ECHA
EUROPEAN CHEMICALS AGENCY

GUIDANCE

Guidance on the Application of the CLP Criteria

Part 4: Environmental hazards

and

Part 5: Additional Hazards

Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures

Version 4.0
Nov 2024

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Guidance on the Application of CLP Criteria

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European Chemicals Agency

P.O Box 400, FI-00121 Helsinki, Finland

DOCUMENT HISTORY

Version	Comment	Date
Version 1.0	First edition	August 2009
n.a.	Please note that change between the version published in August 2009 and that of April 2011 are not recorded in this document history.	April 2011
Version 2.0	Revision of the Guidance addressing content in relation to the environmental criteria chapters and Annexes following the 2nd Adaptation to Technical Progress to the CLP Regulation (Commission Regulation (EU) No 286/2011). The ECHA Secretariat revised the Guidance *Part 4 – Environmental hazards* and *Annexes* of the guidance document referring to the revised criteria for the long-term aquatic hazard for substances and mixtures and added new *Part 5 – Additional hazards* referring to the hazard class 'hazardous to the ozone layer'. As well, a number of examples have been included in the respective Parts and Annexes to illustrate the revisions performed. The update includes the following: • All green boxes in Part 4 that are impacted by the 2nd ATP were updated. As the CLP legal text uses commas instead of dots to define numbers smaller than 1, the green boxes now show commas as well. • Revision of Part 4, by providing guidance on the application of the new long-term aquatic hazard criteria for substances and mixtures. • Section 4.1.3 Classification of substances hazardous to the aquatic environment and section 4.1.4 Classification of mixtures hazardous to the aquatic environment were substantially revised, for example by addition of new references, as well as the new/revised examples to illustrate relevant topics in the Part 4. • New Part 5 – Additional hazards was added (please note that Part 5: Labelling was deleted from the Guidance in previous non-recorded versions and covered via a new Guidance on Labelling and Packaging in accordance with Regulation (EC) No 1272/2008 published in April 2011). • Most of the I.3 sub-sections in Annex I – Aquatic toxicity were revised. • In Annex II – Rapid degradation the terminology was modified. • Most of the Annex IV – Metals and Inorganic Metal Compounds was substantially modified and revised, as well as in sub-section IV.7 new examples were added.	April 2012

Version 2.1	Corrigendum of Part 4: Environmental hazards and its related Annexes I-V. The corrigendum includes the following: • The list of abbreviations was updated. • Update or deletion of outdated references to Guidance on information requirements and chemical safety assessment, Endpoint specific guidance (Chapter R.7a) within Annexes I-V. • A footnote informing the reader that with effect from 1 September 2013, Directive 98/8/EC had been repealed by Biocidal Products Regulation (EU) No 528/2012 was added. • In Part 1, Part 4 and Annexes modal verbs 'shall' were replaced with 'must' where appropriate. • A footnote related to respiratory sensitisation and skin sensitisation in Table 1.1 was removed. A correction to Example D, sub-chapter 4.1.4.7.5 was applied, namely a reference to CLP, Annex I, point (b) (ii) of Table 4.1.0 was introduced. In addition, the result of a summation method calculation was corrected.	November 2013
Version 2.2	Corrigendum to take account of the end of the transition period of the 4th ATP (as foreseen in version 4.0 above): • change the colour of relative legal text boxes from orange to green; • in Part 2, to delete section 2.2.1 Flammable gases and section 2.3.1 Flammable Aerosols (outdated text) and renumber sections 2.2.2 Flammable gases (including chemically unstable gases) and 2.3.2 Aerosols accordingly; • in Part 3, to delete the "outdated text" in sections 3.7.4.1 and 3.7.5.1 in Reproductive Toxicity. In addition, minor editorial errors were corrected and minor reformatting was made.	June 2015
Version 2.3	Partial revision of the Guidance to update the content mainly following the 8th Adaptation to Technical Progress to the CLP Regulation (Commission Regulation (EU) No 286/2011). Revision of few specific additional topics. The update includes the following: (i) Throughout the document: • Revision of legal references and legal text quotations. • Renumbering of some sections. • Deletion of sections regarding the reclassification of substances and mixtures previously classified in accordance with the DSD or DPD.	July 2017

