where radiolabelling would constitute a hazard, such as in human toxicokinetic studies. Substances labelled with stable isotopes are usually separated by HPLC and quantified by mass spectrometry. MS/MS is a commonly used method, where compounds are separated by reverse-phase HPLC, directly ionised via electrospray ionisation, and introduced into a mass spectrometer (Kito and Ito, 2008). The same authors also discuss quantitative techniques based on stable isotope labelling techniques in the same article. Stable isotope-enriched metabolites can also be quantified by automated tandem mass spectrometry (MS/MS) (Zang *et al*. 2003). GC-MS/MS is used for more volatile substances such as the plant terpene methyl eugenol, which was (D3) deuterium labelled. This gave a LOD of 50 µg/kg for Methyl-eugenol in solid food samples and 1 µg/l for liquids, which is of the same order as that obtained with radiolabelled samples (see Table 6.1 below). The kinetic isotope effect, whereby the reaction rate of a chemical reaction is changed when one of the atoms in the reactants is replaced by one of its isotopes, is usually sufficiently small for ^{15}N and ^{13}C replacement in most substances for it to be ignored, although it can be appreciable for deuterium. Stable isotope enriched intermediates can be obtained from 96% to 99% atom enrichment.

Synthesis of stable isotope enriched test substances encompasses several of the problems and constraints encountered with radiolabelling (see Section 8.1.3). However, stable isotope labelled chemical intermediates are generally less expensive than their radiolabelled counterparts and are not subject to additional regulations such as the UK Radioactive Substances Act. Deuterium labelled compounds may also be made by hydrogen-deuterium exchange methods analogous to those used for tritium (see Section 8.1.4).

8.1.2 Radioactive Isotopes

Synthesis of a substance using radioactive isotopes as the building blocks is the most common method of producing a radiolabelled substance. Radiolabelled ^{14}C is the most prevalent, followed by ^{3}H (tritium). This is principally because most substances that are assessed for environmental fate and behaviour studies, which inevitably contain carbon and usually hydrogen. Other isotope options include ^{35}S, and ^{32}P or ^{33}P, which are mainly utilised for human health and pharmaceutical assessment. ^{32}P and ^{33}P, are both β- emmiters of considerably higher energy than either ^{35}S or ^{14}C, so they can be counted with very high efficiency (ca. 99.5%) using Cherenkov counting in a normal liquid scintillation counter. However, they have fairly short half-lives (14.2 days and 25.5 days respectively) that do not lend themselves to the time scales of environmental fate studies. Another radioactive isotope that is often used as a tracer is ^{125}I, which is used to label high molecular weight biological substances, e.g. peptides and proteins but the size of the iodine atom and the resultant steric isotope effect precludes its use as a label for small molecules.

The separation and detection techniques applicable to stable isotope labelling and radiolabelling are listed in Table 8.1, along with a comparison of the advantages of each technique.

Table 8.1: Comparison of Stable and Radioactive Labelling

STABLE/ RADIOACTIVE	NUCLIDES USED	CHROMATOGRAPHY METHODS	DETECTION AND QUANTIFICATION METHODS	ADVANTAGES
Stable Isotope	^{2}H, ^{13}C, ^{15}N,	HPLC, GC*	MS, electrospray MS/MS	Not subject to radioactive substances regulations. Less expensive than radiolabelled methods. Fairly low LOQ
Radioactive Isotope	^{3}H, ^{14}C, ^{32}P, ^{33}P, ^{35}S	HPLC, UHPLC	Many, see Tables 9.2 and 9.3	Quantification by radio-counting is simple. Self-calibrating quantification. Large choice of quantification methods. Very low LOQ (depends on method).

* GC methods can be used to separate small stable isotope-substituted compounds, particularly ^{1}H/^{2}H, but also ^{12}C/^{13}C (Cartoni *et al*. 1967, van Hook 1969)

8.1.2.1 Radioactive Decay

Both ^{14}C and ^{3}H are β^{-}-emitters. β^{-} decay, involves a proton being transformed to a neutron via the generation and loss of an electron and a neutrino. The respective stable daughter nuclei formed from the decay of ^{14}C and ^{3}H are ^{14}N and ^{3}He. The properties of tritium and ^{14}C are important for their uses in fate and behaviour studies are shown in Table 8.2 below.

Table 8.2: Radionuclide half-life and other characteristics.

RADIONUCLIDE	T½ (Y)	B^{-} ENERGY (KEV)	COUNTING BY END WINDOW GM TUBE POSSIBLE	LSC COUNTING EFFICIENCY (MAX) %	TYPICAL (SPECIFIC ACTIVITY PER MOL)	RADIOLYSIS	ISOTOPE EXCHANGE POSSIBLE
^{3}H	12.3	18.6	No	65	mCi	High	Yes
^{14}C	5.740	156	Yes	97	µCi	Low	No

8.1.2.2 Units of Radioactivity

Radioactive flux was originally defined as the quantity of radioactive particles per second emitted by one gram of Radium and named the Curie (Ci). This is currently defined as 3.7×10^{10} dps (disintegrations per second). Consequently, 1 Ci = 3.7×10^{10} dps = 2.22×10^{12} dpm (disintegrations per minute). 1 dps (the Becquerel, Bq) is the modern SI unit for radioactive flux, so 1 Ci = 3.7×10^{10} Bq. Although a rather arbitrary unit, it is worth recognising the non-SI Curie is still used extensively today when measuring radioactivity, especially by the largely American suppliers of radiochemicals such as Perkin Elmer, VWR, American Radiolabeled Chemicals Inc., which still supply their products using Ci units for quantities and Ci/mass or mol for specific activity. The conversion of these two units is shown in Table 8.3.

Table 8.3: Conversion of Ci and Bq units of radioactivity

UNIT OF RADIOACTIVITY	1	10^{-3}	10^{-6}	10^{-9}	10^{-12}
Curie	Ci	mCi	µCi	nC1	pCi
Becquerel	37 GBq	37 MBq	37 kBq	37Bq	37 mBq

8.1.2.3 Properties of ^{3}H and ^{14}C and Consequences for Use as Tracers

The respective radioactive-decay constants for ^{3}H and ^{14}C (5.80×10^{-2} and $1.25 \times 10^{-4} y^{-1}$) means that pure tritium is 464 times more radioactive than pure ^{14}C. The accepted values of the specific activity of substances containing a single isotope replacement of tritium or ^{14}C are 28.6 Ci and 0.0625 per mmol respectively. The consequence for this is tritiated compounds are typically synthesised with a specific activity in the range 10-200 Ci/mmol, whereas ^{14}C compounds are synthesised in the range 50-500 mCi/mmol, so that tritiated compounds as supplied are about 1000-fold more radioactive than ^{14}C. These high concentrations are usually diluted prior to use and may also be radio-diluted by adding a “non-radiolabelled” compound, reducing the specific activity.

8.1.3 Radiochemical Synthesis

The synthesis of radiolabelled substances for fate and behaviour studies is the most expensive parts of the whole project. The use of radiolabelled test substance is a requirement in the compulsory studies required for registration and authorisation of pharmaceuticals and PPP. Most commonly, medicinal and pesticide substances are ^{14}C labelled due to the radionuclide’s long half-life and chemical stability. They are synthesised on a case-by-case basis with the label being introduced in one of the last steps in any multi-stage synthesis. Consequently, the synthesis route to make the labelled substance and the standard route used to make the product may differ. The chemistry used to make radiolabelled derivatives can be complex, however, the emerging field of late stage functionalisation, which can introduce important chemical groups in the very last steps of the synthesis, may make high yielding routes to labelled substances more accessible and cheaper. The details of the route and method used to make the radiolabelled test compounds are rarely reported in publicly available sources in any detail, although in the case of PPP (or pharmaceuticals) the summary document may refer to in-house proprietary reports or simply include details of the contract laboratory that did the radiosynthesis. For reports in the open scientific literature, many will be published in the specialist *Journal of Labelled Compounds and Radiopharmaceuticals*. For details on methods of tritium and ^{14}C labelling see Voges *et al*. (2006). For studies required for REACH registration, the in-house synthesis of radiolabelled substances is out of the question for many registrants; however, there are several contract laboratories offering these services, although costs are high.

For any environmental fate and behaviour report that uses a radiolabelled test substance the following information must be included:

- Type of radiolabel, usually ^{14}C or 3H.
- Position of the radiolabel in the chemical structure.
- Specific radiochemical activity in units of Ci or Bq/mass or mol.
- Radiochemical purity (usually by HPLC), preferably >98% and the method used to determine this.

8.1.4 Hydrogen-Tritium Exchange

The problems of ^{14}C radiolabelling described above have been addressed by the pharmaceutical industry as their requirements for labelled compounds are higher than other industries and includes actual pharmaceutical ingredients, pharmaceutical impurities and metabolites. The exchange of hydrogen atoms with tritium (or deuterium), particularly aromatic hydrogens, in organic compounds catalysed by such metals as platinum and palladium has been known for a long time. However, the yields in the early days were not particularly high and often gave rise to unacceptable degrees of radiolysis in the case of tritium. Consequently, these problems, particularly with respect to the nature of the catalysts used, have been addressed by the pharmaceutical industry, so that metal-catalysed exchange with tritiated water and tritium gas has now been extensively used to prepare a wide range of tritium-labelled compounds in support of pharmaceutical research. For a review see Hesk *et al*. (2010).

8.1.5 Other Important Factors for Consideration in Radiochemical Labelling

There are several considerations in the choice between ^{14}C and ^{3}H, or other isotopes. The labelled compound must be as pure as possible, as quite small amounts of labelled impurities can significantly affect the results. The location of the label also needs careful consideration. For example, if the test molecule can be cleaved during metabolism (e.g. two rings are joined by a labile moiety) both halves need to be radiolabelled. The specific activity of the substances should be as high as possible, this is especially important when working with low concentrations.

Other important considerations regarding environmental fate studies using radiolabelled compounds are the identification of metabolites and the treatment of phase II metabolites (conjugates):

- Radiolabelled metabolites are generally present in quantities too small for structural determination by techniques as NMR or even MS (although this may sometimes be possible through sample pooling). This can be overcome with high specific activity of the test substance and subsequently the metabolites, or unlabelled metabolites will need to synthesised as certified reference standards.
- Metabolites are frequently conjugated to sugars or sulfate. These need to be hydrolysed using an enzyme or acid prior to identification of the metabolite and this can be problematic if the nature of the conjugate is unknown.

8.2 Identification and Quantification of Radiolabelled Substances.

Labelled compounds should be separated by HPLC or TLC and detected and quantified by LSC in the cells of a detector. Residues associated with solids that are quantified by combustion analysis of solid samples in an O_2 atmosphere.

8.2.1 Pre-analysis Sample Clean-up and Concentration

Prior to separation and quantification by HPLC and radio-counting, samples extracted in environmental fate studies, such as fish tissue, water, soil and sediment samples are subjected to a system of pre-analysis clean-up and concentration. The corresponding methods are described in sections 7.3 and 7.4 and, in the case of radiolabelled samples are usually solid phase extraction (SPE) or solid phase micro-extraction (SPME). The use of radiolabelled materials enables the extraction process to be easily checked for sample losses and the concentration step inherent in these methods improves the LOQ. Solid phase extraction can also, in some cases, remove interfering co-extracted materials such as phosphorescent compounds (Section 8.2.2.1)

8.2.2 Liquid Scintillation Counters

Independent of the nature of radioactive decay (β^-, β^+, γ or α emission) radioactivity can be measured and quantified by scintillation counting. In this process radioactive emissions are measured indirectly by transforming their energy into a directly proportional amount of visible energy (photons). Currently, scintillation fluids comprise either a combination of components e.g. 2,5-diphenyloxazole (PPO) and di(phenyl-5-oxazolyl-2)-1,4-benzene (POPOP), which when activated by 382-430 nm light emit in the visible range (500-505 nm) and is detectable by photo-multiplier tubes. More recently, primary scintillants that emit visible light have been developed such as 1-phenyl-3-mesityl-2-pyrazoline (PMP), which emits visible light at λ_{max} = 430 nm without the need for a secondary scintillator.

Commercial liquid scintillation cocktails contain the scintillants dissolved in aromatic solvents such as toluene or xylene and high proportions of non-ionic surfactants such as Triton-X100 so that they will accept large amounts of aqueous samples and still give a single liquid phase. Where possible, toluene and xylene have been replaced with, less toxic and less flammable solvents such as di-isopropylnaphthalene (DIPN), phenylxylylethane (PXE) or linear alkyl benzene (LAB).

In a typical LS counter, the sample, which is contained in a 7- or 20-ml glass or HDPE vial, is surrounded by two PM tubes, light guides and reflectors (so-called 4π geometry counting). The counting chamber is also surrounded by lead shielding to reduce the background radiation. Noise in the electronic circuitry is compensated for by anti-coincidence counting in which events occurring at the same time in each PM tube are ignored. Un-quenched efficiencies (cpm x 100/dpm) of modern LS counters are 92-97% for ^{14}C and ca. 65% for tritium.

Quantification of $^{14}CO_2$ using liquid scintillation counting in samples trapped by strong bases such as sodium or potassium hydroxides needs particular consideration, primarily due to the poor compatibility of the scintillation cocktail and the strong alkali used to trap the CO_2. Organic bases such as ethanolamine and the quaternary ammonium hydroxide benzethonium hydroxide, are claimed to have better compatibility with liquid scintillation cocktails. Recommended CO_2 trapping reagents and compatible LS cocktails are described in an Application Note by Perkin Elmer (Perkin Elmer.com).

8.2.2.1 Quenching and Adjustment for Phosphorescence

Quenching is the reduction in counting efficiency caused by two main mechanisms: chemical and colour quenching. Chemical quenching is caused by electronegative solvents or solutes in the mixture that affect the energy transfer process by capturing π-electrons associated with the aromatic solvent and thus reduce the availability of π electrons necessary for efficient energy transfer to the scintillant molecule. Colour quenching is caused by coloured compounds in the sample, which attenuate the light emitted by the primary scintillant and reduce its intensity. Chemical quenching is compensated for by performing quench curves, in which a constant amount of a radiochemical standard is counted in the presence of a varying amount of a known chemical quench agent such as chloroform, carbon tetrachloride, water, acetone etc. These quench curves will need to be repeated for each radionuclide and in most modern LS counters the curves are stored electronically. When samples are counted in the normal way, they are then quench curve-corrected automatically by the LS counter.

Colour quenching, in which appreciable amounts of coloured material are unavoidably extracted from the matrix can be more of a problem. The alternatives are to chemically bleach the colour (which may cause chemical quenching as well), to perform a colour quench correction curve using a dye or to dilute the sample if the level of radioactivity is high enough to quantify in the diluted sample.

The low energy β-particles of tritium are more affected by quenching than the higher energy particles of ^{14}C. Phosphorescence is the phenomenon by which a photon excites a molecule into an excited singlet state and instead of decaying via a triplet state and instantly re-emitting a photon it decays via a number of "forbidden" excited states in a time dependent (minutes to hours) manner. This is particularly a problem when extracting plants, where appreciable amounts of phosphorescent coloured compounds can be co-extracted from the matrix. This is unlikely to be a problem in environmental fate studies where the extracted matrix is fish tissue but can be an issue when extracting soils and sediments, as dissolved organic matter of plant origin is both coloured and phosphorescent. To reduce the degree of phosphorescence, samples are normally stored overnight in the fridge for the phosphorescence to decay before counting.

8.2.3 Radioactivity Monitoring and Quantification in Liquid Chromatography (HPLC and UHPLC)

It greatly enhances the analysis of radiolabelled substances if the eluent of HPLC chromatographic separations can be directly and quantitatively monitored for radioactivity. For HPLC this accomplished by in-flow post column addition of scintillation fluid, prior to entering detection cells. Historically, sensitivity of detection was enhanced using a large volume flow cell, which could lead to undesirable effects such as peak tailing and spreading. These larger flow cells are not compatible with newer microbore HPLC (particularly UHPLC) systems, which use very small volumes of mobile phase. These problems have been largely overcome with modern technology in which the geometry of the system has been redesigned to use a much smaller components and cell volumes e.g. the MIRA Star® (RayTest) and Beta-RAM-5® or Beta-Ram-6® (Lablogic). An alternative is to use solid state external scintillators in the post-column flow (heterogeneous counting), although these are not suitable for detection of the very low energy β-particles emitted by tritium, for which homogeneous LSC is the only option. This technology has also been miniaturised so it is suitable for micro-bore UHPLC (e.g. the MIRA Star® µ-HPLC-LS for ^{14}C).

To increase sensitivity synchronised accumulating radioisotope detectors in which multiple solid scintillation detector cells are connected in series for signal accumulation have been developed for on-line HPLC. These systems are superior to a single counting cell and can achieve an LOD of 10 Bq. (Baba *et al*. 1982). This has been commercialised and specifically designed for micro-HPLC (RAMONA Star® Quattro; www.raytest.com). Another development in homogeneous LSC detection has been the stopped-flow techniques used by the Beta-RAM detectors (see above) in which the flow is stopped upon detection of radioactivity (peak detection) prior to LSC using a homogeneous method. The Aim Research v.ARC 3 Radio-LC System for UHPLC and HPLC claims an efficiency of >95% for ^{14}C and >50% for ^{3}H when measuring un-quenched samples (Aim Research). For an overview of methods for detecting and quantifying radioactivity in HPLC eluants see Kiffe *et al*. (2008) and Zhu (2007).

An alternative for eluant monitoring for HPLC is post column collection of fractions to multiwell plates. Radioactivity is then measured externally using TopCount SSC® instrument (Börnsen, KO, 2000). This method allows µL fractions to be collected in multiwell plates, where each well contains scintillation gel. This method has been used for metabolism studies with LumaPlates™, which incorporate a solid state scintillant in the plate base (Bruin *et al*. 2006, Kiffe *et al*. 2003). This takes longer per run but has a superior LOD, which is reported to be 15 dpm for ^{14}C and a 10 minute count time (Zhu *et al*. 2005a, 2005b). See Table 8.6. A very low LOD can be important in following some OECD Guidelines such as OECD 309 (Aerobic Mineralisation in Surface Water), as this states: "A low concentration in this test means a concentration (e.g. less than 1 µg/l to 100 µg/l) which is low enough to ensure that the biodegradation kinetics obtained in the test reflect those expected in the environment." Consequently, methods are required to accurately quantify very low levels of radioactivity in order to carry out this test satisfactorily. A benefit here is that additional analytical runs can be performed to collect numerous fractions in a well plate that does not contain scintillation fluid. These fractions may then be prepared for specific analysis using LC/MS.

8.2.4 Radioactivity Monitoring in Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) with normal phase (usually silica gel), reverse phase or one of several newly developed phases is a long-established, rapid and simple chromatography system with moderate separation efficiency. The very low energy β^--emitter **^{3}H** on TLC traces can be quantified by using windowless GM tube detectors such as on the MARITA Star® or RITA Star® radioactivity TLC analyser for ^{3}H (RayTest.com) or the higher energy β^--emitter **^{14}C** thin window monitors for ^{14}C with the MARITA Star® or RITA Star® radioactivity TLC analyser for ^{14}C (RayTest.com).

The most robust and reliable detection and quantification system for radiolabelled regions on TLC plates is phosphor-imaging, which is a form of solid-state liquid scintillation counting where radioactive material can be localised and quantified. It has largely replaced traditional X-ray film imaging. Although it has a poorer spatial resolution than film imaging, it has greater sensitivity, faster image development, reusable detection plates and an enhanced linear dynamic range. This equipment is generally available and can be used to locate and quantify all low energy β-emitters, including tritium. Equipment is available from several suppliers, for example EAG Industries. For a review see Poole (2014) and Clark (2000).

8.2.5 Accelerator Mass Spectrometry

Accelerator Mass Spectrometry (AMS), in which ^{14}C or ^{3}H metabolites are measured directly by MS has an extremely low LOD, equivalent to about 0.05 Bq of radioactivity; however, although it has been used by the pharmaceutical industry for ADME, it is presently an expensive technique and lacks widespread availability.

8.2.6 Combustion Analysis of Unextractable Radioactivity

Unextractable radiolabelled materials in fate and behaviour studies are bound to the matrix so tightly that they are not extracted with methodology used in the study. In order to obtain a mass balance, they are usually quantified by combustion analysis in which the sample of homogeneous sample of matrix is combusted using a catalyst under a stream of oxygen. The resultant $^{14}CO_2$ is passed through an alkaline scintillation cocktail, which absorbs the CO_2. Tritium-labelled compounds, which produce ^{3}H2O can be quantified in a similar way, although the organic base is omitted from the scintillation cocktail.

8.2.7 Limits of Quantification (LOQ) for Radioactivity Counting

The LOQ values for radioactivity quantification depend on several factors, so it is difficult to generalise and draw conclusions for any one particular method and the method itself will also influence the LOQ. Other factors that will affect the LOQ are specific radiochemical activity, identity of radionuclide, counting efficiency, counting geometry, count time and quenching. Radioactive decay is a random process, so counting low-level radioactivity requires long counting times and although it is perfectly possible to quantify low levels of radioactivity, the counting uncertainty % = f x √N, where N = number of counts/min. and f a proportionality constant. Consequently, the counting uncertainty is proportional to the square root of the counts/min. In static LSC it might be possible to count samples for an hour or longer to improve the counting statistics but for HPLC flow-through detectors the equivalent count time is much shorter**.** This this has been solved to a degree by the use of stopped-flow HPLC technology in which the flow through to the detector is stopped upon peak detection, thus increasing the residence time in the scintillation detector (see Section 8. 2.1 above).

Nonetheless, it is illustrative to show what likely values for the LOQ can be obtained for fate and behaviour experiments using either tritiated or ^{14}C labelled compounds. Table 8.4 has been constructed using values for commercially available ^{3}H or ^{14}C glucose with specific activities of 20 and 0.060 Ci/mmol respectively. It also assumes the radiolabelled material has not been radio-diluted with unlabelled material. The data are based on information generated from the TopCount and LumaPlate counting system (Bornsen, 2000) and the equation used to measure the LOD for the system is below (from Zhu, 2005a).

$$\mathrm{LOD} = 2.71/T \times E + 4.65 \times \sqrt{(B/T \times E)}$$

Where T = count time (10 min.),
B = background cpm,
E = counting efficiency, 90.9% for ^{14}C and 40.2% for ^{3}H

It was assumed that LOQ = 3 x LOD, and the substance is glucose MWt =180.

Table 8.4: Calculated LOQ Values for TopCount, LSC and RFD.

		TOPCOUNT HPLC	TOPCOUNT UHPLC	LSC	RFD
NUCLIDE	SP. ACT. CI/MMOL (	LOQ µG/L (1 ML COUNTED)	LOQ µG/L (10 µL COUNTED)	LOQ µG/L (1 ML COUNTED)	LOQ µG/L (1 ML COUNTED)
^{3}H	20	0.1	10	0.20	10
^{14}C	0.06	21	2100	42	2100

Clearly, the LOQ values represent the most favourable values that could be obtained and indicate that radiolabelled compounds of this specific activity could be radio-diluted significantly and still obtain LOQ values well within the requirements for fate and behaviour studies. It also shows the superiority of the TopCount post-fractionation system applied to HPLC over normal in-line RFD systems (ca. 100-fold), where the count time is limited.
The detection and quantification methods available for in line radioactivity and post-separation counting radioactivity are presented in Tables 8.5 and 8.3, respectively.

Table 8.5: Detection and quantification methods for in line radioactivity in different chromatographic separation systems.

DETECTION METHOD	SUITABLE RADIONUCLIDE	CHROMATOGRAPHY TECHNIQUE(S)	COMMERCIAL SYSTEM EXAMPLE	ADVANTAGES
Homogeneous liquid scintillator	$^{3}H/^{14}C$	HPLC	RAMONA liquid scintillator	Better sensitivity and lower background
Heterogeneous Solid scintillator	^{14}C	HPLC	RAMONA integral solid scintillator	Better peak resolution. No need for scintillant solution
Synchronised Accumulating Radioisotope Detector. Liquid scintillator	$^{3}H/^{14}C$	HPLC	RAMONA Star quattro® HPLC-LS for Tritium	Better LOD than single detector systems
Synchronised Accumulating Radioisotope Detector. Solid scintillator	^{14}C	HPLC	RAMONA QUATTRO® integral solid scintillator	Better LOD than single detector systems
Systems with very small flow cells dead volume. Liquid and	$^{3}H/^{14}C$	UHPLC	MIRA Star® µ-HPLC-LS for Tritium	Suitable for very low eluant volumes

DETECTION METHOD	SUITABLE RADIONUCLIDE	CHROMATOGRAPHY TECHNIQUE(S)	COMMERCIAL SYSTEM EXAMPLE	ADVANTAGES
solid scintillator				
Systems with very small flow cells dead volume. Solid scintillator	^{3}H/^{14}C	UHPLC	MIRA Star® μ-HPLC-LS for ^{14}C	Suitable for very low eluant volumes
Liquid chromatography accurate radioisotope counting (LC-ARC - stopped flow counting)	^{14}C	HPLC or UHPLC	ARC 3® Radio-LC System for UPLC and HPLC	Stated to have the highest in-line radioisotope detection sensitivity and resolution

Table 8.6: Detection and quantification methods for post separation counting radioactivity in different chromatographic separation systems.

DETECTION METHOD	SUITABLE RADIONUCLIDE	CHROMATOGRAPHY TECHNIQUE(S)	COMMERCIAL SYSTEM EXAMPLE	ADVANTAGES
"TopCount" SSC	^{3}H/^{14}C	UHPLC	TopCount®/ LumaPlate	Low background so low LOD. Very High sensitivity.
Windowless GM counting	^{3}H/^{14}C	TLC	RITA Star ®TLC analyser for Tritium	High sensitivity
Thin window GM counting	^{14}C	TLC	RITA Star® TLC analyser for ^{14}C	High sensitivity for ^{14}C but not suitable for ^{3}H
Phosphor-imaging	^{14}C	TLC	Molecular Dynamics Typhoon FLA 7000®	High sensitivity and good spatial resolution

9. Chromatography and stable isotope detection

Chromatography is used to separate the test substance and metabolites from the extracted samples. Different chromatography techniques are discussed in Sections 9.1 - 9.3. Eluents containing the different moieties, which should be time separated and resolved from each other, flow directly into a detector or fraction collection vessel. Detectors may be destructive e.g. MS or non-destructive e.g. UV or radio-detection cell. The latter lend themselves to be used in conjunction with fractionation. These detectors are discussed in Sections 9.4 and 9.5.

This section covers:

- Liquid chromatography techniques that can be performed in conjunction with the methods for detection of radiolabelled compounds covered in Chapter 9.
- Other chromatography methods that are suitable for use with stable isotope labelled and non-labelled materials.
- Detection methods that are suitable for identification and quantification of stable isotope labelled and non-labelled materials.
- Detection techniques that can be used to identify unknown metabolites already detected by radioactivity monitoring techniques.

Literature on the analysis of trace contaminants in the environment has been a valuable source of information on analytical methods and procedures that could be relevant for environmental fate laboratory studies. Examples of the use of various techniques have been found. However, most papers did not discuss advantages and disadvantages of the different techniques or problems encountered during the studies. Where comparisons were found, these were often promoting a particular technique; even-handed comparisons were lacking. Advantages and disadvantages presented below are based on our critical review of the literature and our own experience.

There are the general pathways to analysis.

- Radiolabelling (See Chapter 8)
    - Fraction counted by liquid scintillation counter or similar.
    - Fraction counted by liquid scintillation count then analysed by mass spectroscopy method or nuclear magnetic resonance.
    - High performance liquid chromatography with radioactivity monitor detector (HPLC -RAM).
- Stable isotope labelling
    - Combustion to $^{13}CO_2$ then Isotope Ratio Mass Spectrometry.
    - Quantification and identification by a chromatography mass spectroscopy method such as GC-MS, LC-MS, LC-MS/MS, LC-HRMS.
- Unlabelled methods are unable to determine non-extractible residues (NER) except by difference which would include losses to air.
    - Quantification of parent.
    - Quantification of parent and limited identification of metabolites. Unlikely to be better than semi-quantitative without suitable calibration standards for the metabolites.

9.1 Fractionation methods

Most of the general pathways to analysis deal with the fractionation of the sample by chromatography into well-plates, this is a common method for separation in radiochemistry. The choice of technique depends upon the type of labelling of the substance, the properties of the substance and the availability of equipment. Table 9.1 shows methods that are used for fractionating samples. Fractionation could also permit another dimension to analysis, whether this is chromatography or spectroscopy (NMR or MS). For example, a complex substance could be first separated in this way and the fractions identified by NMR. We have not found any examples of this type of application in the literature relating specifically to laboratory bioaccumulation and bioconcentration tests. Although it is a commonly used in the analyses of complex natural substances, pharmaceutical metabolites and metabolic profiling of human urine (McGill *et al.,* 2019).

Table 9.1: Chromatography methods that are used for fractionating samples.

	APPLICATION	ADVANTAGES	DISADVANTAGES
High pressure liquid chromatography (HPLC)	Separation of non-volatile compounds	• See above. • Probably the preferred fractionating method.	• See above
Ultra performance liquid chromatography (UHPLC)	Separation of non-volatile compounds	• See above. • Very rarely has been used in this manner.	• Very small elution volumes. • Uncommon in test laboratories.
Thin Layer Chromatography (TLC)	Separation of mixtures on mainly silica based TLC plates. Although cellulose, ion exchange resin, controlled porosity solid plates could be used.	• Detection of radiolabelled compounds now performed special densitometers. • Simple. • Cheap. • Excellent fractionation technique. • Excellent preparative method.	• Migration characteristics very sensitive to conditions. • Thin layers easily damaged. • Moderate quantitative precision (5-10%)
Size Exclusion Chromatography (SEC)	Determination of peptides, proteins, oligomers and polymers by molecular size.	• Routinely used with a fraction collector. • Useful to separate a substance by molecular weight.	• Uncommon in test laboratories unless regularly test polymers. • Superseded by high performance size exclusion chromatography (HPSEC).
Supercritical fluid chromatography (SFC)	Determination of volatile compounds by partition with CO_2 and CO_2 modified with solvent to change polarity.	• Excellent selectivity. • Speed. • Able to separate large molecules. • Better resolution than HPLC.	• Specialist equipment that is unlikely to be present in test laboratories. • More complex and expensive instruments than GC or HPLC.

9.1.1 Radiolabelled material

Liquid Chromatography (LC) or Thin Layer Chromatography (TLC) are used for fractionation (see Section 5) and the detectors used are summarised in Figure 9.1. Gas Chromatography (GC) is considered not possible on grounds of safety and they are not sensitive enough.

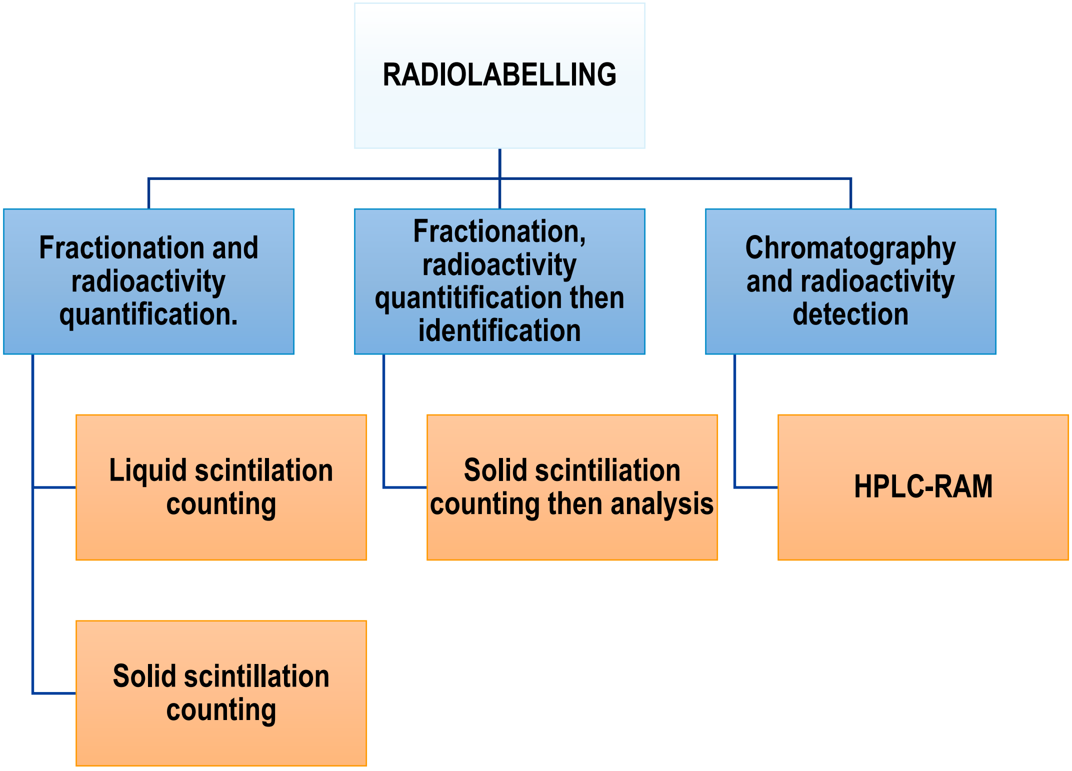

Figure 9.1: Summary of the available techniques for the quantification of radiolabelled constituents.

9.1.2 Isotope Ratio Mass Spectrometry (IRMS)

This could be considered to be the stable isotope equivalent of radiometric methods used for radiolabelled constituents. The fractions separated by a chromatography technique are combusted to $^{13}CO_2$ and the isotope ration determined by a dedicated mass spectroscopy detector. This then can only be used to determine the amount of stable isotope labelled material. It would be essential to perform this to obtain the amount lost to non-extractable residue for the mass balance calculation. However, it is unable to determine the identity of what is in the fraction. This would have to be done by other analytical methods such as LC-MS, GC-MS, MS/MS and NMR. It also means that the analysis would be qualitative and for identification of the composition of the fractions rather than quantitative.

9.1.3 Stable isotope labelled

The full range of techniques listed in Table 9.1 are possible; typically, LC or GC are used because these are well understood and available to the test laboratory (see Section 5). The choice of technique depends on the properties of the substance and the availability of equipment:

- For fairly non-polar and fairly volatile substances Gas Chromatography (GC) is ideal as it requires solvent exchange, so no water is present, and it has superior resolving power over all other chromatography techniques.
- For polar substances, High Performance Liquid Chromatography (HPLC) and its newer cousin Ultra High Performance Liquid Chromatography (UHPLC) are typically used. Derivatisation to stable and volatile non-polar derivatives could also be consider followed by GC. This has been performed for decades for long chain carboxylic acids (fatty acids), amines and amines.
- For ionic or ionisable substances, ion pairing liquid chromatography should probably be considered so that the substances can be run on reverse phase High Performance Liquid Chromatography (HPLC) and Ultra High Performance Liquid Chromatography (UHPLC) equipment that is probably more commonly used in a test laboratory. Ion Chromatography or Capillary Electrophoresis (CE) are excellent techniques but may not be available to the test laboratory.

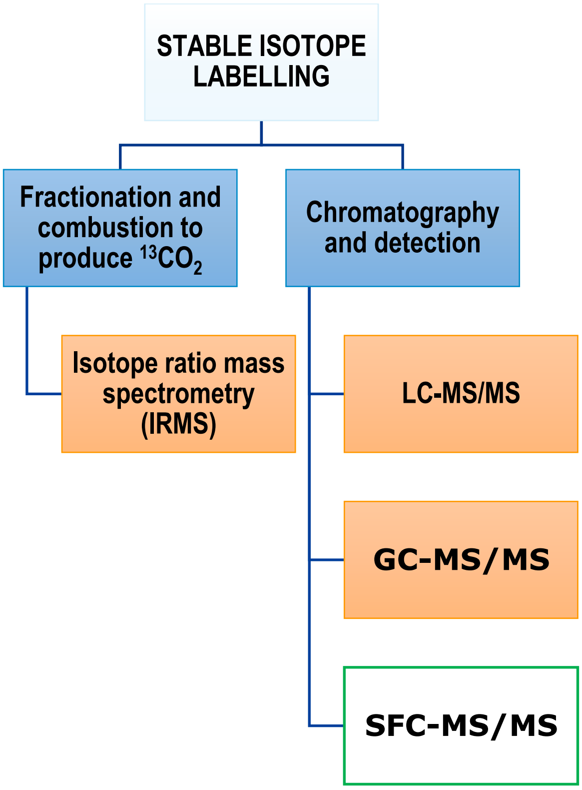

Figure 9.2: Quantification and identification by stable isotope labelling methods.

The methods in Figure 9.2 are listed hierarchically, with the orange boxes indicating the methods which, in the present author's view, should be considered in the first instance. These methods are generally the most suitable, widely available and cost effective, and applicable to a wide range of substances. Where these methods are not suitable for the substance under consideration, the techniques in the green outlined box could be attempted.

9.1.4 Non-labelled material

Non-labelled methods that would only quantify the disappearance of parent. The methods in Figure 9.3 are listed hierarchically, with the orange boxes indicating the methods which, in the present author's view, should be considered in the first instance. These methods are generally the most suitable, widely available and cost effective, and applicable to a wide range of substances. Where these methods are not suitable for the substance under consideration, the techniques in the green outlined box could be attempted.

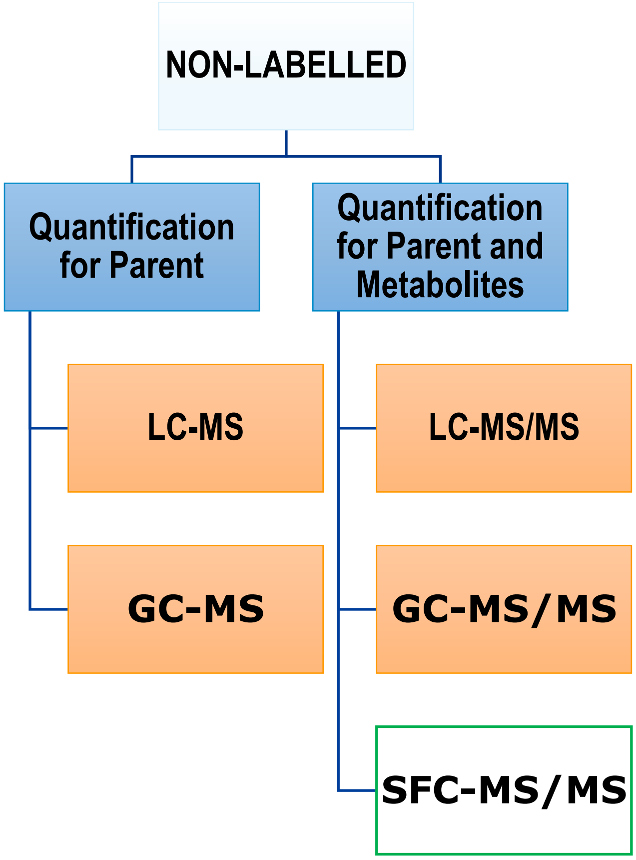

Figure 9.3: Quantification of the disappearance of the parent substance on unlabelled substances.

9.2 Chromatography

The different chromatographic techniques that exist are summarised in Table 9.2. The advantages and disadvantages of the different separation techniques are given in Table 9.3 and 9.4.

Table 9.2: Classification of chromatographic methods*.

	STATIONARY PHASE	MOBILE PHASE	SORPTION MECHANISM	GENERAL APPLICATIONS
Planar Chromatography				
Paper Chromatography (PC)	Paper (cellulose)	Liquid	Partition	Analysis of mixtures
Thin Layer Chromatography (TLC)	Silica, cellulose, ion exchange resin, controlled porosity solid	Liquid	Adsorption	Analysis of mixtures
Liquid Chromatography				
High performance liquid chromatography (HPLC)	Solid or bonded phase	Liquid	Modified partition	Determination of non-volatile compounds
Ultra-high performance liquid chromatography (UHPLC)	Nano particle solid phase	Liquid	Modified partition	Determination of non-volatile compounds
Ion Exchange Chromatography (IEC) Ion Chromatography (IC)	Ion exchange resin or bonded phase	Liquid	Ion Exchange	Determination of non-volatile anions and cations.
Capillary Electrophoresis (CE)	Solid	Liquid	Electrically charged molecules	Determination of polar macromolecules such as proteins.
Size Exclusion Chromatography (SEC), Gel Permeation Chromatography (GPC), Gel Filtration Chromatography (GFC)	Controlled porosity solid such as silica or polymeric gel	Liquid	Molecular size (Stokes' Radius)	Determination of peptides, proteins and polymers
Chiral Chromatography (CC)	Solid chiral selector or pre-column chiral reactions	Liquid	Selective adsorption of chiral isomers	Separation and determination of chiral compounds
Gas Chromatography				
Gas Liquid Chromatography (GLC)	Liquid phase on a wall or solid support	Gas [He, H2 or N_2]	Partition	Determination of volatile compounds or gasses
Supercritical fluid chromatography (SFC)	Solid or bonded phase	CO_2	Partition	Determination of volatile compounds

* Summerfield (2010)

Table 9.3: Advantages and disadvantages of main chromatography techniques.

	APPLICATION	ADVANTAGES	DISADVANTAGES
High performance liquid chromatography (HPLC)	Separation of non-volatile compounds including ionic and polymeric samples.	• Universally the technique of choice particularly when dealing with water soluble metabolites. • Available in all test laboratories. • Complimentary to GC. • The technique of choice for radiolabelled compounds.	• Column performance very sensitive to method of packing. • Long run times, up to 60 minutes unless microbore columns used.
Gas Chromatography (GC)	Separation of volatile and thermally stable compounds below about 400°C.	• Available in all test laboratories. • Rapid and simple. • Able to cope with 100 or even 10 000 constituents. • Very small samples (nanograms) • Relative precision 2-5%.	• Analytes must be volatile and thermally stable below about 400°C. • The most commonly used detector is the flame ionising detector and this is not selective. • Not used with radiolabelled compounds on safety grounds and not sensitive enough.
Ultra performance liquid chromatography (UHPLC)		• Speed with run times of minutes compared to 20-60 minutes for HPLC. • Column efficiency approaching that of the GC. • Uses 10-100 more pressure than conventional HPLC. • Lower consumption of mobile phase. • Can use Reverse Phase (RP), Normal Phase (NP) or HILIC Columns • Detection limits improve with decreasing bore diameter due to less dispersion and higher efficiency. • Has been only recently used with radiolabelled compounds.	• Not widely used by test laboratories. • Specialist instruments to deal with the high pressure. • Stringent sample clean-up to avoid particulates or precipitates blocking the columns. • Not as well suited to fractionation as conventional HPLC due to very small elution volumes.

Table 9.4: Advantages and disadvantages of secondary chromatography techniques that are unlikely to be available to test laboratories.

	APPLICATION	ADVANTAGES	DISADVANTAGES
Ion Chromatography (IC)	Determination of non-volatile anions and cations including amino acids on ion exchange resin or bonded phase.	• Higher matrix tolerance • Many detection options and wide range of analytes • High selectivity • Low detection limits	• Specialist equipment that is unlikely to be present in test laboratories. • Separation accompanied by large excess of eluting electrolyte.
Capillary Electrophoresis (CE)	Separation of mainly charged materials by differential migration through a capillary column in an applied potential gradient. The migration rates depend upon the size, shape and charge of the species.	• Characterisation and quantification of biologically active materials in complex matrices. • Able to separate macromolecules such as proteins and peptides.	• Specialist equipment that is unlikely to be present in test laboratories unless routinely test proteins and peptides. • Mobility are very sensitive to supporting medium.
High performance size exclusion chromatography	Determination of peptides, proteins and polymers by molecular size.	• Can be used to separate proteins and polymers.	• Specialist equipment that is unlikely to be present in test laboratories unless routinely test polymers.

Other chromatography techniques that lend themselves to fractionating the samples and their characteristics are discussed in Section 9.3.

9.2.1 Selection of Liquid Chromatography Method

The selection of the type of liquid chromatography performed depends upon the solubility, molecular weight and polarity as shown in Figures 9.4, 9.5, 9.6 and 9.7.

The initial consideration for substances with a molecular weight of less than 2000 g/mol is their volatility as shown in Figure 9.4. Polar, ionic and ionisable analytes are non-volatile.

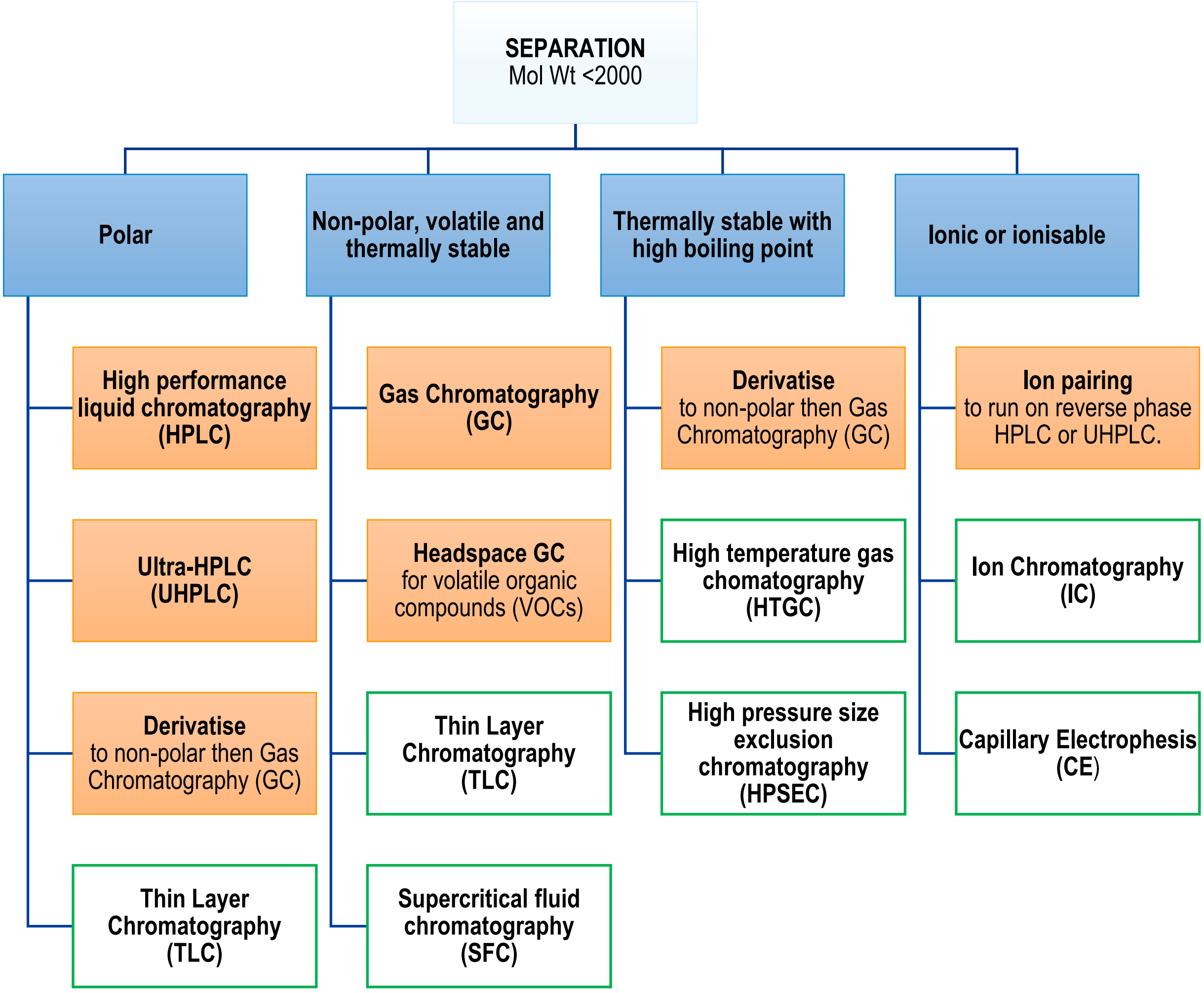

Figure 9.4: Choosing the chromatography separation technique.

The methods in Figure 9.4 are listed hierarchically, with the orange boxes indicating the methods which, in the present author's view, should be considered in the first instance. These methods are generally the most suitable, widely available and cost effective. Where these methods are not suitable for the substance under consideration, the techniques in the green outlined box could be attempted.

The second consideration their solubility in water (See Figure 9.5) or in organic solvents (See Figure 9.6).

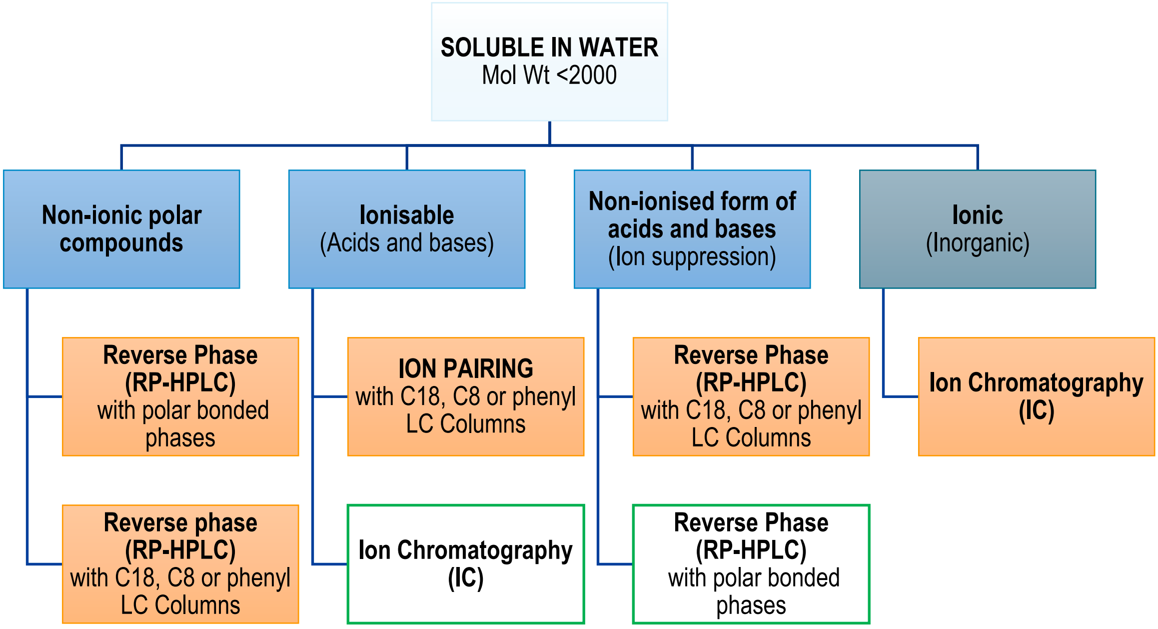

Figure 9.5: Selection guide for the liquid chromatography of substances soluble in water with molecular weight less than 2000 g/mol. Separation method would be chosen as a function of the molar mass, solubility and polarity.

The methods in Figure 9.5 are listed hierarchically, with the orange boxes indicating the methods which, in the present author's view, should be considered in the first instance. These methods are generally the most suitable, widely available and cost effective. Where these methods are not suitable for the substance under consideration, the techniques in the green outlined box could be attempted.

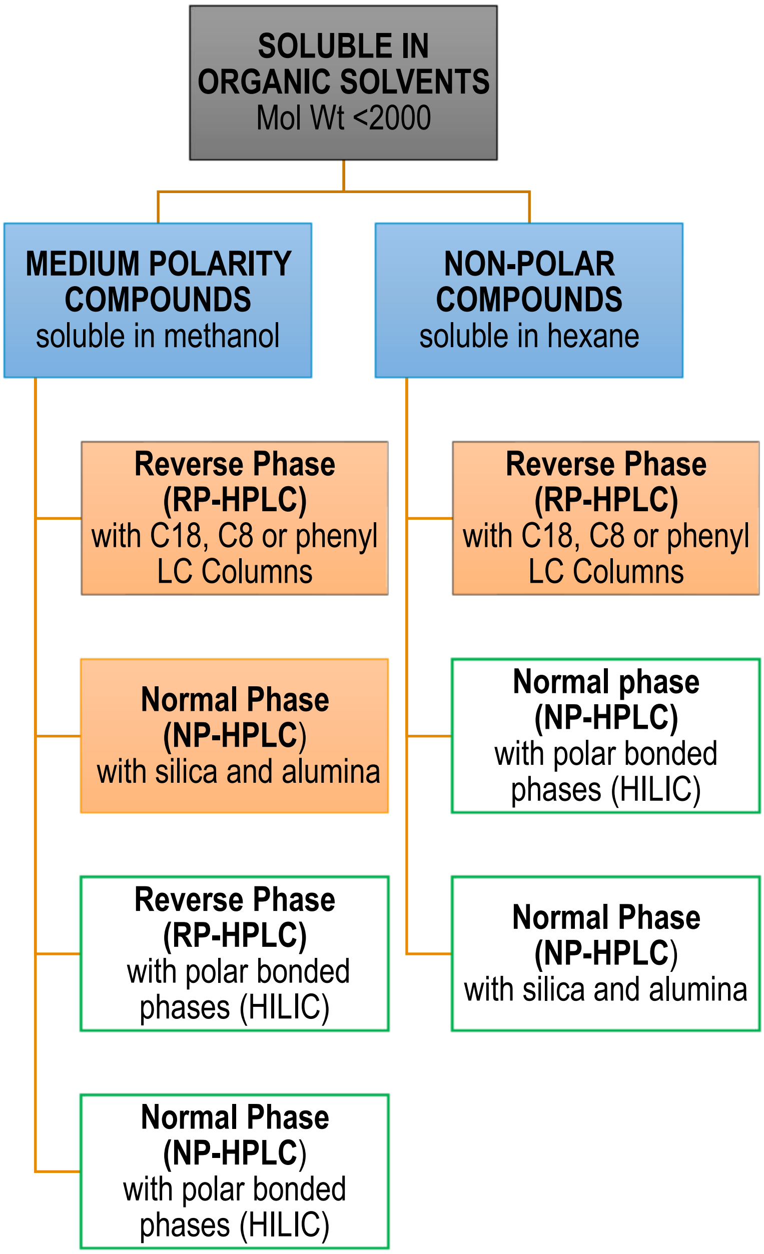

Figure 9.6: Selection guide for the liquid chromatography of substances soluble in organic solvents with molecular weight less than 2000 g/mol. Separation method would be chosen as a function of the molar mass, solubility and polarity.

The methods in Figure 9.6 are listed hierarchically, with the orange boxes indicating the methods which, in the present author's view, should be considered in the first instance. These methods are generally the most suitable, widely available and cost effective. Where these methods are not suitable for the substance under consideration, the techniques in the green outlined box could be attempted.