	(ii) Minor changes to Part 4 to update the terminology when referring to short-term (acute) and long-term (chronic) studies	
Version 3.0	Part 4 has been updated to provide clarifications, correct errors, delete information considered irrelevant, and add text on new OECD TGs. The update includes the following: • Section 4.1.3.3.1: The paragraph referring to the absence of chronic data has been amended to reflect general data availability and established practice; • Section 4.1.3.3.1; Additional text was added to clarify cases where data on a degradation product may need to be considered; • Section 4.1.3.3.1: A reference to a topic the guidance annexes do not directly comment on was deleted; • At the end of section 4.1.3.3.2: Some text was added to further define instances where Category Chronic 4 may apply; • Section 4.1.3.3.3: Text was added on the fact that M-factors are considered part of the classification; • Section 4.1.4.5: Clarification on deriving classification when using toxicity values calculated from the additivity formula was added; • Section 4.1.4.7.5: Correction to Example D and explanation added; • Section 4.1.7 was deleted as any reference to reclassification from DSD is out of date; • Annex I.2: General statements on most commonly occurring issues during aquatic toxicity testing have been added; • Annex I.2.1.1: General considerations on OECD TG 236 were added; • Annex I.2.1.2: General considerations regarding various relevant OECD TGs added; • Annex I.2.2.1: A more recent change in the respective OECD TG (202) is reflected; • Annex I.2.2.1: Clarifications on invertebrate data beyond *Daphnia Magna* were added; • Annex I.2.2.2: A more recent change in the respective OECD TG protocol (202 part II) is reflected; • Annex I.2.2.2: Clarifications on invertebrate data beyond *Daphnia Magna* were added; • Annex I.2.3.2: Clarifications on aquatic macrophyte data were added; • Annex I.2.3.2: CLP preference on algae as the preferred test species deleted; • Annex I.3.2: Clarification on use of surrogate approach for chronic classification added; • Annex I.4: Clarification on use of data for difficult to test substances added;	January 2024

	• Annex II.2: Two first sentences of the paragraph have been deleted as they were vague and did not offer any added value; • Annex II.2.3.6: An additional statement that soil degradation data can be used under certain conditions in the absence of aquatic degradation data has been added; • Annex II.2.3.7: Clarification on the use of anaerobic degradation data has been added; • Annex II.3.1: Clarification on the general guidance for complex substances has been added (also change in I.4.5); • Annex II.3.5: Clarification text on presence of both positive and negative ready biodegradability tests has been added; • Annex III.2.1: Footnote 29 has been deleted as it is out of date; • Annex III.2.1.2: Clarification text on radio-labelled substances has been added; • Annex III.2.2.2, Table III.1 has been deleted; • Annex III.2.2.2, Guidance on (Q)SAR BCFs and their use has been added; • Annex III.5: Text added to emphasise that a conclusion on bioaccumulation is required under CLP and that a conclusion as "inconclusive" is still possible, albeit not preferred; • Annex III.5: More explicit wording added on the conclusion on bioaccumulation, based on the available data; • Annex IV.3: Rapid removal footnote amended to reflect CARACAL decision on rapid removal; • Annex IV.5.4: Correction of error regarding loading rates used to determine chronic M-factors; • Annex IV.5.6: Correction of how alloys are considered under CLP to remove reference to 'special preparations' and accurately reflect CLP Art. 2(27); • Table IV.1: Correction of criteria error on determining M-factors for readily soluble metal compounds; Besides these changes, typos, spelling errors and other formatting issues, such as homogenisation of referencing (both within the document and to external sources), have been addressed. Note, such changes are not substantial and do not alter the content.	
Version 4.0	Introduction of Guidance on Environmental Endocrine Disruptors (section 4.2). Introduction of Guidance on PBT/vPvB and PMT/vPvM (section 4.3). Guidance re-presented as various parts.	November 2024

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LIST OF ABBREVIATIONS

Standard term / Abbreviation	Explanation
(Q)SAR	(Quantitative) Structure Activity Relationship
ADME	Absorption, Distribution, Metabolism, Excretion
AMA	Amphibian Metamorphosis Assay
AOP	Adverse Outcome Pathway
ASTM	American Society for the Testing of Materials
ATP	Adaptation to Technical Progress (ATP) to the CLP Regulation
BAF	Bioaccumulation Factor
BAT	Bioaccumulation Assessment Tool
BCF	Bioconcentration Factor
BMF	Biomagnification factor
BOD	Biological Oxygen Demand
BP	Biocidal Products
BPP™	Biodegradation Probability Program
BPR	Biocidal Products Regulation (Regulation EU 528/2012)
BSAF	Biota-Sediment/Soil Accumulation Factor
C&L	Classification and Labelling
CA	Competent Authority
CERAPP	Collaborative Estrogen Receptor Activity Prediction Project
CLP	Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures[1]

[1] Regulation (EC) No 1272/2008 of the European Parliament and Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC and amending Regulation (EC) No 1907/2006 [OJ L 353, 31.12.2008, p. 1].