When dealing with macromolecules or substances that are insoluble in water or solvents, then other methods need to be employed (See Figure 9.7).

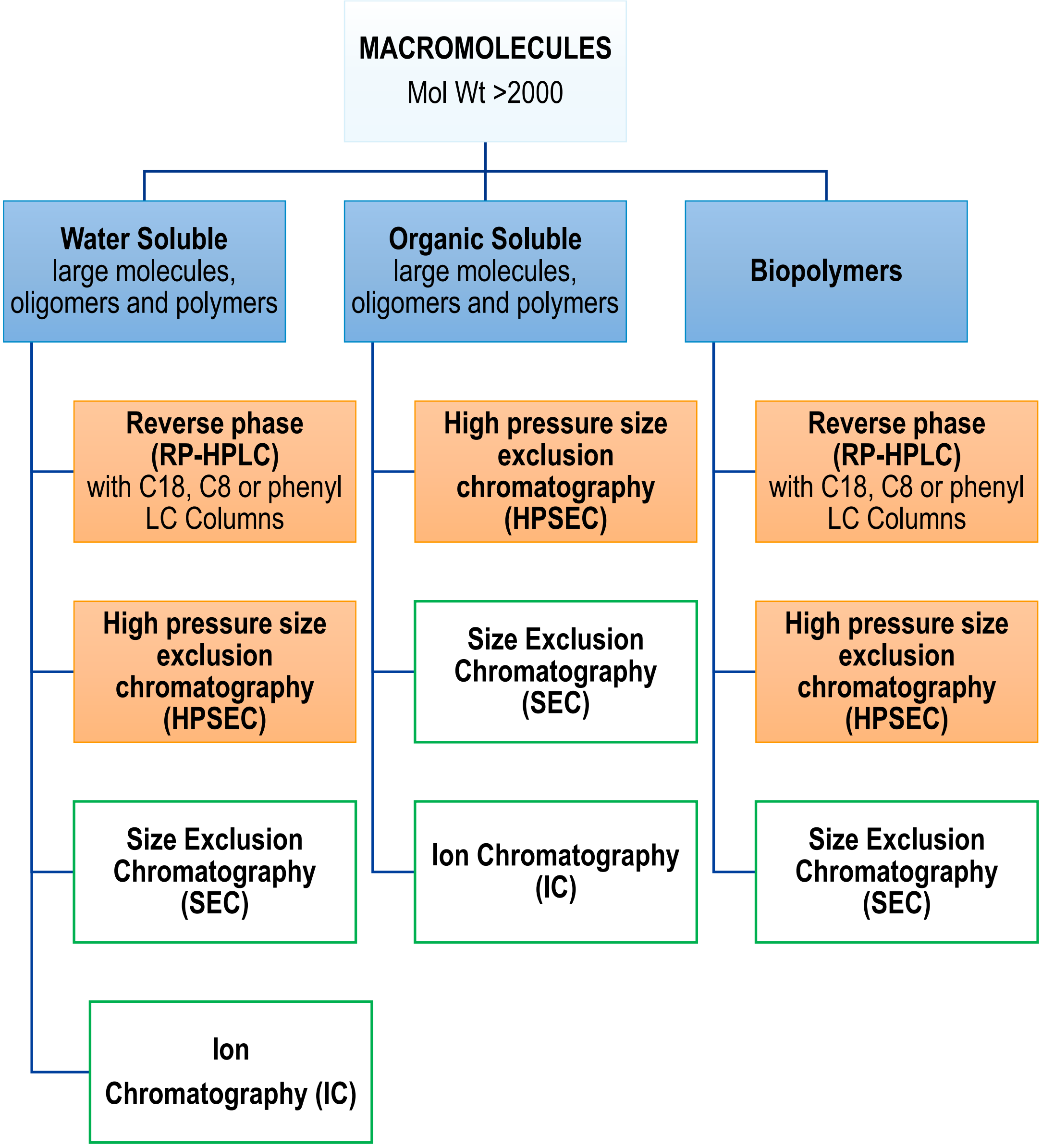

Figure 9.7: Selection guide for the liquid chromatography of macromolecules with molecular weight greater than 2000 g/mol. Separation method would be chosen as a function of the molar mass, solubility and polarity.

The methods in Figure 9.7 are listed hierarchically, with the orange boxes indicating the methods which, in the present author’s view, should be considered in the first instance. These methods are generally the most suitable, widely available and cost effective. Where these methods are not suitable for the substance under consideration, the techniques in the green outlined box could be attempted.

9.2.2 High Performance/Pressure Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) has been the main method used to separate compounds according to their polarity. Ganorkar and Shirkhedkar (2017) reviewed the development of the method from 1970 and assess the methodologies, design of experiments, applications and future prospects. Table 9.5 shows the main types of HPLC column.

Table 9.5: Liquid Chromatography Columns*.

COLUMNS	NOTES
Reverse Phase HPLC Columns	Stainless steel tubing, typically 5-30 cm in long stainless-steel columns with 2-5 mm inner diameter . The stationary phase is chemically or physically bonded to the packing material, normally 3-10 micron silica particles. A relatively non-polar stationary phase of surface modified silica support (e.g. octadecyl silanised (ODS) C_{18}; C_6 phenyl or fluorinated phenyl) and polar mobile phase, such as binary or tertiary mixtures of water with acetonitrile, or methanol. Reversed-phase chromatography is the most common form of liquid chromatography, primarily due to the wide range of analytes that can dissolve in the mobile phase.
Normal Phase HPLC on Hydrophilic Interaction Liquid Chromatography (HILIC) Columns	Polar stationary phase and non-polar organic solvent, such as n-hexane or isopropyl ether as the mobile phase. It separates by polarity so poorly discriminates homologues. The stationary phase is a bonded siloxane with a polar functional group, such as diols, cyano-, amino- or amide- groups. The solvent elution in reversed-phase is usually the opposite of that in normal phase liquid chromatography. In other words, polar compounds are eluted first. HILIC columns have practically replaced silica columns.
High performance liquid affinity chromatography (HPLAC)	Immobilised biological related agent (e.g. protein or receptor)

* Summerfield (2010)

An example of good practice in the use of HPLC is given by Albaseer *et al.* (2011) in their analysis of analytical artefacts, sample handling and preservation methods for environmental samples of synthetic pyrethroids. The following precautions were considered useful for reliable analysis and avoidance of artefacts and are generally applicable, particularly for highly sorptive substances:

- Extract obtained after the pre-concentration step should be analysed immediately, as its high concentration may cause analyte loss (e.g. by degradative interactions or irreversible sorption to the sample container).
- Blank sample injection should be done at reasonable intervals, if several samples are to be analysed in the same session.
- Injection syringe should be rinsed after each injection by a suitable solvent.

9.2.2.1 Derivatisation for Liquid Chromatography

Most examples of derivatisation performed for liquid chromatography are to improve the selectivity of the quantification, for example adding a chromophore or fluorophore. However, it is also possible to use derivatisation to change the polarity of analytes to provide better separation or allow a different (available) column to be used. Some examples of derivatising agents are shown in Tables 9.6 and 9.7 below.

Table 9.6: Derivatisation for Liquid Chromatography for UV detection.

TARGET ANALYTES	DERIVATISING AGENT	REFERENCE
Amines, amino acids and peptides	• Acyl chlorides (e.g. benzoyl chloride, p-nitrobenzoyl chloride) • Arylsulfonyl chlorides (e.g. toluenesullfonyl chloride, benzenesulfonyl chloride) • Nitrobenzenes (e.g. 1-fluoro-2,4-dinitrobenzene) • Isocyanates (e.g. phenyl isocyanate) • Isothiocyanates (e.g. dimethylaminobenzene-4'-isothiocyanate.)	Karty *et al.* (2004)
Carboxylic acids	• Phenacyl bromide. • Methylphthalimide. • p-nitrobenzyl-N,N'-diisopropylisourea. • Phenyl isocyanate.	
Hydroxy compounds	• Acyl chlorides (e.g. benzoyl chloride). • Phenyl isocyanate.	
Carbonyl compounds	• 2,4-dinitrophenylhydrazine. • 3-methyl-1-phenyl-2-pyrazoline-5-one.	

Table 9.7: Derivatisation for Liquid Chromatography for fluorescence detection.

TARGET ANALYTES	DERIVATISING AGENT	REFERENCE – NONE FOUND
Amines, amino acids and peptides	• Sulfonyl chlorides (e.g. 2,5-dimethylaminonaphthalene-1-sulfonyl chloride).	
Carboxylic acids	• Isocyanates/isothiocyanates (Fluorescein isothiocyanate). • Fluorescamine	
Hydroxy compounds	• Diacetyldihydrofluorescein.	
Carbonyl compounds	• Dansylhydrazine. • Semicarbazide. • 2-aminopyridine. • 1,2-diphenylethylenediamine.	
Nitro-polyaromatic hydrocarbons	• Online reduction with Pd/Pt yielding corresponding amine.	

9.2.3 Ultra-High Performance Liquid Chromatography (UHPLC)

UHPLC is a liquid chromatography technique that uses small particle size and high pressure to achieve much improved resolution when compared to HPLC. It is only in the last decade that its promise has started to be realised with the commercial introduction of improved capillary column preparation techniques that have led to columns with unprecedented performance in speed, as reviewed by Blue *et al*. (2017).

There is a large amount of published literature relating to UHPLC and examples of its use in both laboratory studies (see Section 4) and in environmental samples have been found. This technique shows much promise for use in environmental fate studies. However, the majority of the published examples relate to medicinal substances or plant protection products and the technique may not yet be routinely available in laboratories testing industrial chemicals. Table 9.8 summarises types of UHPLC column.

Ultra-Performance Liquid Chromatography (UPLC) is a method trademarked by Waters, who commercialised the method. This technique uses a very small particle size stationary phase in order to increase the efficiency and speed of separation of the test substances (and metabolites).

Fekete *et al.* (2014) and Fekete *et al.* (2015) outlined the following advances in UHPLC in the last few years:

- The use of short narrow-bore columns packed with sub-3 µm core-shell and porous sub-2 µm particles.
- Increasing the mobile phase temperature in liquid decreases analysis time but may not improve the separation efficient as measured by high plate count.
- Fully porous particles down to sub-2µm as used in UHPLC.
- Superficially porous particles (SPP) from 1.3 to 5µm were introduced in 2007 are considered by the authors to be the most promising strategies for high resolution and high throughput separations.
- Silica-based monoliths available since 2000, may be more competitive if the upper pressure limit of the column can be extended to 400-600 bar.
- The extension of system pressure in UHPLC beyond 1000 bar has been a powerful strategy to maximise efficiency but its impact on throughput remains limited and frictional heating effects should be considered with care.

Fekete *et al.* (2014) also outlined the challenges in terms of extra-column variance, dwell volume, maximum system pressure, detector data acquisition rate, and injection cycle time.

Table 9.8: Ultra-HPLC (UHPLC) columns.

	COLUMN PARAMETERS
Microbore HPLC	0.5-1 mm ID columns (compatible with normal HPLC systems)
Capillary HPLC / Capillary UHPLC	0.1-0.5 mm ID Columns
Nano-HPLC	<0.1 mm ID Columns. Packed fused silica capillaries with internal fits to keep the stationary phase.

9.2.4 Gas chromatography (GC)

Gas chromatography (GC, also known as gas liquid chromatography, GLC) has been a standard separation technique for many decades. There are many examples of its use in environmental fate studies (see Section 5) and for the analysis environmental samples. These are not reviewed here because this is a standard technique; for the same reason, no recent reviews of the method were found.

GC cannot be used for radiolabelled substances but may be used for stable isotope labelled or non-labelled substances. It has superior selectivity to liquid chromatography methods (although UHPLC may now approach it) and has the advantage that no water is present, therefore, hydrolysis/degradation reactions in solution cannot occur.

In effect, GC separates by the boiling point of the substance and can be used for volatile substances providing that they do not decompose upon heating or react with the stationary phase. Polar non-volatile compounds can be analysed after suitable derivatisation. Depending upon the volatility of the analytes, different methods of sample introduction can be performed such as headspace GC for very volatile substances and pyrolysis-GC for involatile substances. The later has mainly been used for oligomers and polymers. High temperature gas chromatography could be used for high boiling constituents. Table 9.9 summarises the different kinds of gas chromatography column.

Table 9.9: Gas chromatography columns*.

	DESCRIPTION	ADVANTAGES	DISADVANTAGES
Narrow Bore Capillary Columns	15-100 m columns of thin fused silica (0.1-0.35 mm ID) with internal surfaces of the column treated or silanised. Coiled around a lightweight metal support. • He or H2 carrier flow rate of 1-5 ml/min. • Sample capacity < 0.1 µl	Provides much higher separation efficiency than packed columns.	Resolving power for up to 100-1000 components depending upon the length of the column. Easily overloaded by the sample.
Wide Bore Columns (megabore)	Made from 0.53 mm internal diameter silica tube with lengths varying from 5-50 m. • Carrier gas flow rates as high as 15 ml/min (helium). • Sample capacity of 0.1 to 10 l µl	Higher capacity than narrow bore capillary columns.	Resolving power for up to 100 components depending upon the length of the column.
Packed Columns	1-3 m stainless steel or glass columns (2-4 mm ID) packed with a porous inert support on which the stationary phase is deposited (3-25%) • N_2 carrier flow rate of 10-40 ml/min. • Sample capacity 0.1-20 µl.	Primarily for routine analysis and not trace analysis. Could be used for prep-GC.	Limited efficiency and resolving power for up to 20 components.

* Summerfield (2010)

9.2.4.1 Derivatisation for GC

The aim of GC derivatisation is to produce a non-polar derivative from a polar substance so that it can be separated by GC. Table 9.10 summarises derivatisation methods for GC; it includes examples of their use in environmental analysis where these were found during literature searching.

Table 9.10: Derivatisation for GC.

ANALYTES	1. DERIVATIZATION TECHNIQUE	TARGET SUBSTANCE FORMED	REFERENCE
Phthalates	2. Alkaline hydrolysis 3. Acidification the obtained phthalic acid (PA) and alcohols 4. Extracted with organic solvent 5. Derivatised by silylation	R-O-CO-SiMe$_3$	Net *et al.* (2015)
Carboxylic acids R-COOH	• Methylation by CH2N$_2$; BF$_3$/methanol. • Silylation with chlorotrimethylsilane.	R-COOMe R-O-CO-SiMe$_3$	
Alcohols / hydroxy groups R-OH	• Methylation with methyl iodide/Ag$_2$O. • Silylation with trimethylsilyl donor such as HMDS or BSTFA. • Acylation with (R′-CO)$_2$O.	R-O-CO-Me R-O-CO-SiMe$_3$ R-O-CO-R′	Bielicka-Daszkiewicz *et al*. (2004)
Amines R-NH2	• Silylation with trimethylsilyl donor such as HMDS or BSTFA. • Acylation with (R′-CO)$_2$O.	R-O-SiMe$_3$ R-O-CO-R′	
Thiols and mercaptans R-SH	• Silylation with trimethylsilyl donor such as HMDS or BSTFA. • Acylation with (R′-CO)$_2$O.	R-O-SiMe$_3$ R-O-CO-R′	
Derivatisation of Chemical Warfare Agents (CWA)	Emphasised the importance of derivatising techniques to convert reactive groups into non-polar derivatives for ease of gas chromatography.		Popiel and Sankowska (2011)

9.3 Other Chromatography Techniques

9.3.1 Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) has been mainly been used in connection with radiochemistry. Examples can be found in Section 5.

9.3.2 Ion Chromatography

Ion chromatography is a liquid chromatography technique that may be used for ionised or ionisable substances. The method is summarised in Table 9.11. Its use for environmental trace analysis of polar organic pollutants is reviewed in Reemtsma (2003). Analysis of ionising substances is discussed further in Section 10.4.

Table 9.11: Anion or Cation Ion Chromatography (IC) Columns

STATIONARY PHASE	Consists of porous-layer beads that have cation or anion exchange sites.
MOBILE PHASE	Contains electrolytes such as Na_2CO_3 or $NaHCO_3$ for anions and HCl or $CH3SO_3H$ for cations.
SUPPRESSOR	The detection of low levels of ionic solutes in the presence of an eluting electrolyte is not feasible unless the latter is removed by the suppressor and converted to the barely conductive carbonic acid (H2O & CO_2). This leaves the solute ions as the only ionic species enabling them to be sensitively detected.

9.3.3 Capillary Electrophoresis (CE)

Capillary electrophoresis (CE) is a liquid chromatography technique that may be used for ionised or ionisable substances. Chang *et al.* (2016) reviewed the use of capillary electrophoresis (CE) to determine pesticides and their metabolites in environmental samples. They considered CE to be a fast, reliable, and environmentally friendly method that can accurately analyse dilute, complex samples containing both parent substances and their metabolites. Analysis of ionising substances is discussed further in Section 10.4.

9.3.4 Size exclusion / Gel Permeation Chromatography (GPC)

Size exclusion and Gel Permeation Chromatography (GPC) does not have the resolving power to be used directly as a separation technique in environmental fate studies. However, it could be used for fractionating a complex substance prior to further separation. We have not found examples in the literature of the use of this technique in environmental fate laboratory studies or environmental trace analysis.

9.3.5 High pressure size exclusion chromatography (HPSEC)

High pressure size exclusion chromatography (HPSEC) is a technique that can be used for separation of very high molecular weight species (>2000 Da). Conte and Piccolo (1999) used high pressure size exclusion chromatography (HPSEC) to separate humic substances. This is an example of a difficult substance that is almost impossible to separate in any other manner. It has a far higher efficiency than SEC but still does not compare to HPLC separation. We have not found examples in the literature of the use of this technique in environmental fate laboratory studies or environmental trace analysis.

9.3.6 Supercritical fluid chromatography (SFC)

Supercritical fluid chromatography (SFC) is a form of chromatography that uses a supercritical fluid such as carbon dioxide as the mobile phase. Fekete *et al*. (2015) stated that SFC columns packed with 2.7 µm SPP particles represented a good compromise between throughput and resolution. In order to obtain the highest possible kinetic performance in SFC, there was a need to further reduce extra-column band broadening down to 10 µL or less and the system upper pressure limit should be extended to about 800 bar, to have a sufficient flexibility, particularly when working with a mobile phases containing a relatively high amount of organic solvent (e.g.

20-40% MeOH). This is a relatively new technique that can provide good separation of compounds that a not stable for GC analysis. It is also a good method for enantiomeric separations. However, it is unlikely to be available in test laboratories. There are no examples of its use in laboratory environmental fate studies and only a small number in environmental trace analysis in the literature (for example, Fekete *et al.* 2015 and Patel *et al.* 2016).

9.4 Hyphenated Chromatography Methods

Use of hyphenated methods of chromatography (LC-LC, GCxGC, comprehensive GC), or separation by one chromatography technique followed by another, has been reported for complex substances to increase the resolving power and reduce peak co-elution, however the use of such techniques appears to be predominantly within academia. As yet, there appears to be little evidence of inter-laboratory reliability, and their expense has meant that they have not been used much outside the research setting (with the exception of 2 D-TLC).

The use of GCxGC is specifically mentioned in REACH R11 (ECHA,2017) in regard to the characterisation of petroleum substances:

> "*For most petroleum substances, the complexity of the chemical composition is such that it is beyond the capability of routine analytical methodology to obtain complete characterisation… For the purposes of a PBT assessment of petroleum substances, when required, it is suggested that an analytical approach using GCxGC is used when feasible. This method offers a high resolution that may also be helpful in being more precise as to the exact type of structures present, (Forbes et al., 2006), in contrast to more generic methods based on Total Petroleum Hydrocarbon (e.g. TNRCC Method 1005). Still other methods could be used to characterize the composition of petroleum substances as the GCxGC method has the caveat that it can only be used for carbon numbers up to around C30.*"

Despite the technology of GCxGC being over two decades old, it should be noted that our experience indicates that the use of GCxGC is very limited for many reasons:

- To date, there are no standard methods (such as ASTM) or accepted trained algorithms for peak alignment in GCxGC chromatograms. It is hoped that this will be addressed in the next decade by ASTM and other recognised standards authorities.
- There are large interlaboratory differences in quantification due to in the main the algorithms used to determine the identity of the constituents.
- The addition of heteroatoms increases the polarity of the constituents. The ability of the algorithms to determine and quantify is still problematic after two decades.
- The upper limit of the analytical method in carbon number is C30.

There are examples of GCxGC being used in laboratory environmental fate studies, these studies focussed on the analysis of non-steroidal anti-inflammatory drugs and PAHs (Idowu *et al.* 2018 and Marsik *et al.* 2017).

9.5 Mass Spectroscopy (MS)

Mass spectroscopy (MS) is a very powerful tool that can be employed in many ways. These include:

- Determination of the identity of a radioactive moiety directly or after fractionation.
- Connection in line or parallel with a chromatography method.
- Can be used for both identification and quantification.

MS is appropriate for quantification if a suitable standard is available i.e. for parent substance or known, available metabolite. However, quantification of unknowns (most metabolites) is problematic. Some modes of ionisation can be very selective in the types of compounds they can observe. It is not always obvious what this selectivity will be (unlike for example UV-vis detection where lack of chromophore means that a substance will not be seen). Many MS techniques will have difficulty detecting polar compounds. This is problematic for detection of metabolites as many metabolic processes generate more polar molecules.

Different types of MS are described in Sections 9.5.2 - 9.5.5. For known metabolites, identification/quantification can be carried out using low resolution MS (Section 9.5.2). This is a standard technique combined with GC or LC separation and there are many examples of its use in environmental fate studies (see Section 5).

HRMS can also be a very helpful technique for identification of unknown transformation product and various techniques that allow greater identification power than low resolution MS are summarised in Sections 9.5.3 - 9.5.5. Examples of the use of these techniques are given in the sections below.

9.5.1 Artefact formation in Mass Spectrometry

Keller *et al*. (2008) states that with the invention of electrospray ionization and matrix-assisted laser desorption/ionization, scientists employing modern mass spectrometry naturally face new challenges with respect to background interferences and contaminants that might not play a significant role in traditional or other analytical techniques (Keller *et al*., 2008). Mass spectroscopy will only detect ionised species, consequently non-ionised species are not detected. A stark example of this was given by Rodgers *et al*. (2019) who demonstrated that only one sixth of the signal from weathered crude oil was singly ionised by electron impact MS. The remainder either gave no signal or had multiple ionisations.

Pseudo-molecular ion formation occurs in soft ionisation techniques of electrospray ionisation mass spectrometry (ESI-MS) with the formation of dimer, dimer adduct and other adducts. The formation of particular pseudo-molecular ions depends on ion affinity and molecular structure of the analyte as well as the solvent/buffer conditions used (Schug and McNair, 2002). Adduct formation with ammonium and sodium ions is poorly understood and complicated to control. (Kruve and Kaupmees, 2017). These are a few examples in the literature:

- Mullins *et al.* (2019) described the significant formation of in-source fragmentation, dimer and dimer adducts result in minimal "mother ion" [M-H]- signal for the ESI- of hexafluoropropylene oxide-dimer acid (HFPO-DA).
- Schug and McNair (2002) described the dimer formation of six acidic anti-inflammatory pharmaceuticals that contain a carboxylic acid moiety and various adducts of [2 M-H]- and [2 M-(2H+Na)]- formed.
- Kruve and Kaupmees (2017) concluded that an appropriate choice of additive may increase sensitivity by up to three orders of magnitude.
- Erngren *et al.* (2019) demonstrated adduct formation in electrospray ionisation-mass spectrometry with hydrophilic interaction liquid chromatography (HILIC) was strongly affected by the inorganic ion concentration of the samples. They warned that where internal standards were not used or properly matched, great care must be taken to ensure minimal variation of inorganic ion concentration between samples. Also, the use of alkali metal ion adducts as quantitative target ions in relative quantitative applications should be avoided if proper internal standards were not used.

9.5.2 Low Resolution Mass Spectroscopy

Electron ionization (EI), a hard ionization technique, is the typical method of choice for analyses of small (<1000 Da), nonpolar and volatile compounds coupled to a gas chromatograph (GC) or liquid chromatograph (LC). This involves ionization by electrons with about 70 eV energy and so yields very reproducible mass spectra with a large number of fragments. However, these spectra frequently lack the molecular ions (M^+) due to the high internal energy transferred to the precursors and this lack of molecular weight information is one of the greatest limitations of this ionization method in the determination of unknowns. To reduce the complexity from the matrix, the mass spectrometer is operated in single ion mode (SIM) where only the data for that particular ion is collected (normally +/- 0.5 m/z on a low-resolution MS). Therefore, compounds that do not have this mass (mass over charge, m/z) would not be detected by the mass spectrometer.

Other types of mass spectrometry are:

- Time-of-flight detector mass spectrometry (TOF-MS) is a very fast detector and commonly used for GC and LC.
- Quadrupole Mass Spectrometry (Q-MS) is a moderate resolution MS that is now increasingly commonly used with liquid chromatography (LC-MS).

9.5.3 Soft ionisation techniques

Soft ionization techniques are routinely to ionize thermolabile, low molecular weight, polar organic analytes. These methods usually yield ions with no unpaired electrons and the resulting $[M+H]^+$ or $[M-H]^-$ species are referred to as protonated or deprotonated molecules. Their low internal energy results in negligible fragmentation in a single stage MS experiment so the intact accurate mass for sensitive, fragile compounds and large biomolecules as well as their detailed structures could be determined when high pressure liquid chromatography (HPLC) is used.

There are numerous soft ionisation / chemical ionisation techniques that predominantly produce the molecular ion which makes determination of the analyte much easier, reduces noise and certainly improves detection limits. The mode used depends upon the polarity and acidity of the analytes, some of which may not be suitable due to matrix and sample type. These techniques are summarised in the sections below. Electrospray ionization is far more widespread than any other ionization type. (Steckel and Schlosser, 2019).

9.5.3.1 Chemical Ionisation (CI)

Using this technique, the molecular fragmentation is negligible. Chemical Ionisation (CI) techniques are summarised in Table 9.12.

Table 9.12: Chemical Ionisation (CI) techniques.

IONIZATION	SPECIFICITIES	CHARACTERISTICS	REFERENCE
Atmospheric pressure chemical ionization (APCI)	Useful for small, thermally stable, semipolar compounds such as lipids and polar PAH metabolites.	• Chemical ionisation performed under atmospheric pressure. • Used after LC and CE separation. • Unlike ESI, ions do not carry multiple charges.	
Positive [(+) ESI] electrospray ionisation	Weak acid / weak basic species with at least one positive charge with hetero atoms (e.g. quaternary ammonium salts)	• Dimer formation at high concentrations or if analyte has a tendency to form complexes. • Neutral species such as ketones, aldehydes of alcohols cannot be detected. • Only electrospray-compatible solvents (e.g., acetonitrile, water, methanol, ethanol) containing 0.1% formic or acetic acid to enhance protonation or solvent mixtures can be used for dissolving samples during sample preparation. • Deionised should be used for aqueous solutions to effectively minimize the intensity of sodium adducts in the spectra. • Low-binding, high-quality plastic sample tubes to decrease the otherwise always present plasticizer contamination. • Larger molecules with several charge -carrying functional groups such as proteins and peptides in (+)ESI can exhibit multiple charges resulting from $[M+nH]^{n+}$.	Steckel and Schlosser, 2019
Negative [(-) ESI] electrospray ionisation	Selective ionisation of highly acidic species that have at least one negative charge.		
Desorption electrospray ionization (DESI)	Permits fast monitoring of organic contaminants (e.g. drugs, explosives and biopolymers).	• An ambient ionisation technique in which solvent electrospray is directed at a sample which a voltage is applied.	Manikandan *et al.* (2016)

9.5.3.2 Photon ionisation (PI)

This technique permits a wide range of adjustable ionisations. The dominant ion is $[M+H]^+$. Table 9.13 summarises photon ionisation (PI) techniques.

Table 9.13: Photon ionisation (PI).

IONIZATION	SPECIFICITIES	CHARACTERISTICS	REFERENCE
Matrix-assisted laser desorption ionization (MALDI)	Solid substrates	• Cannot be attached to chromatography • Not a suitable method for OECD 305 studies.	
Atmospheric pressure photoionization (APPI)	Polar compounds such as lipids.	• Useful for less polar compounds (e.g., lipids)	
Resonance-enhanced multi-photon ionization (REMPI)	Uses a tuneable laser source to cause the ionisation.	• Has been used for the online analysis of dioxin precursors and PAHs in waste incineration flue gas.	Heger *et al.* (1999)
Proton-transfer-reaction (PTR)	Volatile organic compounds	• Proton transfer to gaseous organic compounds from the formation of free hydronium ions ($H3O^+$) produced by hollow cathode discharge with water vapour.	
Dielectric-barrier-discharge ionization (DBDI)	Large biomolecules.	• Formation of a low temperature plasma between two insulated electrodes.	

9.5.4 Tandem MS (MS/MS)

Tandem MS (MS/MS) uses selected reaction monitoring (SRM) to select an ion of a particular mass in the first stage MS. An ion product of a fragmentation reaction of the precursor ion is selected in the second MS for detection. Liquid Chromatography tandem mass spectrometry (LC-MS/MS) has become a key technique for environmental analysis as it permits the analysis of wide range of polar and non-volatile compounds with increased specificity, confidence of identification, while reducing sample preparation to a minimum (Rosen, 2007) The modern use of machine learning approaches to substructure discovery or metabolites could be considered the most important advance in MS/MS over the last few years. (Rogers *et al.,* 2019). A number of examples of the use of MS/MS in laboratory environmental fate studies were found (see Section 5). This is a powerful technique but does require expertise in the interpretation of results.

9.5.5 High Resolution MS

High Resolution Mass Spectrometers can resolve the mass/charge ratio of an ion to 4 decimal places in comparison to low resolution where accuracy is 1 to 2 decimal places. The use of HRMS allows for exact mass determination of the parent substance and/or fragments. High Resolution MS are expensive and require specialised operators. Examples are:

- Quadrupole time-of-flight mass spectrometers (QTOF-MS) with their higher mass resolution are ideal for the determination of molecular formulas of unknown compounds and for screening purposes.
- Triple-quadrupole mass spectrometers are highly suited for sensitive quantification and for qualitative analyses.
- Ion traps are especially suited for structure elucidation.
- Orbitrap^{TM} MS has been used in proteomics, metabolomics, environmental, food and safety analysis. This has been reviewed by Hu *et al*. (2005) and Perry *et al*. (2008)
- High Resolution-Electrospray Ionization-Mass Spectrometry (HR-ESI-MS) and Fourier-transform ion cyclotron MS (FT-ICR-MS) are other examples of high-resolution mass spectroscopy methods.

9.6 Detectors

Radiolabelled compounds are used almost exclusively analysed on high performance liquid chromatography or TLC based systems coupled to a detector. Gas chromatography-based techniques can be used for non-radiolabelled applications, also coupled to a detector. A summary of what kind of detectors can be used with each method of chromatography and their advantages/disadvantages is shown in Table 9.14 and 9.15. Radioactivity detectors, Mass Spectrometry (MS) and Nuclear Magnetic Resonance (NMR) are further discussed in Sections 8, 9.5 and 9.6.3, respectively.

It is important to be aware that different detectors can have different selectivity and sensitivity to different analytes. This can be significant when considering complex substances or transformation products.

9.6.1 Liquid chromatography detectors

Table 9.14: Commonly used detectors coupled to HPLC / LC (adapted from Summerfield, 2010 and Swartz, 2010).

	ADVANTAGES	DISADVANTAGES	ANALYTICAL LOD	LINEAR RANGE
"Universal" Detectors				
Ultra violet-visible or diode array detector (UV-Vis or DOD)	Universal at low wavelength. Non-destructive	Position of absorbance bands influenced by pH , solvent and temperature.	ng	10^5
Refractive index detector (RI)	Original detector for HPLC. Low cost. Non-destructive. Universal detector	Cannot use gradient elution. Low sensitivity. Poor stability to temperature and flow changes.	µg	10^2
Evaporative light scattering detector (ELSD)	Detects most non-volatile analytes. Better sensitivity than RI. Can be used with strong absorbing solvents, e.g. acetone.	Requires the use of volatile buffers. Limited dynamic range.	high ng	10^3

	ADVANTAGES	DISADVANTAGES	ANALYTICAL LOD	LINEAR RANGE
Corona charged aerosol detector (CAD) / Corona discharge detector (CDD)	Highest sensitivity of the universal type detector. Wide dynamic range. Detects any non-volatile or semi-volatile analyte. Consistent response. Ease of use.	Destructive. Requires the use of volatile buffers	low ng	10^4
Mass Spectroscopy				
Mass spectrometer (MS) • Single quadrupole • Time of flight (TOF)	Selective. Most commonly used.	Destructive. Normally operated in SIM mode so may not observe metabolites.	ng	10^2
High resolution mass spectroscopy (HRMS) • Triple quadrupole • Quadrupole-TOF • Tandem Mass spectrometer LC-MS/MS	Very selective. Could be used with cold and radiolabelled compounds.	Very expensive and needs very experienced analysts.	ng	10^3
Electrochemical				
Conductivity	Charged analytes, inorganic ions, or organic chemicals (e.g. amines, amino acids). Normally used in Ion Chromatography (IC).	Requires suppression of mobile phase background conductivity and electrode fouling. Requires special Ion Chromatography system and columns.	pg	10^2–10^3
Electrochemical Detector (EC) / Amperometric	Only oxidizable and reducible analytes are detected e.g. aromatic amines, phenols, indols, thiol and nitro-derivatives.	Mobile phase must be conductive; Not all compounds are detected.	pg	10^4–10^5
Spectroscopy				
Fluorescence detection (FLD)	Very selective. And sensitive. Works well with gradients.	Influenced by pH and solvent. Often requires derivatisation.	pg	10^3
Nuclear magnetic resonance spectroscopy (NMR)	Very selective	Can be used in quantification of complex substances. Very expensive and specialised.	Unknown	Not known

9.6.2 Gas chromatography detectors

Gas chromatography is not used with radiolabelled samples. The US EPA (2007) Method 8270E provides procedures for analysis of solid, water and wipe samples for detection and measurement of semi-volatile organic compounds, using gas chromatography/mass spectrometry (GC-MS). The US EPA (2007) Method 8260 D provides procedures for analysis of solid, water and wipe samples to detect and measure selected volatile organic compounds, using purge-and-trap GC-MS. Table 9.15 shows commonly used detectors coupled to GC.

Table 9.15: Commonly used detectors coupled to GC; 1 ng = 10-12 g (adapted from Summerfield, 2010 and Nießner and Schäffer, 2017)

GC DETECTOR	COMMENTS	SELECTIVITY	ANALYTICAL LOD	LINEAR RANGE
Common detectors				
Flame ionization detector (FID)	Destructive detector and the most commonly used with GC.	C	0.1 ng	10^7
Thermal conductivity detector (TCD)	Robust and cheap but not as sensitive as FID. Non-destructive detector so can be used for preparative GC.	Universal	10 ng	10^5
Flame photometric detector (FPD)	Mainly used in pesticide analysis.	P, S	0.1 ng (P)	10^4
Electron capture detector (ECD)	Needs a licence to handle the radioactive source. Commonly used in pesticide analysis.	Halogens, R-NO_2, R-CN	10^{-6} ng	10^5
Mass Spectrometry				
Low Resolution Mass spectrometer (MS)	Specific compounds are monitored in single ion mode (SIM). • Single quadrupole • Time of flight (TOF)	Very selective in SIM mode	10 ng (TIC) 0.1 ng (SIM)	10^4
High Resolution Mass Spectroscopy (MS/MS or HR-MS)	Expensive and mainly in research laboratories. • Quadrupole-TOF (Q-TOF) • Quadrupole-Orbitrap™-HRMS • Triple quadrupole • Ion Trap	Very selective	10^{-4} ng	10^6
Rarely Used				
Fourier Transform Infrared (FT-IR)	Excellent for organic mixtures such as fragrances, solvents, carbonyls etc. Non-destructive so can be used for preparative GC.	Selective	1000 ng	10^3
Alkali FID (AFID) / Nitrogen-Phosphorus Detector (NPD)	Short detector life and rarely used.	P, S, N, halogens	0.01 ng (P) 0.1 ng (N)	10^4 10^4
Electrolytic conductivity detector (ELCD)	Needs a clean hydrogen source. Rarely used.	Halogen, S, N	10^{-6} ng	10^5
Photoionization detector (PID)	Gas detector using the UV irradiation of GC effluent	Benzene, toluene etc.	Unknown	
Thermoionic ionization detector (TID)	Used in Pyrollysis-GC where nitro-groups (nitro-PAHs or explosives) or halogens are formed.	Halogens, N	Unknown	

9.6.3 Nuclear magnetic resonance (NMR)

Nuclear magnetic resonance (NMR) is a very powerful tool for the identification, and potentially for the quantification, of compounds. Unlike mass spectrometry, NMR has the advantage of being non-destructive. It has been used in environmental analysis after fractionating by chromatography (SFC, TLC, LC, HPLC) and can be used to characterise complex mixtures using multi-dimensional NMR. The selectivity is enhanced by using different nuclei in combination including ^{1}H, ^{2}H, ^{13}C, ^{14}N, ^{15}N, ^{17}O, ^{31}P, ^{32}Si, ^{35}Cl, ^{37}Cl.

NMR may be a useful detection technique, especially when dealing with complex substances. For example:

- Zeltst *et al.* (2019) characterised polar compounds using supercritical fluid chromatography–nuclear magnetic resonance spectroscopy (SFC–NMR
- Kuhn *et al.* (2019) used an integrated approach for mixture analysis using MS and NMR techniques.
- Jenne *et al.* (2019) used targeted NMR experiments: an example of selective ^{13}C-12C bond detection in complex mixtures by ^{13}C labelling of algae. Then the *daphnia* eat the algae that were suspended in the NMR.
- McGill *et al.* (2019) applied novel sold phase extraction-NMR protocols for metabolic profiling of human urine.

The purity, quantity and cleanliness of a sample required to perform NMR is not standard for any laboratory due to paucity of sample size, matrix from which it is extracted, and quantity of material generated. Additionally, this technique has a high cost.

10. Summary of specific issues for "difficult" substances

Some (combinations of) physico-chemical properties can lead to technical challenges when conducting aquatic toxicity studies. Substances which have one or some of these properties are termed 'difficult substances' in OECD 23 (2019), and guidance is provided on how to tackle the challenges associated with performing aquatic toxicity studies with these substances. A subset of these 'difficult' properties that the authors of this report consider could cause issues in environmental fate studies is listed below in Table 10.1. This table also lists the Section of this report in which further information about the substance type can be found.

Table 10.1: Properties of "difficult" test chemicals that could cause issues in bioaccumulation or degradation studies. After OECD 23 (2019).

PROPERTY	NATURE OF DIFFICULTY	SEE SECTION
Hydrophobic	• Maintaining exposure concentrations • Analysing exposure concentration	10.1
Poorly or sparingly water-soluble	• Achieving/maintaining required exposure concentrations • Analysing exposure concentration	10.3
Colloids	• Achieving, maintaining and measuring exposure concentrations	10.1
Adsorbing	• Maintaining exposure concentrations • Analysing exposure concentration	10.1
Surfactants	• Assessing phase behaviour in test solution • Maintaining exposure concentrations • Achieve water saturation • Analysing exposure concentration	10.3
Complexing	• Distinguishing complexed and non-complexed fractions in test solution • Depletion of nutrients in test solution	11.4
Ionised	• Distinguishing ionised and non-ionised fractions in test solution • Defining exact exposure concentrations	11.4
Hydrolytically unstable	• Maintaining exposure concentrations • Achieve water saturation • Toxicity of breakdown products	11.6
Volatile	• Maintaining exposure concentrations • Achieve water saturation • Analysing exposure concentration	11.5
Multi-component substances	• Chemical characterisation (e.g. identification and quantification of individual chemical components and determination of their properties) • Preparing representative test solutions • Defining exact exposure concentrations	10.2
Toxic at low concentrations	• Achieving/maintaining required exposure concentrations • Analysing exposure concentration	6.2.1

The other properties of difficult substances defined by OECD 23 (2019) that are not envisaged to be a problem in bioaccumulation or degradation studies are shown in Table 10.2.

Table 10.2: Properties of "difficult" test chemicals that are unlikely to lead to difficulties in bioaccumulation or degradation studies. After OECD 23 (2019).

PROPERTY	NATURE OF DIFFICULTY
Photo-degradable	Maintaining exposure concentrations Toxicity of breakdown products
Biodegradable	Maintaining exposure concentrations Toxicity of breakdown products
Subject to corrosion/ transformation	Achieving, maintaining and measuring exposure concentrations Toxicity of breakdown products
Oxidisable	Achieving, maintaining and measuring exposure concentrations Toxicity of modified chemical structures or breakdown products

It is particularly important to understand the identity and physicochemical properties of the substance (and potential transformation products) prior to performing environmental fate studies. This was discussed in detail in Section 6.

The following discussions include the key issues for selected difficult substance types based upon experience and the literature review.

10.1 Hydrophobic substances and sorption to particulates and surfaces

When designing tests, it is important to consider the sorption of substances to particulates and surfaces. The OECD 23 (2019) guidance on difficult substances discusses the problems of sorption of a test substance to test vessels but does not consider problems associated with suspended particulate matter (<0.7 micron) that would pass through filters. The OECD TG 305 limits the acceptable values for particulate matter (dry matter, not passing a 0.45 µm filter) and total organic carbon to 5 mg/l and 2 mg/l, respectively. If an aquatic bioaccumulation study is performed with a hydrophobic substance, the potential for adsorption to food, faeces and suspended particulate matter must be considered. Good study design requires that all food residue and faecal matter are removed from the test system at regular intervals on a daily basis and should ideally be present for short periods of time only, therefore the largest losses observed during these studies would be expected to be sorption to the walls of the test vessel.

Losses from the water phase when dealing with sediments and soils could increase with the sorption to particulates and suspended particulate matter. For example, polycyclic aromatic hydrocarbons (PAHs) become attached to the surface of dispersed particles (Li *et al*., 2014, and Oln *et al*., 2014). Particles (> 0.7 micron) and suspended particulate matter (SPM) in water (<0.7 micron) can cause a difference in partition behaviour in separation.

Albaseer *et al*. (2011) and Petrovic (2014) review problems associated with sorption of analytes and their recommendations are discussed in Section 7.2 of this report.

10.2 Complex substances (UVCB/multi-constituent)

For a complex substance, the 'substance' to be tested in the environmental fate studies must be identified. This may be the whole substance, specific constituent(s) or fraction(s) of the substance. Guidance on selection of the appropriate test substance is given in ECHA Guidance R11 (ECHA, 2017).

Each constituent in a multi-constituent substance or UVCB substance adds complexity to the analytical methodology and to the interpretation of the study results. Where it is appropriate to test specific constituents of the substance, each additional constituent selected will also add to the cost of the study, particularly for radiolabelled studies, where the cost associated with synthesis of radiolabelled constituents is significant. Throughout ECHA Guidance R11, it is stated that the feasibility and proportionality of efforts should play a part design of the test, e.g. in selecting the 'substance' to be tested. Several approaches to selection and assessment of the appropriate test 'substance' are described in R.11.4.2.2.2. These essentially focus on achieving an assessment of the persistence and bioaccumulation potential of the multi-constituent/UVCB based on a 'worst-case' assessment of the constituents or fractions.

If testing whole substance, constituents (and impurities in the case of multi-constituent substances) potentially have a wide range of physical chemical properties and subsequently very different degradation pathways:

- This would affect their extraction efficiency and partitioning between the water and the organic content of the soil/sediment/fish.
- There would be large chromatographic differences between polar and non-polar compounds.
- It should be noted that synthesised multi-constituent and UVCB substances would contain a proportion of the starting materials. These could be confused with metabolites from the constituents.
- The sensitivity of the analytical method required to identify and quantify multiple constituents, and potential metabolites of these constituents may be a significant challenge.
- Analytical reference standards are unlikely to be available for individual constituents, increasing the difficulty of quantification.

The partitioning of constituents of variable solubility in a multi-constituent substance can also be a challenge, where the soluble constituents are dissolved preferentially. This is even more complex for Unknown or Variable Composition, Complex Reaction Products and Biological Materials (UVCB) such as petroleum-derived products (Letinski *et al*., 2016), wood pulp extracts or essential oils.

10.3 Poorly soluble substances

10.3.1 Poorly soluble liquids

A poorly soluble liquid as defined by ECHA Guidance R11 (ECHA, 2017) has a water solubility of less than 1 mg/l at 20°C. Poorly soluble liquids are often highly sorptive compounds that adhere to a solid surface so extracting and analysing them is a challenge. Many of these compounds adhere to glass surfaces as well as particulates and suspended particulate matter. Therefore, polytetrafluoroethylene (PTFE), borosilicate (i.e. Pyrex®) glassware should be used to minimize adsorption to glass surfaces. Care should also be taken to identify if the laboratory glass and plastic ware could be a potential source of contamination in analysis. This is critically important when non-radiolabelled techniques are employed.

The challenges for this kind of substance often relate more to generation and maintenance of a homogeneous test solution, and taking representative samples, than to the analytical stage (ECETOC 1996 and ECETOC 2014). Therefore, careful consideration should be made as to whether a homogeneous solution can be achieved. A preliminary study may need to be carried out in order to determine this.

The behaviour of a poorly soluble liquid in water depends on their density, the temperature and the sorption to the test system:

- Poorly soluble liquids that are less dense than water often form a skin on the surface. Then the top layer will have a higher concentration of the substance.
- Poorly soluble liquids with density similar to water form droplets that are dispersed throughout the water column. This can lead to high variation in measured concentrations and the results may be outside the normally accepted range of 80-120%. (OECD 23, 2019) in bioaccumulation study with aqueous exposure.
- Poorly soluble liquids that have a density greater than water form droplets on the bottom of the water column and are only dispersed kinetically by stirring or disturbance. Higher concentrations are found in the bottom sample. If the substance has a similar refractive index to the test solution this would be very difficult to observe.
- A poorly soluble liquid may form a dispersion of that liquid substance. This dispersion can form larger liquid droplets over time. This can lead to measured concentrations greatly exceeding the theoretical solubility. This has been observed for practically insoluble PAHs in water (Redman *et al.*, 2012). These droplets may act as a reservoir of test material (Redman, 2015) and can physically interact with the test organisms. Various factors influence the droplet size and longevity including dispersion method, composition and use of co-solvent.

10.3.1.1 Use of co-solvent

Spiking of water is may be performed in conjunction with a co-solvent. For example, OECD TG 307 for aerobic conditions mentions solvents as possible 'chemical reagents used' and describes how to deal with them without setting a concentration limit. This can result in a false observation of the solubility of the chemical, where more of the substance seems to be dissolved than is really the case (Redman, 2015). It takes time for the solvent shell around the chemical to be fully replaced by water. This can lead over time to the formation of droplets, aggregation or deposition upon solid surfaces such as the glassware or to the fish itself. This creates a heterogeneous solution, potentially stratification and hence contributes to the loss of the analyte. If the solvent volumes used is significant, a separate phase may be formed in the test system, and the preferential distribution of the test substance into this phase may limit the bioavailability of the substance in the compartment of interest.

Therefore, maintaining the dosing or maintaining homogeneous solution is difficult with poorly soluble liquids especially when going below 1 µg/l. In addition, clear, colourless liquids can be difficult to distinguish from the aqueous media and there is no simple method (such as Raleigh Tyndall Scattering as used with solids) to determine whether the poorly soluble liquid has dissolved.

Additional problems can arise where soluble substances are present as well as the insoluble fraction (for example, as impurities of a mono-constituent substance or constituents of complex substances, such as multi-constituent or UVCB).

The following substance types can themselves act as co-solvents, or can react to form substances which become co-solvents, so increasing the amount of less soluble constituents:

- Substance that hydrolyse and release smaller polar molecules such as methanol, ethanol, etc. could increase the solvent loading.
- Substances that have ionisable groups can influence the pH of the solution making other constituents more or less soluble.
- Substances provided in a salt form may be difficult to test, as the conjugate acid/base may not be stable enough during the laboratory assessment period.

10.3.1.2 Passive dosing

Passive dosing is a method of maintaining freely dissolved concentrations of hydrophobic organic chemicals (Log $K_{ow} > 5$) that are poorly soluble, and therefore has the potential to be used to achieve stable aqueous concentrations for an aqueous bioaccumulation study. Passive dosing is mentioned in both OECD 23 (2019) and OECD 264 (2017) and is becoming more widely employed in BCF testing. Mono-constituents, multi-constituent and UVCBs have been dosed in this manner, although it should be noted that when dosing UVCBs with this technique it may be the case that not all of the chemical classes within the UVCB will appear in the solution. (e.g. some insoluble chemicals which are degradable will not appear in the solution). A high purity polymer or analytical grade polymer such as polydimethylsiloxane (PDMS) must be used. The principles of passive dossing are outlined in Annex 6 of OECD 23 (2019) and further information is provided in Appendix 1.

10.3.2 Poorly soluble solids

Low solubility solids are a challenge particularly in extraction from the matrix if their solubility is far less than 1 part per million (1 mg/l). They have similar issues to poorly soluble liquids. However, it is possible to determine whether a solid is dissolved using Rayleigh-Tyndall Scattering.

The lower the solubility, the more difficult it is to be certain that the sample is homogeneous in nature. This is shown by orders of magnitude differences in the extraction efficiency which can in part be corrected for by the addition of internal standards in the extraction phase that have similar physical properties.

10.4 Surfactants

This section covers analysis of test items that have surfactant properties (and is also relevant to substances which demonstrate surface activity). Use of surfactants as aids to analysis is not covered.

Surfactants cover a wide range of analytes with different polarity and acidity of analytes, including:

- Anionic surfactants, which are weakly acidic with carboxylic acid groups or strongly acidic with aromatic sulfonate, haloacetic acid, linear alkylbenzene sulfonate, or aliphatic sulfonate groups.
- Neutral compound classes, namely alkylphenol ethoxylates, alcohol ethoxylates,
- Cationic surfactants are mostly quaternary ammonium compounds.

Surfactants are very difficult substances to extract and measure because of their physical properties. In addition, for multi-constituent substances, the presence of a surfactant can influence other chemicals present in the test system making insoluble substances potentially more bioavailable.

Reemtsma (2003) stated that liquid chromatography-mass spectrometry using atmospheric pressure ionization (LC-API-MS) has drastically changed the analytical methods used to detect polar pollutants and metabolites in water. The selection of LC conditions depends upon the polarity and acidity of the analytes. The following techniques are possible:

- Reversed-phase liquid chromatography
- Ion-pair chromatography
- Capillary electrophoresis,
- Ion chromatography.

A range of techniques has been used to characterise non-ionic and ionic surfactants in soil and water; some examples for non-ionic surfactants are given in Tables 10.3 and 10.4. Kurrey *et al*. (2019) reviewed analytical approaches and challenges, including separation, pre-concentration, analysis and method validation for surface active agents in the environment.

Table 10.3: Examples of detection of non-ionic surfactants in water and soil.

	TECHNIQUES	COMMENT	REFERENCE
Non-ionic surfactants in water	SPE then LC-MS	Non-polar surfactants [alcohol ethoxylates (AEs) and alkylamine ethoxylates (AMEs)] are used as adjuvants in pesticide formulations. Hydrophobic SPE cartridges with Porapak RDX [polymeric poly(divinylbenzene-vinylpyrrolidone) resin] were used to extract alcohol ethoxylates (AEs) and alkylamine ethoxylates (AMEs) from ground water, surface water and soil interstitial water. The detection limits for alcohol ethoxylates were 16–60 ng/l and 35–93% recoveries. Those for alkylamine ethoxylates were 0.3–6 µg/l and 28–96% respectively. The procedure was employed on samples of ground water, soil interstitial water and samples collected from farming areas. Individual AEs were detected at concentration levels ranging from 33 to 189 ng/l water. Detection was by LC-MS.	Krogh (2002)
Non-ionic surfactants in soil	PLE cleaned up by SPE Analysed by LC-APCI-MS	Alkylphenols (octyl and nonylphenol), alkylphenol polyethoxylates (APEOs), and alcohol ethoxylates (AEOs) determined in a Mediterranean forest soil amended with sludges from six wastewater treatment plants (WWTPs). Isolated from soil by pressurized liquid extraction (PLE) using a mixture acetone–hexane (50:50 v/v), the extracts were cleaned up by solid-phase extraction (SPE, C18), and determined by LC-APCI-MS)	Andreu (2007)

	TECHNIQUES	COMMENT	REFERENCE
		using analytical standards for quantification. AEOs and APEOS were identified by monitoring the corresponding ammonium adduct $[M^+NH3]^+$ Octyl and nonyl and the deprotonated molecule $[M–H]^−$ for octyl and nonylphenol. Recoveries by spiking soil samples ranged from 89 to 94%. LOQ = 1 to 100 μg/kg depending upon analyte.	
Alcohol ethoxylates in water	LLE with ethyl acetate. LC-MS/MS in multiple reaction monitoring (MRM) mode	Alcohol ethoxylates (AEs) monitored by m/z = 44 of a single oxyethylene subunit from river water in Poland. LOD of 1-9 ng/l	Zembrzuska (2016b)

Table 10.4: Degradation of Surfactants

	METHODS	NOTES	REFERENCE
Degradation pathways of alcohol ethoxylates	LLE with ethyl acetate. LC-MS/MS in multiple reaction monitoring (MRM) mode	Alcohol ethoxylates under aerobic conditions are biodegraded by two pathways. • Central fission with the formation of PEG. • Ω-oxidation of the oxyethylene chain with the formation of carboxylated AE and subsequent shortening of the oxyethylene chain by a single unit.	Zembrzuska (2016a)
Degradation of surfactants	14C labelled	The biodegradability of dodecyl benzene sulphonate (LAS), nonylphenol-di-ethoxylate (NP2EO) and tridecyl-tetra-ethoxylate (LAE) in soil at 10 and 400 mg/kg.	Gejlsbjerg (2003)
Biodegradation of anionic surfactants and non-ionic surfactants	No analytical mentioned in abstract	The extent and kinetics of the primary biodegradation of alkyl chain: dodecyl ethoxy sulfate (C12-AES), sodium dodecyl sulfate (SDS), dodecyl alcohol ethoxylate (C12-AEO) and dodecyl benzenesulfonate (C12-LAS). Primary biodegradation half-life < 2 days.	Pérez-Carrera (2010)
Degradation of alcohol ethoxylates in marine sediments	No analytical mentioned in abstract	Study of anaerobic degradation and aerobic degradation products/precursors (polyethylene glycols, PEGs) in marine sediments.	Traverso-Soto (2016)
Degradation non-ion and anionic surfactants	No analytical mentioned in abstract	Linear alkylbenzene sulfonates (LAS), alkyl ethoxysulfates (AES), nonylphenol ethoxylates (NPEOs) and alcohol ethoxylates (AEOs) in different aquatic ecosystems. Urban wastewater discharges and industrial activities were identified as the main sources for these compounds and their metabolites. • LAS, AES and carboxylic metabolites remained in the dissolved form (87–99%). • NPEOs and AEOs were mostly associated with particulate matter (65–86%), so their degradation in the water column was limited due to their lower bioavailability.	Lara-Martín (2008)

10.5 Ionising substances

The key issue for ionising substances is the extraction of the analyte from water and ionic interaction with soil/sediment organic and inorganic material. Various methods have been developed for efficient extraction of ionising substances from water and solid matrices including ion-pairing, ion exchange resins, and ionic pressurised liquid extraction columns. Some of these techniques are discussed in Section 9 of this report. Some examples of the analysis of ionic surfactants are given in Section 9.3.2. The selection of an extraction method appropriate to the test substance and matrix is the crucial step in analytical method development.