CMC	Critical Micelle Concentration
CMR	Carcinogenic, Mutagenic, Reprotoxic
COD	Chemical Oxygen Demand
ComPARA	Collaborative Modelling Project for Androgen Receptor Activity
CRED	Criteria for Reporting and Evaluating Ecotoxicity Data
CSA	Chemical Safety Assessment
CTA	Comparative thyroid assay
DFOP	Double First-Order
DOC	Dissolved Organic Carbon
D_{OW}	Octanol-water distribution coefficient
DPD	Directive 1999/45/EC on the classification and labelling of Dangerous Preparations[2]
DSD	Directive 67/548/EEC on the classification and labelling of Dangerous Substances[3]
EAMA	Extended Amphibian Metamorphosis Assay
EATS	Estrogen, Androgen, Thyroid and Steroidogenic
EC number	European Community number
EC_{10}	Effect Concentration that causes a measurable adverse effect to 10% of the test organisms comparing to the control group
ECHA	European Chemicals Agency, Helsinki (https://echa.europa.eu/)
eChemPortal	Global Portal to Information on Chemical Substances
ECOTOX	ECOTOXicology Knowledgebase
ECx	Effect Concentration, associated with x% response

[2] Directive 1999/45/EC of the European Parliament and of the Council of 31 May 1999 concerning the approximation of the laws, regulations and administrative provisions of the Member States relating to the classification, packaging and labelling of dangerous preparations [OJ L 200, 30.7.1999, p. 1].

[3] Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances [OJ 196, 16.8.1967, p. 1].

ED	Endocrine disruptor or endocrine disrupting
ED ENV	Endocrine disruptor for the environment
ED HH	Endocrine disruptor for human health
EffD	Effective Dose
EFSA	European Food Safety Authority
ELS	Early life stages
EPI	Estimation Programs Interface
ER	Estrogen receptor
EROD	Ethoxyresorufin-O-deethylase
ERV	Ecotoxicity Reference Value
ESR	Existing Substances Regulation
FDA	Food and Drug Administration of United States
FE	first estrus
FFLCTT	Fish full lifecycle toxicity test
FOMC	First Order Multi-Compartment
FSTRA	Fish short term reproduction assay
GCL	Generic Concentration Limit
GHS	Globally Harmonised System of Classification and Labelling of Chemicals[4]
GLP	Good Laboratory Practice

[4] Globally Harmonised System of Classification and Labelling of Chemicals (GHS), Fifth revised edition, United Nations, New York and Geneva, 2013.

GSI	Gonadosomatic index
H	Henry's Law constant
HC	Hazard Class
HPLC	High-Performance Liquid Chromatography
HPT axis	Hypothalamic–Pituitary–Thyroid axis
HS model	Hockey-Stick model
IC	Inorganic Carbon
IPCheM	Information Platform for Chemical Monitoring
IR&CSA	Guidance on Information Requirements and Chemical Safety Assessment, ECHA (http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_en.htm)
ISO	International Organisation for Standardization
IUPAC	International Union of Pure and Applied Chemistry
IVIVE	in Vitro to In Vivo Extrapolation
K_d	Distribution coefficient
KE	Key Event
KER	Key event relationship
K_F	Freundlich adsorption coefficient
K_{OA}	Octanol-Air partition coefficient
K_{OC}	Organic Carbon- water partition coefficient
K_{ow}	The n-octanol/water partition coefficient
LBD	Ligand binding domain
LD_{50}/LC_{50}	Median (50%) lethal dose/concentration
LDL	Low-Density Lipoprotein (cholesterol)
LFER	Linear Free-Energy Relationships

LO (A) EL/C	Lowest Observed (Adverse) Effect Level/Concentration
LoE	Lines of Evidence
LOQ	Level of Quantification
MCI	Molecular Connectivity Indices
M-factor	Multiplying factor
MIE	Molecular initiating event
MoA	Mode of Action
MRL	Maximum Residue Level
MTC	Maximum tolerated concentration
MW	Molecular weight
n.a.	Not available
NAFTA	North American Free Trade Agreement
NAMs	New Approach Methodologies
NERs	Non-Extractable Residues
NITE	Japanese National Institute of Technology and Evaluation
NO(A)EC	No Observed (Adverse) Effect Concentration
NOEC	No Observed Effect Concentration
NOELR	No Observed Effect Loading Rate
NR	Nipple retention
OC	Organic Carbon
ODP	Ozone Depleting Potential
ODS	Ozone Depleting Substances
OECD	Organisation for Economic Co-operation and Development
OECD CF	OECD Conceptual Framework