10.6 Volatile compounds

Volatile organic compounds (VOCs) are defined as difficult substances, and bioaccumulation and degradation simulation studies may not be possible with very highly volatile compounds. Study design for testing with such compounds is extremely challenging and significant adaptations to the test system may be required. In addition, volatile compounds can be lost during all stages of sample handling and extraction. Chary and Fernandez-Aiba (2012) reviewed the determination of volatile organic compounds in drinking and environmental waters. Ketola *et al.*, (1997) compared different methods for the determination of volatile organic compounds in water samples. We have found examples of OECD TG 305 studies on volatile substances, including siloxanes, hydrocarbons and Persistent Organic Pollutants (POPs) in the literature.

Reviews

- Chary and Fernandez·Aiba (2012) reviewed the determination of volatile organic compounds in drinking and environmental waters.
- Keto *et al.* (1997) compared of different methods for the determination of volatile organic compounds in water samples.

EPA Standard Methods

- US EPA (2007) *Method 8270E* provides procedures for analysis of solid, water and wipe samples for detection and measurement of semi-volatile organic compounds, using gas chromatography/mass spectrometry (GC-MS)
- US EPA (2007) *Method 8260 D* provides procedures for analysis of solid, water and wipe samples to detect and measure selected volatile organic compounds, using purge-and-trap GC-MS.

Examples

- Starokozhev *et al*. (2011) investigated the partitioning mechanism for volatile organic compounds in a multiphase system.
- Amaral *et al*. (2010) analysed ultra-trace amounts of volatile organic contaminants in groundwater by vacuum extraction.
- Kistemann *et al*. (2008) determined vinyl chloride (VC) and precursor volatile organic compounds (VOC) in groundwater.
- Belanger *et al*. (2007) evaluation the use of microwave-assisted process technology for head space analysis of volatile organic compounds (VOCs).
- Kavcar *et al*. (2006) analysed volatile organic compounds (VOCs) in drinking water.

10.7 Transformation products with “difficult” properties

Transformation products could have any of the difficult properties described in Table 10.1. It is unlikely that a metabolite would be more insoluble/sorptive than the parent substance. However, it is possible that metabolites that are more volatile or more reactive than the parent or are ionisable or have surfactant properties when the parent substance does not (or vice versa), could be formed.

Techniques for handling volatile or ionising substances would be similar to those outlined for parent substances with these properties. It may be important to be aware of the potential for formation of such products to avoid, for example, losses by volatilisation (Section 6.3 briefly reviews *in silico* methods for metabolite prediction). If the test substance and its degradation products have very different properties, they may require different extraction methods, separation and detection methods, increasing the complexity of the analytical process.

Reactive groups present in transformation products could react with the substrate or itself in the form of aggregates and oligomers. These could be lost as non-extractable residue (NER) that could not be extracted and therefore analysed. In addition, if a transformation product can undergo condensation/aggregation/polymerisation reactions, then extraction, freezing or solvent reduction can change the properties of the analyte. It is again important to understand the potential for formation of such products and select appropriate methods for extraction, storage and concentration.

11. Conclusion and Recommendations

11.1 Regulatory context and requirements of the OECD test guidelines

For substances containing an organic moiety, all regulatory frameworks require an assessment of the persistence (P) and potential for bioaccumulation (B) of the constituents, any impurities and additives present at ≥0.1% (w/w)) and relevant transformation/degradation products. The degradation rates obtained in soil, sediment, or surface water (e.g. those obtained in studies conducted according to OECD 307, 308 and 309), and bioconcentration factors in aquatic species (e.g. OECD 305) are directly compared against threshold values for 'P' and 'B' as set out in REACH Regulation (EC No. 1907/2006).

The elements of the respective OECD test guidelines (and equivalent alternatives) are set out in Section 3 of this report. In general, the guidelines contain very little advice or guidance on the selection and validation of appropriate extraction, separation and detection techniques. The OECD test guidelines, and the regulatory framework legislation and guidance documents, set out the requirements for identification and quantification of transformation and degradation products. The literature review of current practice in bioaccumulation and degradation simulation studies carried out for this project included studies published in the academic literature and those in EFSA Draft Assessment Reports (DARs) (see Section 5). During the scoping phase of the project, the use of data published for these endpoints in ECHA disseminated dossiers was considered; however, following assessment of a limited number of dossiers, it was concluded that the data presented in the disseminated dossier may be insufficient for the present reviewers to draw any meaningful conclusions regarding extraction, separation and detection techniques used within these studies. Also, studies published in the academic literature may not necessarily be conducted in the context of any regulatory framework.

It is therefore difficult to comment on the compliance of such studies with all aspects of the guidelines, particularly in regard to identification (and quantification) of metabolites or transformation/degradation products formed during the study. In the majority of studies reviewed, there was no reference to any attempt to identify these transformation/degradation products. In contrast, degradation studies (particularly soil degradation studies) reported in the EFSA DARs include both identification and quantification of metabolites, and a proposed route of degradation. Applicants for authorisation of PPPs are required to submit a validated analytical method for the active substance and relevant metabolites in soil, sediment, water and tissue, and they are expected to be able to provide analytical reference standards for active substance and metabolites. Therefore, the data that is generated (for degradation studies in particular) for PPPs generally appears to be of high quality as a consequence of this.

11.2 Use of radiolabelled, stable-isotope labelled, or non-labelled test substance

The first consideration when conducting a bioaccumulation or degradation study should be to determine whether radiolabelled, stable isotope labelled, or non-labelled test material will be used.

The OECD test guidelines (OECD 305, 307, 308 and 309) do not differentiate between the use of stable isotope labelling and radiolabelling, however, the selection of the isotopic labelling technique impacts the choice of any further extraction, separation and detection techniques. The most common stable isotopes used in environmental studies are ^{2}H (deuterium), ^{13}C and ^{15}N. Substitution with radioactive isotopes is by far the most usual method used to label substances. ^{14}C-labelling appears to be preferred followed by ^{3}H, but ^{32}P or ^{35}S which may also be useful.

The choice of isotope label is influenced by several factors: principally the chemical structure of the test substance itself, but also the half-life and specific activity of a radioactive isotope. Whilst detection and quantification methods applicable to stable and radioactive isotope labels have a low LOQ, in general the use of a radioactive isotope allows a greater choice of detection techniques, and the LOQ is an order of magnitude lower than that of a stable isotope method. Therefore, radiolabelling of the test substance should be considered first.

The use of radiolabelled compounds is recommended in both the ECHA guidance (R.7b and R.7c Section R.7.10.4) and in the draft guidance under preparation by the EMA (EMEA/CHMP/SWP/4447/00 Rev. 1). It allows the mass balance requirement of the studies to be fulfilled by enabling the quantification of non-extractable residues, and the use of radiolabelled substance specific chemical analyses can also facilitate the identification and quantification of any transformation products.

The minimum information requirements for test substances under the various regulatory guidance discussed in Section 3 (i.e. type and position of radiolabel, specific activity, and radiochemical purity) are sufficient to allow the reliability of a study to be assessed. The specific activity of the substance should be as high as possible, particularly when working with low concentrations in degradation simulation studies. The supplier's recommendations on acceptable storage conditions and storage period of any radiolabelled compounds should be adhered to.

Liquid Scintillation Counting (LSC) remains the most common and widely available method for quantifying labelled compounds. In addition to bulk measurements, radioactive fractions/regions of interest are generated using separation techniques such as HPLC and or TLC. If it is not possible to directly measure the radioactive content on-line or using an imager, then fractions can be quantified directly using an off-line scintillation counting technique. This includes the collecting fractions of the HPLC eluant in a 96-well plate and counting the radioactivity externally. This technique has a superior LOD and may be used in conjunction with specific analysis such LC-MS with samples that do not contain scintillation materials. Direct quantification and identification of test substance and transformation/degradation products may be achieved via QToF. However, an alternative would be to make further use of fractionation by conducting a second-dimension analysis with high-resolution MS, MS/MS (or even NMR, if available) in order identify the transformation/degradation products.

Quantification of radiolabelled compounds has the advantage of being an absolute method, in comparison to all other quantitative analytical methods which are calibrated using an analytical standard. Particularly where quantification of transformation and degradation products are required, it may be difficult or impossible to obtain an analytical standard for calibration of an unlabelled or stable isotope labelled transformation/degradation product. Using a radiolabelled compound, quantification of the transformation products is still absolute. Similarly, quantification of non-extractable residues (NERs) is possible with radiolabelled test compounds, whereas using unlabelled methods it is difficult to obtain a mass-balance as the NERs cannot be quantified.

Stable-isotope labelling can, however, reduce the cost and technical difficulties associated with radiolabelling. Quantification of transformation/degradation products and a mass balance can be calculated using IR-MS analysis although, since this is a destructive method, identification of transformation and degradation products is consequently more difficult. The determination of NERs with stable isotope labelled compounds does not appear to be widely employed, however, combustion of residues to produce $^{13}CO_2$ and subsequent detection by a suitable mass spectrometer is feasible.

The use of isotopically labelled test substances significantly increases the cost of any environmental fate study. The use of radiolabelled drugs or pesticides is required for authorisation of pharmaceuticals and plant protection products, and therefore these industries, unlike industries for industrial chemicals, often have the capacity to synthesise radiolabelled test compounds in-house. Since the synthetic route for radiochemical synthesis usually significantly differs from the industrial synthesis route, and the chemistry involved in synthesis of radiolabelled derivatives can be complex, the use of radiolabelled compounds may, in some cases, not be feasible. In this case, the use of a non-labelled test substance in a bioaccumulation or degradation study may be unavoidable.

When using a non-labelled test substance, any mass-balance requirement cannot be met. Analytical methods for identification and quantification of the test substance itself are usually well developed, however identification of transformation and degradation products are challenging and quantification of these products even more difficult (particularly when their identity is unknown). It may be feasible to make some attempt to quantify any transformation/degradation products, however this is likely to depend on how chemically distinct the products are from organic substances naturally occurring in the matrix (soil, sediment or fish tissue). Precise quantification of non-labelled transformation/degradation products is unlikely to be possible without the identification and subsequent synthesis of these products as analytical standards for calibration.

11.3 Selection of appropriate sampling and extraction techniques

The sampling and extraction stages of any study are well-known to have greater error than the separation and detection stages, issues such as efficiency and selectivity of the method for different components, potential for artefact formation, and method validity require careful consideration. However, these areas are often given less attention and further guidance to address these aspects may be needed. Careful consideration of these stages of the process has the potential to improve the reliability of test results without the same cost and availability issues as use of new separation or detection techniques.

The sampling strategy should be seriously considered as part of test design as this is often the largest source of experimental error and great care must be taken to obtain a homogenous and representative sample (see Section 7.1). The use of passive sampling is not commonplace in laboratory fate studies. Whilst this powerful tool is still in its infancy it has the potential to be very useful, particularly in measuring aqueous concentrations in bioaccumulation studies conducted with poorly soluble substances. Some examples of the use of passive sampling in other matrices are discussed in Section 7.1.3, however this is an area which would benefit from further research.

Direct analysis of the test substance in the matrix (soil, sediment, water or fish tissue) is frequently difficult or impossible, particularly given the low concentrations present in the matrix in bioaccumulation and degradation studies. Prior to analysis, extraction from the matrix and sample clean-up is likely to be required, and the method developed should be validated for use. Traditional techniques which can be applied for the extraction of substances from a water phase are liquid-liquid extraction (LLE) or solid-phase extraction (SPE). Where liquid-liquid extraction is a nonselective extraction technique using larger volumes of solvent, SPE is a faster extraction technique using a solid phase extractant and requiring a much smaller amount of solvent, and the substances are generally concentrated by a factor of 10 in the final extract. Solid phase microextraction (SPME) is the most commonly used microextraction technique, having the advantages that no solvent is needed, small sample volumes are needed and can be used for numerous substances due to a wide choice of fibres.

Techniques for the extraction of substances from water in bioaccumulation (OECD 305) or degradation studies (OECD 309) reported in the peer-reviewed literature included LLE, SPE, SPME or direct analysis. Direct analysis of water samples was only reported when ^{14}C-labelled substances were used, LLE, SPE or SPME were used for non-labelled substances. LLE and SPE were used for a broad range of substances with different properties as a result of the availability of solid phases with different properties or non-selective character of LLE. Although both techniques are widely applicable, SPE is a faster and ‘greener’ technique than LLE. SPME was reported to be used for poorly soluble substances, and a study comparing the results from LLE to SPME showed similar results for organochlorine substances. This indicates that microextraction techniques can be a suitable extraction technique from an aqueous matrix and having the advantages of microextraction (see Section 7.3) and that the use of these techniques should be further investigated.

Extraction techniques for substances from solid matrices such as fish, soil and sediment can range from Soxhlet extraction and liquid-solid extraction by agitation to more modern techniques such as Pressurised Liquid Extraction (PLE), Supercritical fluid extraction (SFE), Microwave assisted extraction (MAE) and Ultrasound-assisted extraction (UAE). Where Soxhlet and liquid-solid extraction are time consuming and require larger volumes of extraction solvent, more recently used extraction techniques such as PLE and UAE are fast and require smaller amount of solvent.

A review of the literature on studies extracting substances from solid matrices showed that PLE or UAE were commonly used for fish, soil and sediment. Soxhlet extraction was used to a lesser extent as an extraction technique for soil while numerous studies stated that a solvent extraction was applied for soil and sediment without further details on the specific technique used. Although not provided by all studies, extraction efficiencies using PLE, UAE, and Soxhlet extraction were shown to be satisfactory for various substances (see Section 5), although the recovery can be influenced by extraction parameters such as solvent type, extraction time, or temperature. Given the benefits of automation or large batch analysis, reduced use of solvent and faster extraction, PLE or UAE are preferred extraction techniques for solid matrices.

11.4 Separation and Detection

The analysis of sample extracts containing labelled or non-labelled substances can be performed using several chromatography techniques for the separation of the test substances from degradation products or other matrix components and different type of detectors for identification and quantification. When using radiolabelled substances, HPLC or TLC are common separation techniques. LSC or RAM detectors may be used with HPLC methods, whilst TLC methods depend on LSC. Based on the peer-reviewed literature, these separation and detection techniques are commonly used for the analysis of test substances, metabolites/degradation products and/or NER in fish, soil, sediment and water.

Using stable isotopes (such as ^{13}C or ^{15}N), test substances can be analysed by GC coupled to an IRMS. With this technique the test substance, degradation products and NER can be analysed in order to generate a mass balance. There are examples in the peer-reviewed literature that this technique was successfully applied in soil and sediment degradation studies. This technique also has a great advantage that stable isotope substances are available at a lower cost than radiolabelled substances, although identification of transformation/degradation products is difficult.

For non-labelled substances, a range of chromatographic techniques can be selected, however, LC or GC are typically used. Commonly connected detectors to these LC or GC based systems are mass spectrometers, although other detectors can be used. Mass spectrometers have the advantage that they are selective and sensitive and can be used in a targeted as well as non-target approach. The examples in the peer-reviewed literature showed that, depending on the properties of the test substance, GC-MS or LC-MS were predominantly used, although there were examples where other detectors were used including FID, ECD or FLD. The examples in the literature showed that transformation or degradation products were predominantly analysed by targeted techniques such as MS/MS, however, in some cases non-target detectors (High Resolution MS) are also being used for the identification of degradation products. The use of soft ionisation in combination with high resolution MS could be further investigated in the identification of transformation/degradation products.

11.5 Quality control of the analytical method

Whether an analytical method used in bioaccumulation or degradation studies is validated and thereby generating reliable and robust data is key for interpreting the outcome of these studies. According to OECD guidelines, information on recovery, repeatability and sensitivity should be provided in these types of environmental fate studies. In the studies obtained in the peer-reviewed literature, information on LOD/LOQ or recovery was not always provided in each of the studies, whereas information on the repeatability was only reported by a few studies. As mentioned earlier, studies published in the academic literature may not necessarily be conducted in the context of any regulatory framework. Therefore, required information on validation parameters was not always provided. When provided, information on recoveries were generally within the range stated in the OECD guidelines and LODs were generally below the minimum required values stated in the guidelines regardless of the extraction, separation and detection technique used.

Although recovery, repeatability and sensitivity are important parameters when validating an analytical method, information on the calibration curve, accuracy and precision of the analytical method and the stability of the analyte in a given matrix under specific storage conditions is also vital. Information on these validation parameters were generally not reported in the obtained literature. It is therefore essential that information on all validation parameters of analytical methods used are provided in order to assess the quality of the data generated in a degradation or bioaccumulation study.

11.6 Key aspects to consider prior to conducting environmental fate studies, and elements of good practice during studies

Besides the extraction, clean up, separation and detection, there are several other important factors that can play a key role in the quality of the data generated by a degradation or bioaccumulation study.

One of the first considerations should be the characterisation of the test substance. The OECD test guidelines specify the minimum purity for the test substance. However, based on the author’s experience, it is important that the test substance (and any synthesised transformation/degradation products to be used as calibration standards) should be characterised using the chromatographic and detection methods to be used in the environmental fate study itself. It is important to identify impurities (including any unreacted starting material) as these may be mistaken for transformation/degradation products.

The availability of reliable data for key physico-chemical properties should be assessed. A reliable measured water solubility value is critical since the applicability of an environmental fate study e.g. OECD surface water simulation test may depend on this value. In the case of OECD 305, a dietary route of administration to the fish should only be performed if it is not feasible to administer the test substance via an aqueous solution; this decision is principally justified based on a knowledge of the water solubility of the test substance and the ability to maintain a stable concentration of the test substance in water, at concentrations which may be determined analytically. Consideration must also be given to the selection of the method used to achieve such constant aqueous concentrations in a BCF study. To maintain freely dissolved concentrations of poorly soluble, hydrophobic organic chemicals, passive dosing may be used. Whilst passive dosing is mentioned in both OECD 23 (2019) and OECD 264 (2017), it does not yet appear to be widely employed in BCF testing, and may be difficult with some substance types, such as multi-constituent substances and UVCBs.

It is important to gather all available information on the expected transformation and degradation products which may be formed during a bioaccumulation or degradation study. Data on the possible transformation and degradation pathways may be available from abiotic degradation and/or toxicokinetic studies. A brief review of a number of *in silico* tools for the prediction of transformation and degradation products was carried out as part of this project, however the majority of published literature in this area relates to tools for drug discovery. Whilst these tools can be helpful in indicating potential transformation/degradation products which may be formed, further research is needed on the applicability of specific *in silico* tools in environmental fate studies in conjunction with the use of additional methods for identification of such transformation/degradation products (such as the soft ionisation, tandem MS/MS and high resolution MS methods discussed in Section 9.5).

The formation of artefacts may be a significant compounding factor in the reliability of data generated in environmental fate studies, and the significance of artefacts increases as the concentration of test substance in the study decreases. Good practice guides are available from instrument manufacturers on avoidance of artefacts in mass spectrometry. There are, however, many opportunities for artefact formation during the sampling, extraction and concentration stages of the analysis; prevention of the formation of such artefacts requires careful consideration of the physico-chemical properties of the test substance, and the possible sources of contamination with the test laboratory, extraction solvents and associated laboratory equipment. Furthermore, the storage conditions and stability of any samples during storage must be considered; in particular, it is good practice to carry out solvent extractions at the time of sampling.

11.7 Specific considerations for ‘difficult-to-test’ substances

Some substance types present particular challenges in environmental fate studies, both in the study design and the analytical methodology. The properties of substances which have the potential to cause issues in bioaccumulation and degradation studies are similar to those which are defined as ‘difficult substances’ in OECD 23 (2019). Where a substance or transformation product possesses such ‘difficult’ properties, the impact of these properties must be considered at all stages of the study. In some cases, the feasibility of the study itself may be defined by these ‘difficult’ properties. The following substance types are considered to present particular difficulties in environmental fate studies:

11.7.1 Poorly soluble, hydrophobic substances

As discussed in earlier sections (see Section 3), a dietary bioaccumulation study may be required where stable aqueous concentrations of hydrophobic/poorly soluble substances cannot be achieved. Similarly, a degradation simulation study in surface water (e.g. OECD 309) is unlikely to be feasible with a very poorly soluble test substance, and a degradation in soil or sediment study should be considered. Where a study is feasible, the potential for sorption of the test substance to particulates and surfaces needs careful consideration.

11.7.2 Complex substances (UVCB / multi-constituent)

At present, there is very little practical consideration for conducting environmental fate (bioaccumulation or degradation) studies with a complex substance such as UVCB or multi-constituent substances. For these complex substances, the 'substance' to be tested in the environmental fate studies must be identified. This may be the whole substance, specific constituent(s) or fraction(s) of the substance. Guidance on selection of the appropriate test substance is given in ECHA Guidance R11 (ECHA, 2017). Nonetheless, the guidance does not acknowledge the technical challenges of selecting and synthesising/isolating individual constituents (and any attempt at radiolabelling these constituents) or in isolating a fraction of the UVCB/multi-constituent substance.

It should further be noted that, given that individual constituents of a UVCB/multi-constituent substance potentially have a wide range of physico-chemical properties, the appropriate extraction and separation techniques for these constituents would be significantly different. Reference standards are unlikely to be available for these individual constituents, and therefore developing and validating an analytical method can be very challenging.

Based on the literature review carried out for this project, identification and quantification of transformation/degradation products has not been attempted in studies with UVCB/multi-constituent substances; in the authors' opinion, this would be extremely difficult to carry out for the majority of such substances. NMR may be a useful method for determining stable isotope labelled constituents or degradation/transformation products, however at present very few contract research laboratories have this facility available for use in environmental fate studies.

As already noted, it is important that any test substance is initially well characterised by the same method to be used for quantification in the study; this is particularly critical for multi-constituent/UVCB substances where the use of different analytical techniques can lead to misinterpretation of the test data.

Automated two-dimensional chromatography techniques such as GC-GC have been recommended in the REACH guidance. However, availability, cost and inter-laboratory reproducibility are problematic for these techniques. An alternative approach could be to fractionate the sample by liquid chromatography (LC) or possibly thin layer chromatography (TLC), followed by a separate analysis of the fractions using an appropriate detector, such as high-resolution mass spectroscopy or nuclear magnetic resonance (NMR). This is another area which would benefit from further research.

11.7.3 Surfactants

Surfactants present particular challenges for extraction and separation, however methods are available to overcome these difficulties (see Section 10.3). The literature review of current practice in guideline bioaccumulation and degradation studies found few examples of studies conducted with surfactant substances; review of the wider literature suggests that the use of SPE and PLE for extraction and clean-up should be explored. In addition, in multi-constituent substances, the presence of components that are surfactants may influence the behaviour of other chemicals present in the test system, and the impact of this should be considered during the initial study design phase.

11.7.4 Volatile compounds

Consideration must be given to loss of parent substance from the test system via other mechanisms, such as volatilisation, to ensure that these are minimised as far as reasonably practical and that such losses can be differentiated from primary (a)biotic degradation processes. The testing of volatile test compounds may require specific adaptations to study design and set-up; the consideration of such adaptations is beyond the scope of the present work. In the context of the analytical methodology, losses of volatile test compounds and transformation products can occur at all stages of sample handling and extraction, however there are fairly well-established methods to handle this type of compound (e.g. US EPA Method 8270E and 8260 D (US EPA, 2007)).

11.8 Summary of Recommendations

Based on the analyses of the literature and the conclusions drawn, the following recommendation are made:

Use and applicability of extraction techniques:

- Regardless of the extraction technique used, efficiency and selectivity of the method for different components, potential for artefact formation, and method validity require careful consideration and method development should be clearly reported.
- Although LLE is still a commonly used extraction technique for extractions from water, SPE is a faster and 'greener' technique than LLE and can be applied to a wide range of chemicals due to the availability of different solid phases, and its use is recommended where applicable.
- The use of microextraction techniques should be investigated for the analysis of water samples.
- For solid phases, PLE or UAE are recommended as they are fast extraction techniques that require small amounts of extraction solvent. These methods are automated or can be operated in larger batches.

Identification and quantification of transformation/degradation products & closing the mass-balance:

- The determination of mass-balance in studies using stable-isotope labelled compounds should be encouraged. The use of stable isotopes in combination with IRMS should be explored in environmental fate studies as an alternative to the use of radiolabelled substances.
- Mass spectrometry is a powerful method for identification and/or quantification. Increased use could be made of soft ionisation, tandem MS/MS and high resolution MS, particularly for degradation products.

Information required to demonstrate method validation:

- Information on quality assurance and quality control of the analytical method should be provided.
- The validation parameters should include information on the calibration curve, accuracy, precision, recoveries (extraction and analytical), sensitivity and stability.

In addition, the following opportunities for further research have been identified:

- Whilst techniques for separation and quantification of radiolabelled test compounds is well established, the use of fractionation techniques could be further developed to include both quantification and identification of test substance and transformation/degradation products.
- Further exploration of the use of passive sampling techniques, both in aqueous media and in soil/sediment matrices, would be beneficial

12. References

Adolfsson-Erici, M., Åkerman, G., McLachlan, M.S., 2012b. In-vivo passive sampling to measure elimination kinetics in bioaccumulation tests. Chemosphere 88, 62–68. https://doi.org/10.1016/j.chemosphere.2012.02.063

Adolfsson-Erici, M., Åkerman, G., McLachlan, M.S., 2012c. Measuring bioconcentration factors in fish using exposure to multiple chemicals and internal benchmarking to correct for growth dilution. Environmental Toxicology and Chemistry 31, 1853–1860. https://doi.org/10.1002/etc.1897

Aim Research http://aimresearchcompany.com/

Albaseer, S.S., Mukkanti, K., Rao, R.N., Swamy, Y.V., 2011. Analytical artifacts, sample handling and preservation methods of environmental samples of synthetic pyrethroids. TrAC Trends in Analytical Chemistry 30, 1771–1780. https://doi.org/10.1016/j.trac.2011.05.010

Alvarez, David A., 2010. Guidelines for the Use of the Semipermeable Membrane Device (SPMD) and the Polar Organic Chemical Integrative Sampler (POCIS) in Environmental Monitoring Studies, Techniques and Methods 1–D4. U.S. Geological Survey. https://pubs.usgs.gov/tm/tm1 d4/pdf/tm1 d4.pdf (Accessed 13/2/2020)

Amaral, H.I.F., Berg, M., Brennwald, M.S., Hofer, M., Kipfer, R., 2010. ^{13}C/12C Analysis of Ultra-Trace Amounts of Volatile Organic Contaminants in Groundwater by Vacuum Extraction. Environ. Sci. Technol. 44, 1023–1029. https://doi.org/10.1021/es901760q

Andreu, V., Ferrer, E., Rubio, J.L., Font, G., Picó, Y., 2007. Quantitative determination of octylphenol, nonylphenol, alkylphenol ethoxylates and alcohol ethoxylates by pressurized liquid extraction and liquid chromatography–mass spectrometry in soils treated with sewage sludges. Science of The Total Environment, Spanish Research on Soil Damage 378, 124–129. https://doi.org/10.1016/j.scitotenv.2007.01.024

Antonio Padilla·Sanchez J, Plaza·Bolanos P, Romero·Gonzalez R, Garrido·Frenich A, Martinez Vidal, JL (2010) Application of a quick, easy, cheap, effective, rugged and safe based method for the simultaneous extraction of chlorophenols, alkylphenols, nitrophenols and cresols in agricultural soils, analyzed by using gas chromatography-triple quadrupole·mass spectrometry/mass spectrometry. J Chromatogr A, 1217(36), 5724-31.

Arnot, J.A., Gobas, F.A., 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environ. Rev. 14, 257–297. https://doi.org/10.1139/a06-005

ASTM E 1022-94 Standard Guide for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Mollusks.

Aulakh JS, Malik AK, Kaur V, Schmitt·Kopplin, P. (2005) A review on solid phase micro extraction-high performance liquid chromatography (SPME-HPLC) analysis of pesticides. Crit Rev Anal Chem 35(1), 71-85.

AusPVMA (2018) Validation of analytical methods for active constituents and agricultural products. Australian Pesticide and Veterinary Medicines Authority; 2018. https://apvma.gov.au/node/1048#Parameters_for_method_validation

Baba, S., Horie, M., Watanabe, K., 1982. Synchronized accumulating radioisotope detector for high-performance liquid chromatography. Journal of Chromatography A 244, 57–64. https://doi.org/10.1016/S0021-9673(00)80122-3

Ball, D.J., Norwood, D.L., Stults, C.L.M., Nagao, L.M., 2012. Leachables and Extractables Handbook: Safety Evaluation, Qualification, and Best Practices Applied to Inhalation Drug Products. Wiley.

Bekele, T.G., Zhao, H., Wang, Y., Jiang, J., Tan, F., 2018. Measurement and prediction of bioconcentration factors of organophosphate flame retardants in common carp (*Cyprinus carpio*). Ecotoxicology and Environmental Safety 166, 270–276. https://doi.org/10.1016/j.ecoenv.2018.09.089

Berijani S, Assadi Y, Anbia M, Milani Hosseini M·R, Aghaee E. (2006) Dispersive liquid-liquid microextraction combined with gas chromatography-flame photometric, detection - very simple, rapid and sensitive method for the determination of organophosphorus pesticides in water. J Chromatogr A, 1123(1), 1-9.

Biel-Maeso, M., González-González, C., Lara-Martín, P.A., Corada-Fernández, C., 2019. Sorption and degradation of contaminants of emerging concern in soils under aerobic and anaerobic conditions. Science of The Total Environment 666, 662–671. https://doi.org/10.1016/j.scitotenv.2019.02.279

Bielicka-Daszkiewicz, K., Dębicka, M., Voelkel, A., 2004. Comparison of three derivatization ways in the separation of phenol and hydroquinone from water samples. Journal of Chromatography A 1052, 233–236. https://doi.org/10.1016/j.chroma.2004.07.067

Birch,H. *et al*. (2010),Passive dosing to determine the speciation of hydrophobic organic chemicals in aqueous samples. Analytical Chemistry, Vol.82/3:1142-1146.

Birch, H., Andersen, H.R., Comber, M., Mayer, P., 2017a. Biodegradation testing of chemicals with high Henry’s constants – Separating mass and effective concentration reveals higher rate constants. Chemosphere 174, 716–721. https://doi.org/10.1016/j.chemosphere.2017.02.003

Birch, H., Hammershøj, R., Comber, M., Mayer, P., 2017b. Biodegradation of hydrocarbon mixtures in surface waters at environmentally-relevant levels – Effect of inoculum origin on kinetics and sequence of degradation. Chemosphere 184, 400–407. https://doi.org/10.1016/j.chemosphere.2017.05.169

Birch, H., Hammershøj, R., Mayer, P., 2018. Determining Biodegradation Kinetics of Hydrocarbons at Low Concentrations: Covering 5 and 9 Orders of Magnitude of K_{ow} and K_{aw}. Environmental Science and Technology 52, 2143-2151. https://doi.org/10.1021/acs.est.7b05624

Birch, H., Redman, A.D., Letinski, D.J., Lyon, D.Y., Mayer, P., 2019. Determining the water solubility of difficult-to-test substances: A tutorial review. Analytica Chimica Acta 1086, 16–28. https://doi.org/10.1016/j.aca.2019.07.034

Blue, L.E., Franklin, E.G., Godinho, J.M., Grinias, J.P., Grinias, K.M., Lunn, D.B., Moore, S.M., 2017. Recent advances in capillary ultrahigh pressure liquid chromatography. J Chromatogr A 1523, 17–39. https://doi.org/10.1016/j.chroma.2017.05.039

Boeckelen A, Niessner R. (1993) Combination of micellar extraction of polycyclic aromatic hydrocarbons from aqueous media with detection by synchronous fluorescence. Fresenius J Anal

Chem, 346(4):435-40.

Böhm, L., Düring, R.-A., Bruckert, H.-J., Schlechtriem, C., 2017. Can solid-phase microextraction replace solvent extraction for water analysis in fish bioconcentration studies with highly hydrophobic organic chemicals? Environmental Toxicology and Chemistry 36, 2887–2894. https://doi.org/10.1002/etc.3854

Börnsen, KO (2000) Using the TopCount® Microplate Scintillation and Luminescence Counter and Deep-Well LumaPlate™ Microplates in Combination with Micro Separation Techniques for Metabolic Studies, Packard Instrument Application Note AN004-TC https://www.researchgate.net/publication/264748484_Using_the_TopCount_R_Microplate_Scintillation_and_Luminescence_Counter_and_Deep-Well_LumaPlate_Microplates_in_Combination_with_Micro_Separation_Techniques_for_Metabolic_Studies

Brooks, S.J., Ruus, A., Rundberget, J.T., Kringstad, A., Lillicrap, A., 2019. Bioaccumulation of selected veterinary medicinal products (VMPs) in the blue mussel (Mytilus edulis). Science of The Total Environment 655, 1409–1419. https://doi.org/10.1016/j.scitotenv.2018.11.212

Bruin, G.J., Waldmeier, F., Boernsen, K.O., Pfaar, U., Gross, G., Zollinger, M., 2006. A microplate solid scintillation counter as a radioactivity detector for high performance liquid chromatography in drug metabolism: Validation and applications. Journal of Chromatography A 1133, 184–194. https://doi.org/10.1016/j.chroma.2006.08.020

Bruzzoniti MC, Checchini L, De Carlo RM, Orlandinl S, Rivoira L, Del Bubba M. (2014) QuEChERS sample preparation for the determination of pesticides and other organic residues in environmental matrices: a critical review. Anal Bloanal Chem, 406(17):4089-116.

Butler, J.D. *et al*. (2013),A novel passive dosing system for determining the toxicity of phenanthrene to early life stages of zebrafish. Science of the Total Environment, Vol.463-464:952-958.

Butler, J.D. *et al*. (2016),Assessing aromatic-hydrocarbon toxicity to fish early life stages using passive-dosing methods and target-lipid and chemical-activity models. Environmental Sciences & Technology, Vol.50/15:8305-8315.

Camenzuli, L., Davis, C.W., Parkerton, T.F., Letinski, D.J., Butler, J.D., Davi, R.A., Febbo, E.J., Paumen, M.L., Lampi, M.A., 2019. Bioconcentration factors for hydrocarbons and petrochemicals: Understanding processes, uncertainty and predictive model performance. Chemosphere 226, 472–482. https://doi.org/10.1016/j.chemosphere.2019.03.147

Campisano, R., K. Hall, J. Griggs, S. Willison, S. Reimer, H. Mash, M. Magnuson, L. Boczek, AND E. Rhodes. Selected Analytical Methods for Environmental Remediation and Recovery (SAM) 2017. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-17/356, 2017.

Carasek, E., Morés, L., Merib, J., 2018. Basic principles, recent trends and future directions of microextraction techniques for the analysis of aqueous environmental samples. Trends in Environmental Analytical Chemistry 19, e00060. https://doi.org/10.1016/j.teac.2018.e00060

Chary, N., Fernández-Alba, A., 2012. Determination of volatile organic compounds in drinking and environmental waters. TrAC Trends in Analytical Chemistry 32, 60–75. https://doi.org/10.1016/j.trac.2011.08.011

Clark, R.P., Shirley, S.G., 1973. Identification of Skin in Airborne Particulate Matter. Nature 246, 39–40. https://doi.org/10.1038/246039a0

Clark, T (2000) http://index-of.co.uk/Tutorials^{-2}/Radioactivity%20 Detection.pdf

Carlos, K.S., Jager, L.S. de, Begley, T.H., 2018. Investigation of the primary plasticisers present in polyvinyl chloride (PVC) products currently authorised as food contact materials. Food Additives & Contaminants: Part A 35, 1214–1222. https://doi.org/10.1080/19440049.2018.1447695

Chang, P.-L., Hsieh, M.-M., Chiu, T.-C., 2016. Recent Advances in the Determination of Pesticides in Environmental Samples by Capillary Electrophoresis. International Journal of Environmental Research and Public Health 13, 409. https://doi.org/10.3390/ijerph13040409

Claßen, D., Siedt, M., Nguyen, K.T., Ackermann, J., Schaeffer, A., 2019. Formation, classification and identification of non-extractable residues of ^{14}C-labelled ionic compounds in soil. Chemosphere 232, 164–170. https://doi.org/10.1016/j.chemosphere.2019.05.038

Corada-Fernández, C., González-Mazo, E., Lara-Martín, P.A., 2018. Evaluation of the anaerobic biodegradation of linear alkylbenzene sulfonates (LAS) using OECD 308 water/sediment systems. Journal of Hazardous Materials 360, 24–31. https://doi.org/10.1016/j.jhazmat.2018.07.087

Commission Communication 2013/C 95/01

Commission Regulation (EU) No 284/2013 (in accordance with Regulation (EC) No 1107/2009)

Commission Regulation (EU) No 518/2012 concerning the making available on the market and use of biocidal products. 22 May 2012.

Cone, E.J., Buchwald, W.F., Darwin, W.D., 1982. Analytical controls in drug metabolic studies. II. Artifact formation during chloroform extraction of drugs and metabolites with amine substituents. Drug metabolism and disposition: the biological fate of chemicals 10, 561–567.

Conte, P., Piccolo, A., 1999. High pressure size exclusion chromatography (HPSEC) of humic substances: Molecular sizes, analytical parameters, and column performance. Chemosphere 38, 517–528. https://doi.org/10.1016/S0045-6535(98)00198-2

Crookes and Fisk (2018). Evaluation of using mobility of chemicals in the environment to fulfil bioaccumulation criteria of the Stockholm Convention. Report prepared for the European Commission.

Dai H, Xiao D, He H, Li H, Yuan D, Zhang C., 2015 Synthesis and analytical applications of molecularly imprinted polymers on the surface of carbon nanotubes: a review. Milcrochim Acta;182(56), 893-908.

Daoussi R, Vess ot S, Andrieu J. Monnier 0. (2009) Sublimation kinetics and sublimation end - point times during freeze-drying of pharmaceutical active principle with organic co-solvent formulations. Chem Eng Res Des, 87(7A):899-907.

Davis, J.W., Gonsior, S., Marty, G., Ariano, J., 2005. The transformation of hexabromocyclododecane in aerobic and anaerobic soils and aquatic sediments. Water Research 39, 1075–1084. https://doi.org/10.1016/j.watres.2004.11.024

de Fátima Alpendurada, M., 2000. Solid-phase microextraction: a promising technique for sample preparation in environmental analysis. Journal of Chromatography A, 9th Symposium on Handling of Environmental and Biological Samples 889, 3–14. https://doi.org/10.1016/S0021-9673(00)00453-2

Demeestere, K., Dewulf, J., De Witte, B., Van Langenhove, H., 2007. Sample preparation for the analysis of volatile organic compounds in air and water matrices. Journal of Chromatography A, Advances in Sample Preparation 1153, 130–144. https://doi.org/10.1016/j.chroma.2007.01.012

Dimitrov S, Nedelcheva D, Dimitrova N and Mekenyan O (2010). Development of a biodegradation model for the prediction of metabolites in soil, Science of The Total Environment, Volume 408, Issue 18, Pages 3811-3816. https://www.sciencedirect.com/science/article/pii/S0048969710001191

Dimpe, K.M., Nomngongo, P.N., 2016. Current sample preparation methodologies for analysis of emerging pollutants in different environmental matrices. TrAC Trends in Analytical Chemistry 82, 199–207. https://doi.org/10.1016/j.trac.2016.05.023

Dionisio, A.C., Rath, S., 2016. Abamectin in soils: Analytical methods, kinetics, sorption and dissipation. Chemosphere 151, 17–29. https://doi.org/10.1016/j.chemosphere.2016.02.058

EAG Industries https://www.eag.com/techniques/imaging/phosphor-imaging/

EC (2010) Guidance document on pesticide residue analytical methods. European Commission. https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_ppp_app-proc_guide_res_post-reg-cont-monitor.pdf

ECETOC, 1996. Aquatic Toxicity Testing of Sparingly Soluble, Volatile and Unstable Substances, Monograph No. 26. European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), pp. 547–548. https://doi.org/10.1016/B978-0-12-386454-3.00505-4

ECETOC, 2014. An evaluation of the challenges and limitations associated with aquatic toxicity and bioaccumulation studies for sparingly soluble and manufactured particulate substances. European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). https://doi.org/10.1016/B978-0-12-386454-3.00505-4

ECHA (2016). Practical Guide - How to Use and Report (Q)SARs, version 3.1. July 2016. https://echa.europa.eu/documents/10162/13655/pg_report_qsars_en.pdf/407 dff11-aa4a-4eef-a1ce-9300f8460099.

ECHA (2017). Guidance on information requirements and chemical safety assessment, Chapter R.11: PBT assessment.

ECHA (2017a). Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7b: Endpoint specific guidance. Version 4.0. June 2017

ECHA (2017b). Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7c: Endpoint specific guidance. Version 3.0. June 2017

ECHA (2017c). Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT/vPvB assessment. Version 3.0. June 2017

ECHA (2018). Guidance on the Biocidal Products Regulation. Volume IV: Environment. Part A: Information Requirements. Version 1.2. May 2018.

EFSA (2007) Opinion on a request from EFSA related to the default Q10 values used to describe the temperature effect on transformation rates of pesticides in soil. EFSA Journal 622:1-32.

El-Amrani, S., Pena-Abaurrea, M., Sanz-Landaluze, J., Ramos, L., Guinea, J., Cámara, C., 2012. Bioconcentration of pesticides in Zebrafish eleutheroembryos (*Danio rerio*). Science of The Total Environment 425, 184–190. https://doi.org/10.1016/j.scitotenv.2012.02.065

El-Amrani, S., Sanz-Landaluze, J., Guinea, J., Cámara, C., 2013. Rapid determination of polycyclic aromatic hydrocarbons (PAHs) in zebrafish eleutheroembryos as a model for the evaluation of PAH bioconcentration. Talanta 104, 67–74. https://doi.org/10.1016/j.talanta.2012.11.025

EMEA (2019). Draft Guideline on the environmental risk assessment of medicinal products for human use (EMEA/CHMP/SWP/4447/00 Rev. 1);

Ende, M., Spiteller, G., 1982. Contaminants in mass spectrometry. Mass Spectrometry Reviews 1, 29–62. https://doi.org/10.1002/mas.1280010105

EPA (1998). EPA 712-C-98-083. Fate, Transport and Transformation Test Guidelines. OPPTS 835.3180. Sediment/Water Microcosm Biodegradation Test.

EPA (1998). EPA 712-C-98-088. Fate, Transport and Transformation Test Guidelines. OPPTS 835.3300. Soil Biodegradation.

EPA (2016). EPA 712-C-16-003. Ecological Effects Test Guidelines. OCSPP 850.1730:Fish Bioconcentration Factor (BCF)

Erngren, I., Haglöf, J., Engskog, M.K.R., Nestor, M., Hedeland, M., Arvidsson, T., Pettersson, C., 2019. Adduct formation in electrospray ionisation-mass spectrometry with hydrophilic interaction liquid chromatography is strongly affected by the inorganic ion concentration of the samples. Journal of Chromatography A 1600, 174–182. https://doi.org/10.1016/j.chroma.2019.04.049

European Medicines Agency (2012) Guideline on bioanalytical method validation. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf

FDA (2015) Analytical Procedures and Methods Validation for Drugs and Biologics. U.S. Department of Health and Human Services, Food and Drug Administration. https://www.fda.gov/files/drugs/published/Analytical-Procedures-and-Methods-Validation-for-Drugs-and-Biologics.pdf

Fekete, S., Kohler, I., Rudaz, S., Guillarme, D., 2014. Importance of instrumentation for fast liquid chromatography in pharmaceutical analysis. J Pharm Biomed Anal 87, 105–119. https://doi.org/10.1016/j.jpba.2013.03.012

Fekete, S., Veuthey, J.-L., Guillarme, D., 2015. Comparison of the most recent chromatographic approaches applied for fast and high resolution separations: Theory and practice. J Chromatogr A 1408, 1–14. https://doi.org/10.1016/j.chroma.2015.07.014

Fifield, F. W., Kealey, D., 2000, Principles and Practice of Analytical Chemistry, 5th Edition, Wiley

Fifield, F. W., Haines, P. J., 2000. Environmental Analytical Chemistry, 2nd Edition. Wiley.

Froeschl B, Stangl G, Niessner R. (1997) Combination or micellar extraction and GC·ECD for the determination or polychlorinated biphenyls (PCBs) in water. Fresenius J Anal Chem 1997;357(6):743-6.

Fischer Scientific, 2019. Common Background Contamination Ions in Mass Spectrometry [WWW Document]. URL https://beta-static.fishersci.ca/content/dam/fishersci/en_US/documents/programs/scientific/brochures-and-catalogs/posters/fisher-chemical-poster.pdf (accessed 12.20.19)

FOCUS (2014). Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Version: 1.1. 18 December 2014

Ganorkar, S.B., Shirkhedkar, A.A., 2017. Design of experiments in liquid chromatography (HPLC) analysis of pharmaceuticals: analytics, applications, implications and future prospects. Reviews in Analytical Chemistry 36. https://doi.org/10.1515/revac-2016-0025

Garcia, S.N., Foster, M., Constantine, L.A., Huggett, D.B., 2012. Field and laboratory fish tissue accumulation of the anti-convulsant drug carbamazepine. Ecotoxicology and Environmental Safety 84, 207–211. https://doi.org/10.1016/j.ecoenv.2012.07.013

Garcia Pinto C, Fernandez Laespada ME, Herrero MartinS, Casas Ferrei ra AM, Perez Pavan JL, Moreno Cordero B (2010). Simplified QuEChERS approach for the extraction of chlorinated compounds from soil samples. Talanta, 81(12), 385-91.

Gejlsbjerg, B., Madsen, T., Thorup Andersen, T., 2003. Comparison of biodegradation of surfactants in soils and sludge–soil mixtures by use of ^{14}C-labelled compounds and automated respirometry. Chemosphere 50, 321–331. https://doi.org/10.1016/S0045-6535(02)00501-5

Ghosh, U., Driscoll, S., Burgess, R.M., Jonker, M.T.O., Reible, D., Gobas, F., Choi, Y.J., Apitz, S.E., Maruya, K.A., Gala, W.R., Mortimer, M., Beegan, C., 2014. Passive sampling methods for contaminated sediments: Practical guidance for selection, calibration, and implementation. Integr. Environ. Assess. Manag. 10, 210-223.

Girardi, C., Greve, J., Lamshöft, M., Fetzer, I., Miltner, A., Schäffer, A., Kästner, M., 2011. Biodegradation of ciprofloxacin in water and soil and its effects on the microbial communities. Journal of Hazardous Materials 198, 22–30. https://doi.org/10.1016/j.jhazmat.2011.10.004

Girardi, C., Nowak, K.M., Carranza-Diaz, O., Lewkow, B., Miltner, A., Gehre, M., Schäffer, A., Kästner, M., 2013. Microbial degradation of the pharmaceutical ibuprofen and the herbicide 2,4-D in water and soil — Use and limits of data obtained from aqueous systems for predicting their fate in soil. Science of The Total Environment 444, 32–42. https://doi.org/10.1016/j.scitotenv.2012.11.051

Giraudo, M., Douville, M., Letcher, R.J., Houde, M., 2017. Effects of food-borne exposure of juvenile rainbow trout (*Oncorhynchus mykiss*) to emerging brominated flame retardants 1,2-bis(2,4,6-tribromophenoxy)ethane and 2-ethylhexyl-2,3,4,5-tetrabromobenzoate. Aquatic Toxicology 186, 40–49. https://doi.org/10.1016/j.aquatox.2017.02.023

Hammershøj, R., Birch, H., Redman, A.D., Mayer, P., 2019. Mixture Effects on Biodegradation Kinetics of Hydrocarbons in Surface Water: Increasing Concentrations Inhibited Degradation whereas Multiple Substrates Did Not. Environmental Science and Technology 53, 3087−3094. DOI: 10.1021/acs.est.9b00638

Hand, L.H., Gougoulias, C., Bramke, I., Thomas, K.A., Oliver, R.G. 2020. Evaluation of the Rhizosphere Contribution to the Environmental Fate of the Herbicide Prometryn. Environmental Toxicology and Chemistry 39, 450-457, https://doi.org/10.1002/etc.4604

Hand, L.H., Moreland, H.J. 2014. Surface water mineralization of isopyrazam according to OECD 309: Observations on implementation of the new data requirement within agrochemical regulation. Environmental Toxicology and Chemistry 33, 730-7268, https://doi.org/10.1002/etc.2490

Heger, H.J., Zimmermann, R., Dorfner, R., Beckmann, M., Griebel, H., Kettrup, A., Boesl, U., 1999. On-Line Emission Analysis of Polycyclic Aromatic Hydrocarbons down to pptv Concentration Levels in the Flue Gas of an Incineration Pilot Plant with a Mobile Resonance-Enhanced Multiphoton Ionization Time-of-Flight Mass Spectrometer. Anal. Chem. 71, 46–57. https://doi.org/10.1021/ac980611y

Hesk, D., Lavey, C.F. and McNamara, P., (2010). Tritium labelling of pharmaceuticals by metal-catalysed exchange methods. Journal of Labelled Compounds and Radiopharmaceuticals, 53: 722-730.

Hinze WL, Pramauro E (1993) A critical-review of surfactant-mediated phase separations (cloud -point extractions)- theory and applications, Crit Rev Anal Chem 24(2),133-77.

Hladik M.L. ,Orlando J.L. , Kuivila K.M., 2009. Collection of Pyrethroids in Water and Sediment Matrices: Development and Validation of a Standard Operating Procedure, Scientific Investigations Report. U.S. Geological Survey.

Hoke, R.A., Ferrell, B.D., Ryan, T., Sloman, T.L., Green, J.W., Nabb, D.L., Mingoia, R., Buck, R.C., Korzeniowski, S.H., 2015. Aquatic hazard, bioaccumulation and screening risk assessment for 6:2 fluorotelomer sulfonate. Chemosphere 128, 258–265. https://doi.org/10.1016/j.chemosphere.2015.01.033

Hoke, R.A., Ferrell, B.D., Sloman, T.L., Buck, R.C., Buxton, L.W., 2016. Aquatic hazard, bioaccumulation and screening risk assessment for ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate. Chemosphere 149, 336–342. https://doi.org/10.1016/j.chemosphere.2016.01.009

Holopainen, S., Nousiainen, M., Sillanpää, M.E.T., Anttalainen, O., 2012. Sample-extraction methods for ion-mobility spectrometry in water analysis. TrAC Trends in Analytical Chemistry 37, 124–134. https://doi.org/10.1016/j.trac.2012.03.014

Horii, Y., Kannan, K., 2008. Survey of Organosilicone Compounds, Including Cyclic and Linear Siloxanes, in Personal-Care and Household Products. Arch Environ Contam Toxicol 55, 701. https://doi.org/10.1007/s00244-008-9172-z

Hou X, Zheng X, Zhang C, M a X, Ling Q, Zhao L. (2014) Ultrasound-assisted dispersive liquid-liquid microextraction based on the solidification of a floating organic droplet followed by gas chromatography for the determination of eight pyrethroid pesticides in tea samples. J Chromatogr B·Anal Technol Biomed Life Sci, 969:123-7.

Hu, Q., Noll, R.J., Li, H., Makarov, A., Hardman, M., Cooks, R.G., 2005. The Orbitrap: a new mass spectrometer. Journal of Mass Spectrometry 40, 430–443. https://doi.org/10.1002/jms.856

Idowu, I., Johnson, W., Francisco, O., Obal, T. Marvin, C., Thomas, P.J., Sandau, C.D., Stetefeld, J., Tomy, G.T.. 2018. Comprehensive two-dimensional gas chromatography high-resolution mass spectrometry for the analysis of substituted and unsubstituted polycyclic aromatic compounds in environmental samples, Journal of Chromatography A, Volume 1579, 106-114. https://www.sciencedirect.com/science/article/abs/pii/S0021967318312937

ISO 14592-1 (ISO 14592-1:2002(E)) Water Quality – Evaluation of the aerobic biodegradability of organic compounds at low concentrations – Part 1: Shake-flask batch test with surface water/sediment suspensions.

Jenne, A., Soong, R., Bermel, W., Sharma, N., Masi, A., Anaraki, M.T., Simpson, A., 2019. Focusing on "the important" through targeted NMR experiments: an example of selective ^{13}C–12C bond detection in complex mixtures. Faraday Discuss. 218, 372–394. https://doi.org/10.1039/C8FD$_0$0213 D

Jiménez-Jiménez, S., Casado, N., García M.A., Marina, M.L., 2019, Enantiomeric analysis of pyrethroids and organophosphorus insecticides Journal of Chromatography A 1605, 360345, https://doi.org/10.1016/j.chroma.2019.06.066

Jonker, M.T.O., Burgess, R.M., Ghosh, U., Gschwend, P.M., Hale, S.E., Lohmann, R., Lydy, M.J., Maruya, K.A., Reible, D., Smedes, F., 2020. Ex situ determination of freely dissolved concentrations of hydrophobic organic chemicals in sediments and soils: basis for interpreting toxicity and assessing bioavailability, risks and remediation necessity. Nature Protocols 15, 1800-1828.

Karty, J.A., Kim, T-Y, Reilly, J.P. (2004) "Utility of chemical derivatisation for peptide mass fingerprinting," In Rosenfeld, J.M., 2004. Sample Preparation for Hyphenated Analytical Techniques. Blackwell Publishing and CRC Press.

Kavcar, P., Odabasi, M., Kitis, M., Inal, F., Sofuoglu, S.C., 2006. Occurrence, oral exposure and risk assessment of volatile organic compounds in drinking water for İzmir. Water Research 40, 3219–3230. https://doi.org/10.1016/j.watres.2006.07.002

Kazmi SR, Jun R, Yu M-S, Chung C and Na D (2019). In silico approaches and tools for the prediction of drug metabolism and fate: A review, Computers in Biology and Medicine, Volume 106, Pages 54-64. https://www.sciencedirect.com/science/article/abs/pii/S0010482519300083

Keller, B.O., Sui, J., Young, A.B., Whittal, R.M., 2008. Interferences and contaminants encountered in modern mass spectrometry. Analytica Chimica Acta, Mass Spectrometry 627, 71–81. https://doi.org/10.1016/j.aca.2008.04.043

Ketola, R.A., Virkki, V.T., Ojala, M., Komppa, V., Kotiaho, T., 1997. Comparison of different methods for the determination of volatile organic compounds in water samples. Talanta 44, 373–382. https://doi.org/10.1016/S0039-9140(96)02072-3

Kiffe, M., Schmid, D., Bruin, G., 2008. Radioactivity Detectors for High-Performance Liquid Chromatography in Drug Metabolism Studies. Journal of Liquid Chromatography & Related Technologies 31, 1593–1619. https://doi.org/10.1080/10826070802126254

Kiffe, M., Jehle, A., Ruembeli, R., 2003. Combination of High-Performance Liquid Chromatography and Microplate Scintillation Counting for Crop and Animal Metabolism Studies: A Comparison with Classical On-Line and Thin-Layer Chromatography Radioactivity Detection. Anal. Chem. 75, 723–730. https://doi.org/10.1021/ac020363c

Kistemann, T., Hundhausen, J., Herbst, S., Claßen, T., Färber, H., 2008. Assessment of a groundwater contamination with vinyl chloride (VC) and precursor volatile organic compounds (VOC) by use of a geographical information system (GIS). International Journal of Hygiene and Environmental Health 211, 308–317. https://doi.org/10.1016/j.ijheh.2007.02.011

Kito, K., Ito, T. (2008). Mass spectrometry-based approaches toward absolute quantitative proteomics. Current Genomics, 9: 263-274.

Knoerr, S.M., Durham, J.A., McNett, D.A., 2017. Development of collection, storage and analysis procedures for the quantification of cyclic volatile methylsiloxanes in wastewater treatment plant effluent and influent. Chemosphere 182, 114–121. https://doi.org/10.1016/j.chemosphere.2017.04.136

Krogh, K.A., Vejrup, K.V., Mogensen, B.B., Halling-Sørensen, B., 2002. Liquid chromatography–mass spectrometry method to determine alcohol ethoxylates and alkylamine ethoxylates in soil interstitial water, ground water and surface water samples. Journal of Chromatography A, 957, 45–57. https://doi.org/10.1016/S0021-9673(02)00077-8

Kruve, A., Kaupmees, K., 2017. Adduct Formation in ESI/MS by Mobile Phase Additives. J. Am. Soc. Mass Spectrom. 28, 887–894. https://doi.org/10.1007/s13361-017-1626-y

Kubo, T., Hosoya, K., Otsuka, K., 2014. Molecularly Imprinted Adsorbents for Selective Separation and/or Concentration of Environmental Pollutants. Analytical Sciences 30, 97–104. https://doi.org/10.2116/analsci.30.97

Kuhn, S., Colreavy-Donnelly, S., Souza, J.S. de, Borges, R.M., 2019. An integrated approach for mixture analysis using MS and NMR techniques. Faraday Discuss. 218, 339–353. https://doi.org/10.1039/C8FD$_0$0227 D

Kurrey, R., Mahilang, M. Deb, M.K., Syriyas, K. 2019, Analytical approach on surface active agents in the environment and challenges Trends in Environmental Analytical Chemistry 21, e00061, https://doi.org/10.1016/j.teac.2019.e00061

Lablogic https://lablogic.com/life-sciences/instruments/beta-ram-5

Lafontaine, A., Gismondi, E., Dodet, N., Joaquim-Justo, C., Boulangé-Lecomte, C., Caupos, F., Lemoine, S., Lagadic, L., Forget-Leray, J., Thomé, J.-P., 2017. Bioaccumulation, distribution and elimination of chlordecone in the giant freshwater prawn Macrobrachium rosenbergii: Field and laboratory studies. Chemosphere 185, 888–898. https://doi.org/10.1016/j.chemosphere.2017.07.099

Lara-Martín, P.A., Gómez-Parra, A., González-Mazo, E., 2008. Sources, transport and reactivity of anionic and non-ionic surfactants in several aquatic ecosystems in SW Spain: A comparative study. Environmental Pollution 156, 36–45. https://doi.org/10.1016/j.envpol.2008.01.005

Lee, J.N., Park, C., Whitesides, G.M., 2003. Solvent Compatibility of Poly(dimethylsiloxane)-Based Microfluidic Devices. Anal. Chem. 75, 6544–6554. https://doi.org/10.1021/ac0346712

Leonard J (2019). Supporting systems science through in silico applications: A focus on informing metabolic mechanisms, Current Opinion in Toxicology, Volume 16, Pages 1-8. https://www.sciencedirect.com/science/article/pii/S2468202019300154#sec4

Lesueur C, Gartner M, Mentler A, Fuerhacker M. (2008) Comparison of four extraction methods for the analysis of 24 pesticides in soil samples with gas chromatography-mass spectrometry and liquid chromatography-ion trap-mass spectrometry. Talanta 75(1), 284-93.

Letinski, D.J., Parkerton, T.F., Redman, A.D., Connelly, M.J., Peterson, B., 2016. Water solubility of selected C9-C18 alkanes using a slow-stir technique: Comparison to structure - property models. Chemosphere 150, 416–423. https://doi.org/10.1016/j.chemosphere.2015.12.038

Li, H., Lu, L., Huang, W., Yang, J., Ran, Y., 2014. In-situ partitioning and bioconcentration of polycyclic aromatic hydrocarbons among water, suspended particulate matter, and fish in the Dongjiang and Pearl Rivers and the Pearl River Estuary, China. Mar. Pollut. Bull. 83, 306–316. https://doi.org/10.1016/j.marpolbul.2014.04.036

Li, J., Liu, H., Wang, C., Yang, J., Han, G. (2018). Stable isotope labeling-assisted GC/MS/MS method for determination of methyleugenol in food samples. J. Sci. Food Agric. 98: 3485-3491.

Liu, X., Lv, K., Deng, C., Yu, Z., Shi, J., Johnson, A.C., 2019. Persistence and migration of tetracycline, sulfonamide, fluoroquinolone, and macrolide antibiotics in streams using a simulated hydrodynamic system. Environmental Pollution 252, 1532–1538. https://doi.org/10.1016/j.envpol.2019.06.095

Liu, X., Xu, X., Li, C., Zhang, H., Fu, Q., Shao, X., Ye, Q., Li, Z., 2016. Assessment of the environmental fate of cycloxaprid in flooded and anaerobic soils by radioisotopic tracing. Science of The Total Environment 543, 116–122. https://doi.org/10.1016/j.scitotenv.2015.11.018

Liu, X., Xu, X., Li, C., Zhang, H., Fu, Q., Shao, X., Ye, Q., Li, Z., 2015. Degradation of chiral neonicotinoid insecticide cycloxaprid in flooded and anoxic soil. Chemosphere 119, 334–341. https://doi.org/10.1016/j.chemosphere.2014.06.016

Lo, J.C., Campbell, D.A., Kennedy, C.J., Gobas, F.A.P.C., 2015. Somatic and gastrointestinal *in vivo* biotransformation rates of hydrophobic chemicals in fish. Environmental Toxicology and Chemistry 34, 2282–2294. https://doi.org/10.1002/etc.3050

Lo J.C., Letinski D.J., Parkerton T.F., Campbell D.A., Gobas F. 2016*. In vivo* Biotransformation Rates of Organic Chemicals in Fish: Relationship with Bioconcentration and Biomagnification Factors, Environmental Science and Technology 50, 13299-13308. DOI:10.1021/acs.est.6b03602

López-López, J.A., Mendiguchía, C., Pinto, J.J., Moreno, C., 2019. Application of solvent-bar micro-extraction for the determination of organic and inorganic compounds. TrAC Trends in Analytical Chemistry 110, 57–65. https://doi.org/10.1016/j.trac.2018.10.034

Manikandan, M., Kazibwe, Z., Hasan, N., Deenadayalan, A., Gopal, J., Pradeep, T., Chun, S., 2016. Biological Desorption Electrospray Ionization Mass Spectrometry (DESI MS) – unequivocal role of crucial ionization factors, solvent system and substrates. TrAC Trends in Analytical Chemistry 78, 109–119. https://doi.org/10.1016/j.trac.2016.02.013

Maia, A.S., Ribeiro, C., Tiritan, M.E., 2020, Analysis of chiral drugs in environmental matrices: Current knowledge and trends in environmental, biodegradation and forensic fields, TrAC Trends in Analytical Chemistry 124, 115783, https://doi.org/10.1016/j.trac.2019.115783

Marsik, P., Rezek, J., Židková, M., Kramulová, B., Tauchen J., Vaněk, T. 2017. Non-steroidal anti-inflammatory drugs in the watercourses of Elbe basin in Czech Republic Chemosphere 171, 97-105. https://www.sciencedirect.com/science/article/pii/S0045653516317751

Maser AC, Hage, DS., 2010 Immunoaffinity chromatography: an introduction to applications and recent developments. Bioanalysis, 2(4), 769-90.

Mayer, P., Parkerton, T.F., Adams, R.G., Cargill, J.G., Gan, J., Gouin, T., Gschwend, P.M., Hawthorne, S.B., Helm, P., Witt, G., You, J., Escher, B.I., 2014. Passive sampling methods for contaminated sediments: scientific rationale supporting use of freely dissolved concentrations.

Integr. Environ. Assess. Manag. 10, 197-209.

McAvoy, D.C., Pittinger, C.A., Willis, A.M., 2016. Biotransformation of tetrabromobisphenol A (TBBPA) in anaerobic digester sludge, soils, and freshwater sediments. Ecotoxicology and Environmental Safety 131, 143–150. https://doi.org/10.1016/j.ecoenv.2015.07.009

McDonough, K., Itrich, N., Schwab, E., Federle, T., 2016. Determination of biodegradation rates for surfactants and a fatty alcohol in aerobic sediment using a simplified test system. Environmental Toxicology and Chemistry 35, 2199–2208. https://doi.org/10.1002/etc.3407

McGill, D., Chekmeneva, E., Lindon, J.C., Takats, Z., Nicholson, J.K., 2019. Application of novel solid phase extraction-NMR protocols for metabolic profiling of human urine. Faraday Discuss. 218, 395–416. https://doi.org/10.1039/C8FD$_0$0220 G

Middleditch, B.S., Hines, H.B., Bastian, F.O., Middleditch, P.R., 1980. 2,5-Di-t-amyl-p-benzoquinone: An antioxidant encountered during the analysis of human tissues. Biomedical Mass Spectrometry 7, 105–108. https://doi.org/10.1002/bms.1200070304

Middleditch, B.S., Zlatkis, A., 1987. Artifacts in Chromatography: An Overview. Journal of Chromatographic Science 25, 547–551. https://doi.org/10.1093/chromsci/25.12.547

Middleditch, B.S., 1989. Analytical Artifacts, 1st Edition. ed, Journal of Chromatography Library. Elsevier Science.

Miller, T.H., Bury, N.R., Owen, S.F., Barron, L.P., 2017. Uptake, biotransformation and elimination of selected pharmaceuticals in a freshwater invertebrate measured using liquid chromatography tandem mass spectrometry. Chemosphere 183, 389–400. https://doi.org/10.1016/j.chemosphere.2017.05.083

Millipore-Sigma, 2017. LC-MS Contaminants: Avoid, identify, minimize. Millipore-Sigma. www.merckmillipore.com

Mohler, R.E., Ahn, S., O’Reilly, K., Zemo, D.A., Devine, C.E., Magaw, R., Sihota, N., 2020, Towards comprehensive analysis of oxygen containing organic compounds in groundwater at a crude oil spill site using GC×GC-TOFMS and Orbitrap ESI-MS, Chemosphere 244, 125504, https://doi.org/10.1016/j.chemosphere.2019.125504

Moreda·Pineiro J, Moreda·Pineiro (2015) A. Recent advances in combining microextraction techniques for sample pre-treatment. TRAC Trends Anal Chem, 71:265-74.

Mullin, L., Katz, D.R., Riddell, N., Plumb, R., Burgess, J.A., Yeung, L.W.Y., Jogsten, I.E., 2019. Analysis of hexafluoropropylene oxide-dimer acid (HFPO-DA) by liquid chromatography-mass spectrometry (LC-MS): Review of current approaches and environmental levels. TrAC Trends in Analytical Chemistry 118, 828–839. https://doi.org/10.1016/j.trac.2019.05.015

Nallani, G.C., Paulos, P.M., Constantine, L.A., Venables, B.J., Huggett, D.B., 2011. Bioconcentration of ibuprofen in fathead minnow (*Pimephales promelas*) and channel catfish (Ictalurus punctatus). Chemosphere 84, 1371–1377. https://doi.org/10.1016/j.chemosphere.2011.05.008

Net, S., Delmont, A., Sempéré, R., Paluselli, A., Ouddane, B., 2015. Reliable quantification of phthalates in environmental matrices (air, water, sludge, sediment and soil): A review. Science of The Total Environment 515–516, 162–180. https://doi.org/10.1016/j.scitotenv.2015.02.013

Neumann, M., Schliebner, I., 2017. Protecting the sources of our drinking water. German Environment Agency. https://www.umweltbundesamt.de/sites/default/files/medien/1410/publikationen/171027_uba_pos_pmt_substances_engl_2aufl_bf.pdf

Nießner, R., Schäffer, A., 2017. Organic Trace Analysis. De Gruyter. https://doi.org/10.1515/9783110441154

OECD 23. (2019) Guidance Document on Aqueous-phase Aquatic Toxicity Testing of Difficult Test Chemicals. ENV/JM/MONO(2000)6/REV1. 8 February 2019

OECD 264. (2017) Guidance Document on Aspects of OECD TG 305 on Fish Bioaccumulation. Series on Testing and Assessment No. 264. ENV/JM/MONO(2017)16. 19-Jul-2017.

OECD 307. (2002) OECD Guidelines for the Testing of Chemicals: Aerobic and Anaerobic Transformation in Soil. Adopted: 24th April 2002

OECD 308. (2002) OECD Guidelines for the Testing of Chemicals: Aerobic and Anaerobic Transformation in Aquatic Sediment Systems. Adopted: 24th April 2002

OECD 309. (2004) OECD Guidelines for the Testing of Chemicals: Aerobic Mineralisation in Surface Water: Simulation Biodegradation Test. Adopted 13th April 2004.

OECD (2019), Guiding Principles and Key Elements for Establishing a Weight of Evidence for Chemical Assessment, Series on Testing and Assessment No. 311, Environment, Health and Safety Division, Environment Directorate.

Overturf, C.L., Overturf, M.D., Huggett, D.B., 2016. Bioconcentration and endocrine disruption effects of diazepam in channel catfish, Ictalurus punctatus. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 183–184, 46–52. https://doi.org/10.1016/j.cbpc.2016.02.001

Padrón, M.E.S, Afonso-Olivares, C., Sosa-Ferrera, Z. , Santana-Rodríguez, J.J. 2014, Review: microextraction techniques coupled to liquid chromatography with mass spectrometry for the determination of organic micropollutants in environmental water samples, Molecules 10320–10349, https://doi:10.3390/molecules190710320

Patel, D.C., Wahab, M.F., Armstrong, D.W., Breitbach, Z.S., 2016. Advances in high-throughput and high-efficiency chiral liquid chromatographic separations. J Chromatogr A 1467, 2–18. https://doi.org/10.1016/j.chroma.2016.07.040

Pawlowski, S., Lanzinger, A.C., Dolich, T., Füßl, S., Salinas, E.R., Zok, S., Weiss, B., Hefner, N., Sloun, P.V., Hombeck, H., Klingelmann, E., Petersen-Thiery, M., 2019. Evaluation of the bioaccumulation of octocrylene after dietary and aqueous exposure. Science of The Total Environment 672, 669–679. https://doi.org/10.1016/j.scitotenv.2019.03.237

Pedersen-Bjergaard, S., Huang, C., Gjelstad, A., 2017. Electromembrane extraction–Recent trends and where to go. Journal of Pharmaceutical Analysis 7, 141–147. https://doi.org/10.1016/j.jpha.2017.04.002

Pérez-Carrera, E., León, V.M., Lara-Martín, P.A., González-Mazo, E., 2010. Influence of the hydrophilic moiety of anionic and nonionic surfactants on their aerobic biodegradation in seawater. Science of The Total Environment 408, 922–930.

https://doi.org/10.1016/j.scitotenv.2009.10.003

Perkin Elmer.com: Perkin Elmer Application Note: LSC in Practice: Radio-Carbon Dioxide (14CO₂) Trapping and Counting https://www.perkinelmer.com/CMSResources/Images/44-73122APP_RadioCarbonDioxideTrappingCounting.pdf

Perry, R.H., Cooks, R.G., Noll, R.J., 2008. Orbitrap mass spectrometry: Instrumentation, ion motion and applications. Mass Spectrometry Reviews 27, 661–699. https://doi.org/10.1002/mas.20186

Petrovic, M., Barcelop, D., 2004, “Environmental organic analytes,” In Rosenfeld, J.M., 2004. Sample Preparation for Hyphenated Analytical Techniques. Blackwell Publishing and CRC Press.

Petrovic, M., 2014. Methodological challenges of multi-residue analysis of pharmaceuticals in environmental samples. Trends in Environmental Analytical Chemistry 1, e25–e33. https://doi.org/10.1016/j.teac.2013.11.004

Piechota P, Cronin MTD, Hewitt M and Madden JC (2013) Pragmatic Approaches to Using Computational Methods To Predict Xenobiotic Metabolism, Journal of Chemical Information and Modeling 53 (6), 1282-1293. https://pubs.acs.org/doi/10.1021/ci400050v

Poole, C., (2014). Instrumental thin-layer chromatography. Elsevier. ISBN: 978-0124172234 pp. 187-188

Popiel, S., Sankowska, M., 2011. Determination of chemical warfare agents and related compounds in environmental samples by solid-phase microextraction with gas chromatography. Journal of Chromatography A 1218, 8457–8479. https://doi.org/10.1016/j.chroma.2011.09.066

Qian, Y., Posch, T., Schmidt, T.C., 2011. Sorption of polycyclic aromatic hydrocarbons (PAHs) on glass surfaces. Chemosphere 82, 859–865. https://doi.org/10.1016/j.chemosphere.2010.11.002

Qin, N., He, W., Kong, X.-Z., Liu, W.-X., He, Q.-S., Yang, B., Wang, Q.-M., Yang, C., Jiang, Y.-J., Jorgensen, S.E., Xu, F.-L., Zhao, X.-L., 2014. Distribution, partitioning and sources of polycyclic aromatic hydrocarbons in the water-SPM-sediment system of Lake Chaohu, China. Sci. Total Environ. 496, 414–423. https://doi.org/10.1016/j.scitotenv.2014.07.045

Ramos, L., Kristenson, E.M., Brinkman, U.A.T., 2002. Current use of pressurised liquid extraction and subcritical water extraction in environmental analysis. Journal of Chromatography A, Sample Handling 975, 3–29. https://doi.org/10.1016/S0021-9673(02)01336-5

Rashid A, Nawaz S, Barker H, Ahmad I, Ashraf M. (2010) Development of a simple extraction and clean-up procedure for determination of organochlorine pesticides in soil using gas chromatography-tandem mass spectrometry. J Chromatogr A, 217(17), 2933- 9.

Raths, J., Kühr, S., Schlechtriem, C., n.d. Bioconcentration, metabolism and spatial distribution of ^{14}C-labelled laurate in the freshwater amphipod *Hyalella Azteca*. Environmental Toxicology and Chemistry n/a. https://doi.org/10.1002/etc.4623

RayTest.com:

https://www.raytest.com/fileadmin/raytest/Downloads/Product_Catalogue_PDF/BETA_radiation.pdf

Redman, A.D., 2015. Role of entrained droplet oil on the bioavailability of petroleum substances in aqueous exposures. Marine Pollution Bulletin 97, 342–348. https://doi.org/10.1016/j.marpolbul.2015.05.068

Redman, A.D., McGrath, J.A., Stubblefield, W.A., Maki, A.W., Di Toro, D.M., 2012. Quantifying the concentration of crude oil microdroplets in oil-water preparations. Environ. Toxicol. Chem. 31, 1814–1822. https://doi.org/10.1002/etc.1882

Redman, A.D. *et al*. (2017), Investigating the role of dissolved and droplet oil in aquatic toxicity using dispersed and passive dosing systems. Environmental Toxicology and Chemistry, Vol.36/4:1020-1028.

Redrup, M.J., Igarashi, H., Schaefgen, J., Lin, J., Geisler, L., Ben M'Barek, M., Ramachandran, S., Cardoso, T., Hillewaert, V., 2016. Sample Management: Recommendation for Best Practices and Harmonization from the Global Bioanalysis Consortium Harmonization Team. AAPS J 18, 290–293. https://doi.org/10.1208/s12248-016-9869-2

Reemtsma, T., 2003. Liquid chromatography–mass spectrometry and strategies for trace-level analysis of polar organic pollutants. Journal of Chromatography A, 1000, 477–501. https://doi.org/10.1016/S0021-9673(03)00507-7

Rezaee M, Assadi Y, Hosseinia, M·RM, Aghaee E, Ahmadi F, Berijani S. (2006) "Determination of organic compounds in water using dispersive liquid-liquid microextraction," J Chromatogr A, 1116 (12), 1-9.

Rodríguez-Cruz, M.S., Pose-Juan, E., Marín-Benito, J.M., Igual, J.M., Sánchez-Martín, M.J., 2019. Pethoxamid dissipation and microbial activity and structure in an agricultural soil: Effect of herbicide rate and organic residues. Applied Soil Ecology 140, 135–143. https://doi.org/10.1016/j.apsoil.2019.04.011

Rosen, R., 2007. Mass spectrometry for monitoring micropollutants in water. Current Opinion in Biotechnology, Energy biotechnology / Environmental biotechnology 18, 246–251. https://doi.org/10.1016/j.copbio.2007.03.005

Rosenfeld, J.M., 2004. Sample Preparation for Hyphenated Analytical Techniques. Blackwell Publishing and CRC Press.

Rouessac, F., Rouessac, A., 2007. Chemical Analysis: Modern Instrumentation Methods and Techniques, 2nd Edition, Wiley. [English translation]

Rouessac, F., Rouessac, A., 2019. Analyse chimique, 9th Edition. ed. Dunod. [French]

Saunders, L.J., Hoffman, A.D., Nichols, J.W., Gobas, F.A.P.C., n.d. Dietary Bioaccumulation and Biotransformation of Hydrophobic Organic Sunscreen Agents in Rainbow Trout. Environmental Toxicology and Chemistry n/a. https://doi.org/10.1002/etc.4638

Schug, K., McNair, H.M., 2002. Adduct formation in electrospray ionization. Part 1: Common acidic pharmaceuticals. Journal of Separation Science 25, 759–766. https://doi.org/10.1002/1615-9314(20020801)25:12<759::AID-JSSC760>3.0.CO;2-M

Schlechtriem, C., Böhm, L., Bebon, R., Bruckert, H.-J., Düring, R.-A., 2017. Fish bioconcentration studies with column-generated analyte concentrations of highly hydrophobic organic chemicals. Environmental Toxicology and Chemistry 36, 906–916. https://doi.org/10.1002/etc.3635

Seiler, T.B. *et al*. (2014), PAH toxicity at aqueous solubility in the fish embryo test with *Danio rerio*using passive dosing. Chemosphere,Vol.112:77−84.

Selcia (2020) Radiolabelling http://www.selcia.com/selcia-radiolabelling

Shang-Qiang, J., Evenson, M.A., 1983. Effects of Contaminants in Blood-Collection Devices on Measurements of Therapeutic Drugs. Clin Chem. 29, 456–461.

Shi, D., Lv, D., Liu, W., Shen, R., Li, D., Hong, H., 2017. Accumulation and developmental toxicity of hexabromocyclododecanes (HBCDs) on the marine copepod Tigriopus japonicus. Chemosphere 167, 155–162. https://doi.org/10.1016/j.chemosphere.2016.09.160

Shi, J., Yang, Y., Zhang, J., Feng, Y., Shao, B., 2016. Uptake, depuration and bioconcentration of bisphenol AF (BPAF) in whole-body and tissues of zebrafish (*Danio rerio*). Ecotoxicology and Environmental Safety 132, 339–344. https://doi.org/10.1016/j.ecoenv.2016.05.025

Shrestha, P., Meisterjahn, B., Hughes, C.B., Mayer, P., Birch, H., Hennecke, D. 2020. Biodegradation testing of volatile hydrophobic chemicals in watersediment systems - Experimental developments and challenges. Chemosphere 238, https://doi.org/10.1016/j.chemosphere.2019.124516

Smith, K.E.C. *et al*. (2010a), Controlling and maintaining exposure of hydrophobic organic compounds in aquatic toxicity tests by passive dosing. Aquatic Toxicolology, Vol.98/1:15-24.

Smith,K.E.C., Oostingh,G.J., Mayer,P.(2010b),Passive dosing for producing defined and constant exposure of hydrophobic organic compounds during *in vitro* toxicity tests. Chemical Research in Toxicology, Vol. 23/1:55-65.

Souza-Silva, É.A., Jiang, R., Rodríguez-Lafuente, A., Gionfriddo, E., Pawliszyn, J., 2015. A critical review of the state of the art of solid-phase microextraction of complex matrices I. Environmental analysis. TrAC Trends in Analytical Chemistry, 71, 224–235. https://doi.org/10.1016/j.trac.2015.04.016

Stangl G, Niessner R. (1995) Micellar extraction - a new step for enrichment in the analysis of napropramide. Int I Environ Anal Chem, 58(1·4)15-22.

Starokozhev, E., Sieg, K., Fries, E., Püttmann, W., 2011. Investigation of partitioning mechanism for volatile organic compounds in a multiphase system. Chemosphere 82, 1482–1488. https://doi.org/10.1016/j.chemosphere.2010.11.033

Steckel, A., Schlosser, G., 2019. An Organic Chemist’s Guide to Electrospray Mass Spectrometric Structure Elucidation. Molecules 24, 611. https://doi.org/10.3390/molecules24030611

Stibany, F. *et al*. (2017), Aquatic toxicity testing of liquid hydrophobic chemicals -Passive dosing exactly at the saturation limit. Chemosphere,Vol.167:551-558.

Summerfield, S., 2010. Part 3: Introduction to Chromatography. Analytical Chemistry Techniques Manual, Loughborough University https://hydra.hull.ac.uk/resources/hull:2406, 1–32.

Swartz, M., 2010. HPLC Detectors: A Brief Review. Journal of Liquid Chromatography & Related Technologies 33, 1130–1150. https://doi.org/10.1080/10826076.2010.484356

Szreniawa-Sztajnert, A., Zabiegała, B., Namieśnik, J., 2013. Developments in ultrasound-assisted microextraction techniques for isolation and preconcentration of organic analytes from aqueous samples. TrAC Trends in Analytical Chemistry 49, 45–54. https://doi.org/10.1016/j.trac.2013.02.015

Teo, C.C., Tan, S.N., Yong, J.W.H., Hew, C.S., Ong, E.S., 2010. Pressurized hot water extraction (PHWE). Journal of Chromatography A, Extraction Techniques 1217, 2484–2494. https://doi.org/10.1016/j.chroma.2009.12.050

Thomas, K.A., Hand, L.H., 2011. Assessing the potential for algae and macrophytes to degrade crop protection products in aquatic ecosystems. Environmental Toxicology and Chemistry 30, 622–631. https://doi.org/10.1002/etc.412

Toräng, L., Nyholm, N., 2005. Biodegradation rates in adapted surface water can be assessed following a preadaptation period with semi-continuous operation. Chemosphere 61, 1–10. https://doi.org/10.1016/j.chemosphere.2005.03.040

Toräng, L., Reuschenbach, P., Müller, B., Nyholm, N., 2002. Laboratory shake flask batch tests can predict field biodegradation of aniline in the Rhine. Chemosphere 49, 1257–1265. https://doi.org/10.1016/S0045-6535(02)00605-7

Torres Padron ME, Afonso·Oiivares C, Sosa·Ferrera Z, Juan Santana-Rodriguez J, (2014) Microextraction techniques coupled to liquid chromatography with mass spectrometry for the determination or organic micropollutants in environmental water samples. Molecules 19(7):10320 - 49.

Traverso-Soto, J.M., Rojas-Ojeda, P., Sanz, J.L., González-Mazo, E., Lara-Martín, P.A., 2016. Anaerobic degradation of alcohol ethoxylates and polyethylene glycols in marine sediments. Science of The Total Environment 544, 118–124. https://doi.org/10.1016/j.scitotenv.2015.11.140

Ulsaker, G.A., Teien, G., 1979. Communications. Gas chromatographic-mass spectrometric analysis of polyethylene bottle packed intravenous solutions contaminated with N-ethylaniline from the rubber part of the two-component closure. Analyst 104, 580–582. https://doi.org/10.1039/AN9790400580

UNEP (2017). United Nations Environment Programme: Stockholm Convention on Persistent Organic Pollutants, Texts and Annexes. Secretariat of the Stockholm Convention (SSC), 2017.

Unice, K.M., Bare, J.L., Kreider, M.L., Panko, J.M., 2015. Experimental methodology for assessing the environmental fate of organic chemicals in polymer matrices using column leaching studies and OECD 308 water/sediment systems: Application to tire and road wear particles. Science of The Total Environment 533, 476–487. https://doi.org/10.1016/j.scitotenv.2015.06.053

US EPA, 1994. SW-846 Test Method 3520C: Continuous Liquid-Liquid Extraction [WWW Document]. US EPA. URL https://www.epa.gov/hw-sw846/sw-846-test-method-3520c-continuous-liquid-liquid-extraction (accessed 12.19.19).

US EPA, 1996. SW-846 Test Method 3510C: Separatory Funnel Liquid-Liquid Extraction [WWW Document]. US EPA. URL https://www.epa.gov/hw-sw846/sw-846-test-method-3510c-separatory-funnel-liquid-liquid-extraction (accessed 12.19.19).

US EPA, 2007. SW-846 Test Method 3535A: Solid-Phase Extraction (SPE) [WWW Document].

US EPA. URL https://www.epa.gov/hw-sw846/sw-846-test-method^{-3}535a-solid-phase-extraction-spe (accessed 12.19.19).

US EPA, 2014. SW-846 Test Method 3511: Organic Compounds in Water by Microextraction [WWW Document]. US EPA. URL https://www.epa.gov/hw-sw846/sw-846-test-method^{-3}511-organic-compounds-water-microextraction (accessed 12.19.19).

Vas G, Vekey K. (2004) Solid-phase microextractlon: a powerful sample preparation tool prior to mass spectrometric analysis, J Mass Spectrom, 39(3), 233-54.

Vasilieva, V., Edelmann, E., Loibner, A.P., Scherr, K.E., 2010, Comprehensive two-dimensional gas chromatography (GCxGC/MS) for monitoring heterocyclic and polycyclic aromatic compounds in industrial soils during biodegradation, Journal of Biotechnology 150, 262, https://doi.org/10.1016/j.jbiotec.2010.09.158

Vergauwen,L. *et al*. (2015),A high throughput passive dosing format for the fish embryo acute toxicity test. Chemosphere, Vol.139:9-16

Verge, K.M., Agnes, G.R., 2002. Plasticizer contamination from vacuum system O-rings in a quadrupole ion trap mass spectrometer. Journal of the American Society for Mass Spectrometry 13, 901–905. https://doi.org/10.1016/S1044-0305(02)00386-0

Voges, R., Heys, J.R., Moenius, T. (2009). Preparation of compounds labeled with tritium and carbon-14. John Wiley & Sons. ISBN 9780470516072

Wang, G., Shi, H., Du, Z., Chen, H., Peng, J., Gao, S., 2017. Bioaccumulation mechanism of organophosphate esters in adult zebrafish (*Danio rerio*). Environmental Pollution 229, 177–187. https://doi.org/10.1016/j.envpol.2017.05.075

Wang, S., Seiwert, B., Kästner, M., Miltner, A., Schäffer, A., Reemtsma, T., Yang, Q., Nowak, K.M., 2016. (Bio)degradation of glyphosate in water-sediment microcosms – A stable isotope co-labeling approach. Water Research 99, 91–100. https://doi.org/10.1016/j.watres.2016.04.041

Wang, W., Yue, L., Zhang, S., Ye, Q., Qi, W., Wang, H., Chen, Z., 2013. Fate of pyribambenz propyl (ZJ0273) in anaerobic soils revealed by position-specific ^{14}C labeling. Journal of Hazardous Materials 258–259, 151–158. https://doi.org/10.1016/j.jhazmat.2013.04.040

Waria, M., O’Connor, G.A., Toor, G.S., 2011. Biodegradation of triclosan in biosolids-amended soils. Environmental Toxicology and Chemistry 30, 2488–2496. https://doi.org/10.1002/etc.666

Wei, X.-F., Linde, E., Hedenqvist, M.S., 2019. Plasticiser loss from plastic or rubber products through diffusion and evaporation. NPJ Materials Degradation 3, 18. https://doi.org/10.1038/s41529-019-0080-7

Wick, A., Jacobs, B., Kunkel, U., Heininger, P., Ternes, T.A., 2016. Benzotriazole UV stabilizers in sediments, suspended particulate matter and fish of German rivers: New insights into occurrence, time trends and persistency. Environmental Pollution 212, 401–412. https://doi.org/10.1016/j.envpol.2016.01.024

Willkowska A, Biziuk M. (2011) Determination of pesticide residues in food matrices using the QuEChERS methodology. Food Chem, 125(3), 803-12.

Woodburn, K., Drottar, K., Domoradzki, J., Durham, J., McNett, D., Jezowski, R., 2013. Determination of the dietary biomagnification of octamethylcyclotetrasiloxane and decamethylcyclopentasiloxane with the rainbow trout (*Oncorhynchus mykiss*). Chemosphere 93, 779–788. https://doi.org/10.1016/j.chemosphere.2012.10.049

Xu, B., Mao, D., Luo, Y., Xu, L., 2011. Sulfamethoxazole biodegradation and biotransformation in the water–sediment system of a natural river. Bioresource Technology 102, 7069–7076. https://doi.org/10.1016/j.biortech.2011.04.086

Yang, Y., Li, T., Yan, L., Yu, Y., Wang, S., Li, C., Wen, Y., Zhao, Y., 2018. Investigation on the relationship between critical body residue and bioconcentration in zebrafish based on bio-uptake kinetics for five nitro-aromatics. Regulatory Toxicology and Pharmacology 98, 18–23. https://doi.org/10.1016/j.yrtph.2018.07.002

Zelst, F.H.M. van, Meerten, S.G.J. van, Kentgens, A.P.M., 2019. Characterising polar compounds using supercritical fluid chromatography–nuclear magnetic resonance spectroscopy (SFC–NMR). Faraday Discuss. 218, 219–232. https://doi.org/10.1039/C8FD$_0$0237A

Zembrzuska, J., Budnik, I., Lukaszewski, Z., 2016a. Parallel pathways of ethoxylated alcohol biodegradation under aerobic conditions. Science of The Total Environment 557–558, 612–619. https://doi.org/10.1016/j.scitotenv.2016.03.112

Zembrzuska, J., Budnik, I., Lukaszewski, Z., 2016b. Monitoring of selected non-ionic surfactants in river water by liquid chromatography–tandem mass spectrometry. Journal of Environmental Management 169, 247–252. https://doi.org/10.1016/j.jenvman.2015.12.034

Zgoła-Grześkowiak, A., Grześkowiak, T., 2011. Dispersive liquid-liquid microextraction. TrAC Trends in Analytical Chemistry, Special Issue 30, 1382–1399. https://doi.org/10.1016/j.trac.2011.04.014

Zhakupbekova, A., Baimatova, N., Kenessov, B., 2019. A critical review of vacuum-assisted headspace solid-phase microextraction for environmental analysis. Trends in Environmental Analytical Chemistry 22, e00065. https://doi.org/10.1016/j.teac.2019.e00065

Zhang, H., Li, X.J., Martin, D.B., Aebersold, R. (2003). Identification and quantification of N-linked glycoproteins using hydrazide chemistry, stable isotope labeling and mass spectrometry. Nature biotechnology, 21: 660-666.

Zhu, M., Zhang, D. and Skiles, G.L. (2005b). Quantification and structural elucidation of low quantities of radiolabelled metabolites using microplate scintillation counting (MSC) techniques in conjunction with LC-MS. In Identification and quantification of drugs, metabolites and metabolizing enzymes by LC-MS. Elsevier, Amsterdam, the Netherlands, pp.195-223.

Zhu, M., Zhao, W. and Humphreys, W.G., 2007. Applications of liquid radiochromatography techniques in drug metabolism studies. In Drug Metabolism in Drug Design and Development: Basic Concepts and Practice, pp.289-317.

Zhu, M., Zhao, W., Vazquez, N., Mitroka, J.G., 2005. Analysis of low level radioactive metabolites in biological fluids using high-performance liquid chromatography with microplate scintillation counting: Method validation and application. Journal of Pharmaceutical and Biomedical Analysis 39, 233–245. https://doi.org/10.1016/j.jpba.2005.03.012

13. Glossary and abbreviations list

Table 13.1: Abbreviations for relative quantity measures as fractions in parts per notation.

UNIT	UNIT		GRAM PER KILOGRAM	GRAM PER GRAM	GRAM PER TONNE	PERCENT
% w/w	percentage by mass	10^{-1}	10^{1} g/kg	10^{-2} g/g	10^{2} kg/t	% w/w
g/kg	grams per kg	10^{-3}	g/kg	mg/g	kg/t	10^{-1}% w/w
ppm	parts per million	10^{-6}	mg/kg	µg/g	g/t	10^{-4}% w/w
ppb	parts per billion	10^{-9}	µg/kg	ng/g	mg/t	10^{-7}% w/w
ppt	parts per trillion	10^{-12}	ng/kg	pg/g	µg/t	10^{-10}% w/w
ppq	parts per quadrillion	10^{-15}	pg/kg	fg/g	ng/t	10^{-13}% w/w

Table 13.2: Abbreviations for mass units.

ABBREVIATION	UNIT	KILOGRAMS	GRAMS
kg	Kilograms	1 kg	1000 g
g	Grams	10^{-3} kg	1 g
mg	Milligrams	10^{-6} kg	10^{-3} g
µg	Micrograms	10^{-9} kg	10^{-6} g
ng	Nanograms	10^{-12} kg	10^{-9} g
pg	Picograms	10^{-15} kg	10^{-12} g
fg	Femtograms	10^{-18} kg	10^{-15} g

13.1 Abbreviations

ABBREVIATION	
AFID	Alkali flame ionising detector (GC)
APCI	Atmospheric pressure chemical ionisation (MS)
APPI	Atmospheric pressure photoionization (MS)
ASE	Accelerated solvent extraction (another name for PLE)
BAF	Bioaccumulation factor
Bq	Becquerel (1 dps)
CAD	Corona charged aerosol detector
CAS	Chemical abstracts service
CD	Conductometric detector
CDD	Corona discharge detector
CEFIC	European Chemical Industry Council
CE-MS	Capillary Electrophoresis-mass spectrometry
CEWAF	Chemically enhanced water accommodated fraction
Cfree	Freely dissolved concentration
CGE	Capillary Gel Electrophoresis
CI	Chemical ionisation
Ci	Curie (a unit of radioactivity, corresponding to 3.7×10^{10} dps = 2.22×10^{12} dpm
CMC	Critical micelle concentration
C_{nom}	Nominal concentration
C_{org}	Concentration in the organism

ABBREVIATION	
cpm	Counts per minute (radioactivity)
CRMs	Certified reference materials
CWAX/TR	Carbowax/template resin-coated fibre
CZE	Capillary Zone Electrophoresis
DAD	Diode array detector
DBDI	Dielectric-barrier-discharge ionization
DDLME	Dispersive liquid–liquid microextraction
DESI	Desorption electrospray ionization
DISPME	Direct-immersion solid phase microextraction
DegT50	Degradation half-life
DT_{50}	Dissipation half-life
DLLE	Dispersive liquid-liquid extraction
DLLME	Dispersive liquid-liquid microextraction
DLS	Dynamic light scattering
dpm	Disintegrations per minute (radioactivity)
dps	Disintegrations per second
DSPE	Dispersive solid phase extraction
DSPME	Dispersive solid phase microextraction
EA-C-IRMS	Elemental analyser – combustion - isotope ratio mass spectrometry
EA-IRMS	Elemental analyser isotope - ratio mass spectrometry
EC	European Commission
EC	Electrochemical detector (HPLC)
ECD	Electron capture detector (GC)
ECHA	European Chemicals Agency
ECx	Effect concentration
EDCs	Endocrine disrupting compounds
EI	Electron impact ionisation (MS)
EINECS	European inventory of existing commercial chemical substances
ELCD	Electrolytic conductivity detector (HPLC)
ELSD	Evaporative light scattering detector (HPLC)
ERA	Environmental risk assessment
ESE	Enhanced solvent extraction
ESI	Electrospray ionization, negative polarity (MS)
EU	European Union
FID	Flame ionization detector (GC)
FLD	Fluorescence detection (HPLC)
FPD	Flame photometric detector (GC)
FT-ICR-MS	Fourier-transform ion cyclotron MS
FT-ICR-MS	Fourier-transform ion cyclotron MS
FT-ICR-MS	Fourier-transform ion cyclotron MS
FWHM	Full width half maximum
GC	Gas chromatography
GCB	Graphitized carbon black
GC-FID	Gas chromatography–flame ionization detection
GC-MS	Gas chromatography–mass spectrometry
GC-NCI-MS	Gas chromatography negative chemical ionization mass spectrometry
GHS	Globally harmonised system of classification and Labelling
GLC	Gas-liquid Chromatography
GLP	Good laboratory practice
HETP	Height equivalent of theoretical plate
HEWAF	High energy water accommodated fraction
HF-LLLME	Hollow fibre liquid-liquid-liquid microextraction
HF-LPME	Hollow-fibre liquid phase microextraction
HILIC	Hydrophilic Interaction Liquid Chromatography
HLB	Hydrophilic-lipophilic-balanced
HOC	Hydrophobic organic chemical / hydrophobic organic contaminants
HPLAC	High performance liquid affinity chromatography
HPLC	High performance liquid chromatography
HPLC-DAD	High performance liquid chromatography-diode array detector
HQ	Hazard Quotient

ABBREVIATION	
HRMS	High resolution mass spectroscopy
HSE	High-pressure solvent extraction
HS-SPME	Headspace solid phase microextraction
IL-USA-DLLME	Ionic liquid-based ultrasound assisted dispersive liquid-liquid microextraction
IMS	Ion mobility spectrometry
IRMS	Isotope ratio mass spectrometry
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
K_{OW}	Octanol-water partition coefficient
K_{sp}	Solubility product constant
LC-MS	Liquid chromatography coupled to mass spectrometer
LC-MS/MS	Liquid chromatography time-of-flight mass spectrometry
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOEC	Lowest observed (adverse) effect concentration
LOQ	Limit of quantification
LPME	Liquid Phase Microextraction
LSC	Liquid scintillation counting
MAE	Microwave-assisted extraction
MALDI	Matrix-assisted laser desorption ionization
MDL	Method detection limit
MDO	Mechanically dispersed oil
ME	Micellar extraction / cloud-point extraction
MEBE	Membrane Enhanced Bioaccessibility Extraction
MECC	Micellar Electrokinetic Capillary Chromatography
MIP	Molecular imprinted polymers
MLD	Method limit detection
MMLLE	Microporous membrane liquid–liquid extraction
MNP	Magnetic nanoparticles
MP	Mobile phase
MQL	Method quantification limit
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS-DLLME	Magnetic stirring assisted dispersive liquid–liquid microextraction
MS-MS	Tandem mass spectrometry
MWCNTs	Multiwalled carbon nanotubes
NER	Non-extractable residues
NI	Negative ionization
NMR	Nuclear magnetic resonance spectroscopy
NOAEL	No-observable-adverse-effect-level
NOM	Natural organic matter
NPD	Nitrogen-Phosphorus Detector
NPs	Nanoparticles
OECD	Organisation for Economic Co-operation and Development
PEC	Predicted environmental concentration
PFE	Pressurized fluid extraction
PI	Photoionization
PID	Photoionization detector
PLE	Pressurized liquid extraction
PNEC	Predicted no-effect concentration
POM-SPE	Polyoxymethylene solid phase extraction

ABBREVIATION	
POPs	Persistent organic pollutants
ppb	parts per billion
ppm	parts per million
ppq	parts per quadrillion
ppt	parts per trillion
Prep-HPLC	Preparative HPLC
PSE	Pressurized solvent extraction (another name for PLE)
PT	Potentiometry titration
PTR	Proton-transfer-reaction
Q	Single quadrupole
QC	Quality control
QIT	Quadrupole-ion-trap
QIT-TOF	Quadrupole ion-trap time-of-flight
QL	Quantitation limit
Q-MS	Quadrupole MS
QqQ	Triple quadrupole mass spectrometer
QSAR	Quantitative structure property relationship
Q-TOF	Quadrupole time-of-flight
QTof-MS	Quadrupole time-of-flight mass spectrometry
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
RAM	Radioactivity monitoring
RAMD	Radioactivity detector (RAM)
RDSE	Rotating disk sorptive extraction
REACH	Registration, evaluation, authorisation, and restriction of chemicals
REMPI	Resonance-enhanced multi-photon ionization
RI	Refractive index detector
RPLC	Reversed-phase liquid chromatography
RSD	Relative standard deviation
SAX	Strong anion exchange
SBSE	Stir bar sorptive extraction
SCX	Strong cation exchange
SEC	Size exclusion chromatography
SFC	Supercritical fluid chromatography
SFE	Supercritical fluid extraction
SIL	Stable isotope labelled
SIM	Selected ion monitoring, Single ion monitoring
SLE	Solid–liquid extraction
SOP	Standard Operation Procedures
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SRM	Selected reaction monitoring
SSA	Specific surface area
SUPRAS	Supramolecular solvents
TCD	Thermal conductivity detector
TEF	Toxicity equivalence factor
TEF	Toxicity equivalence factor
TEQs	Toxic Equivalents
TG	Test guideline
TG-MS	Thermogravimetry-mass spectrometry
TID	Thermionic ionization detector
TLC	Thin layer chromatography
T_m	Melting point temperature
TOF	Time of flight
TOF-MS	Time-of-flight detector Mass Spectrometer
TQ	Triple quadrupole
TU	Toxic Units
UAE	Ultrasound-Assisted Extraction
UA-LLME	Ultrasound-assisted liquid-liquid microextraction
UHPLC	Ultra high performance liquid chromatography
UPLC	Ultra-Performance Liquid Chromatography (Waters PLC trademark) for

ABBREVIATION	
	Ultra-HPLC / UHPLC
UPLC-MS/MS	Ultra performance liquid chromatography coupled to mass spectrometer
UV	Ultraviolet
UVCB	Unknown or variable composition, complex reaction products, and biological materials
UV-DAD	Ultraviolet - Diode array detector
UV-Vis	Ultra violet-visible
VA-DLLME	Vortex-assisted dispersive liquid–liquid microextraction
VOC	Volatile Organic Carbon
WAF	Water accommodated fraction
WAX	Weak anionic exchange
WSF	Water soluble fraction
WWTP	Wastewater treatment plant
XPS	X-ray photoelectron spectroscopy

13.2 Chemical Abbreviations

CHEMICAL ABBREVIATIONS	
AA	Acetic acid
AChE	Acetylcholinesterase
ACN	Acetonitrile
ADONA	Dodecafluoro-3H-4,8-dioxanonanoate
AE/AEO	Alcohol ethoxylates
AES	Alkylethoxysulfates
AMAC	Ammonium acetate
AMF	Ammonium formate
APEO	Alkylphenol ethoxylates
AS	Alkylsulfates
ASo	Alkyl sulfonates
ATAC	Alkyl trimethyl ammonium chloride
BAC	Benzyl ammonium chloride
BaP	Benzo(a)pyrene
BDE	bromodiphenyl ether
BDMAC	Alkyl benzyl dimethyl ammonium chloride
BFRs	Brominated flame retardants
BSAs	Benzenesulfonamides
BSTFA	Bis(trimethylsilyl)trifluoroacetamide
BTEX	Benzene, Toluene, Ethylbenzene, o-Xylene
BTRs	Benzotriazoles
BTs	Benzothiazoles
BZPs	Benzodiazepines
CDEA	Cocamide diethanolamine
CTAB	Cetyltrimethylammonium bromide
DCM	Dichloromethane
DDAC	Dialkyl dimethyl ammonium chloride
DDT	Dichlorodiphenyltrichloroethane
DEEDMAC	Diethylester dimethylammonium chloride
DEET	N,N-Diethyl-meta-toluamide
DEQ	Diesterquaternary
DHTDMAC	Dihydrogenated tallow dimethylammonium chloride
DiSB	Disulfine blue dyes

CHEMICAL ABBREVIATIONS	
DTDMAC	Didecyldimethylammonium chloride
DVB	Divinylbenzene
DVB	Divinylbenzene
EA	Ethyl acetate
FA	Formic acid
FTOH	Fluorotelomer alcohols
GAA	Glacial acetic acid
HBCD	hexabromocyclododecane
HFO	Heavy fuel oil
HFPO-DA	Hexafluoropropylene oxide-dimer acid
HFPO-TA	Hexafluoropropylene oxide-trimer acid
LAS	Linear alkylbenzenesulfonates
MB	Methylene blue dyes
MBSTFA	N-tert-butyl-dimethylsilyl N-methyltrifluoroacetamide
MG	Methylene green dyes
MH-AB	Mixed hemimicelle/admicelle-based
NP	Nonylphenol
NPEC	Nonylphenol ethoxy carboxylates
NPEO	Nonyl phenol ethoxylates
NSAIDs	Nonsteroidal anti-inflammatory drugs
OP	Octylphenol
OPEC	Octylphenol ethoxy carboxylates
OPEO	Octylphenol ethoxylate
OPs	Organophosphates
PA	Polyacrylate
PAHs	Polycyclic aromatic hydrocarbons / polyaromatic hydrocarbons
PAPs	Polyfluorinated phosphate esters
PBDE	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PCDDs	Polychlorinated dibenzodioxines
PCDFs	Polychlorinated dibenzofuranes
PDMS	Polydimethylsiloxane
PEG	Poly(ethylene glycols)
PFAS	Per- and polyfluorinated alkyl substances
PFBA	Perfluorobutanoic acid
PFC	Perfluorinated compounds
PFCAs	Per- and polyfluorinated carboxylic acids
PFECAs	Per- and polyfluorinated ether carboxylic acids
PFESAs	Per- and polyfluorinated ether sulfonic acids
PFHpA	Perfluoroheptanoic acid
PFHxA	Perfluorohexanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PFPeA	Perfluoropentanoic acid
QAC	Quaternary ammonia compounds
SAS	Secondary alkyl sulfate
SDS	Sodium dodecyl sulfate
SPC	Sulfophenylcarboxylates
TBA	Tributylamine
TCC	Triclocarban
TCS	Triclosan
TEA	Triethylamine
TMAC	Dodecyl trimethyl ammonium
TPS	Tetrapropylenebenzenesulfonate

14. Appendix 1: Additional considerations on the use of Passive Dosing

The use of passive dosing as a method of maintaining freely dissolved concentrations of hydrophobic organic chemicals (Log $K_{ow} > 5$) that are poorly soluble is discussed in Section 10.3.1.2, and the principles of passive dossing are further outlined in Annex 6 of OECD 23 (2019).

The stages for passive dosing are:

- Washing of the polymer.
    - Silicone rods were rinsed with deionized water and dried twice with lint-free tissue. Then the rods were then soaked in ethyl acetate for 24 hours, soaked in ethanol for 24 hours, and, finally, dried at 120°C for 2 hours (Hammershoj *et al.*, 2019).
- Application test substance to the polymer.
    - Direct immersion of the polymer into neat liquid test substance (Stibany *et al.*, 2017).
    - A solid could be dispersed as a suspension in methanol (Smith *et al.*, 2010b).
    - Direct syringe injection of test substance into medical grade silicone tubing. This permits the performing of limit exposure at the saturation concentration or as a dose response by varying amount of tubing (Redman *et al.*, 2017).
    - The polymer can be loaded a small volume of test substance dissolved in methanol and then water is added sequentially to push all test chemical into the polymer (Birch *et al.*, 2010).
    - The polymer can be loaded to a certain concentration by equilibrium partitioning from test substance dissolved in methanol. This can be done in many different ways (Smith *et al*., 2010a; Butler *et al*., 2013; Seiler *et al.*; 2014, Vergauwen *et al.*, 2015; Butler *et al.*, 2016).
    - The rods were loaded with liquid neat substance onto 10 and 20 g silicone rods in 100 ml amber glass serum bottles that were then rotated for 48 hours at about 10 rpm to permit the adsorption (Hammershoj *et al.*, 2019).

- Once the test substance is loaded, the polymer is washed with ultrapure water.
    - Non-volatile test substances can be dried in oven to ensure that all solvent is removed before adding it to the test vessels.
    - Volatile test then methanol is removed by washing only and stored in deionised water if they are not immediately used in test media.
    - The rods were wiped twice with lint-free tissue and rinsed once with ultrapure water and transferred to clean bottles(Hammershoj *et al.*, 2019).

If the application of the test substance to the polymer is to be achieved using a solvent suspension, the use of an appropriate solvent is necessary. As noted in OECD 23 (2019), most solvents lead to substantial swelling of the polymer and should therefore be avoided; the use of methanol is accepted in OECD 23, whilst the use of other solvents would require additional experimental validation work. Lee *et al*. (2003) investigated the interaction of solvents with polydimethylsiloxane (PDMS). Moderate to high polar substances caused the PDMS to swell. Solvents used included water, most alcohols (methanol, ethanol, 1-propanol, phenol, ethylene glycol and glycerol), nitriles (acetonitrile), disubstituted amides (NMP, DMF), tetrasubstituted ureas (1,1,3,3-tetramethylurea), sulfoxides (DMSO, tetramethylene sulfone), pyridines, and nitro compounds (nitro-methane). Therefore, these solvents are not suitable for passive dosing.

Solvents that swelled PDMS the most are non-polar solvents including acyclic and cyclic hydrocarbons (pentanes, hexanes, heptane, cyclohexane), aromatic hydrocarbons (xylenes, toluene, benzene), halogenated compounds (chloroform, trichloroethylene), and ethers (diethyl ether, dimethoxyethane, tetrahydro-furan). In addition, there are certain secondary amines (diisopropylamine, dipropylamine) and tertiary amines (triethylamine) that swell PDMS to the greatest extent.