OECD GD 150	Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption
OECD TG	OECD Test Guideline All Test Guidelines are available at the OECD homepage (https://www.oecd.org/en/topics/sub-issues/testing-of-chemicals/test-guidelines.html)
OSPAR	Oslo and Paris Conventions
PAT	Persistence Assessment Tool
PBT	Persistent, Bioaccumulative and Toxic
PBTK	Physiologically Based Toxicokinetic models
PFASs	Per- and Polyfluoroalkyl Substances
PFOS	Perfluorooctane Sulphonic Acid
PMT	Persistent, Mobile and Toxic
PPDB	Pesticides Properties DataBase
PPP	Plant Protection Product
PPPR	Plant Protection Products Regulation (Regulation EC No. 1107/2009)
PPS	Pathway Prediction System
QMRF / QPRF	(Q)SAR Model Reporting Format / (Q)SAR Prediction Reporting Format
RAAF	Read-Across Assessment Framework
RAC	Risk Assessment Committee
REACH	Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals[5]
R_F	Retardation/mobility Factor

[5] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and omission of Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. [OJ L 396, 30.12.2006 p.1.] [Corrigendum: OJ L 136, 29.5.2007 p.3].

RMF	Relative Mobility Factors
SANCO	Directorate-General Health and Consumer Protection
SAR	Structure-activity relationship
SCL	Specific Concentration Limit
SDS	Safety Data Sheet
SFO	Single First-Order Rate
SPM	Suspended Particulate Matter
SPMD	Semi-Permeable Membrane Devices
SPME	Solid-Phase Micro Extraction
SRC	Syracuse Research Corp
SSC	Secondary Sex Characteristics
SSD	Species Sensitivity Distribution
STOT RE	Specific Target Organ Toxic after Repeated Exposure
SVHC	Substances of Very High Concern
T/D	Transformation/Dissolution
T/Dp	Transformation/Dissolution Protocol
T3	Triiodothyronine
T4	Thyroxine
TBG	Thyroxine binding globulin
Test Methods Regulation	Regulation (EC) No 440/2008 laying down test methods pursuant to the REACH Regulation[6]
TG	Test Guideline

[6] Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) [OJ L 142, 31.5.2008, p. 1] [Corrigendum: OJ L 143, 3.6.2008, p. 55].

TH	Thyroid hormone
ThOD / ThCO2	Theoretical Oxygen Demand / theoretical carbon dioxide
TLC	Thin-Layer Chromatography
TMF	Trophic Magnification Factor
TRH	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone
UBA	Umweltbundesamt
UN	United Nations
US EPA	United States Environmental Protection Agency
US EPA	United States Environmental Protection Agency
UVCB	Substances of unknown or variable composition, complex reaction products or biological materials
VCBA	Virtual cell-based assay
VO	Vaginal opening
VP	Vapour Pressure
vPvB	Very Persistent, very Bioaccumulative
vPvM	Very Persistent, very Mobile
VTG	Vitellogenin
WAF	Water Accommodated Fraction
WAT	White adipose tissue
WoE	Weight of Evidence
WS	Water Solubility
WSF	Water soluble fraction
WWTP	Waste Water Treatment Plant

NOTEs to the reader:

In this document, text cited from Regulation (EC) No 1272/2008 is indicated in green boxes in *italic* font.

This symbol highlights text to be noted.

4. PART 4: ENVIRONMENTAL HAZARDS

4.1. HAZARDOUS TO THE AQUATIC ENVIRONMENT

4.1.1. Introduction

Guidance for the application of the criteria covering effects on the aquatic compartment was developed by OECD and incorporated as Annexes 9 and 10 in the 'Globally Harmonised System of classification and labelling of chemicals (UN GHS)' (Fourth revised edition, 2011, https://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev04/English/ST-SG-AC10-30-Rev4e.pdf).

The text in part 4, even more so in some of the Annexes to this chapter, is largely based on the text in UN GHS. The guidance given in Annexes 9 and 10 of UN GHS relates to substances, but not mixtures. Some parts have therefore been slightly revised to take into account recent developments and additional guidance documents provided by ECHA. Furthermore guidance on the classification of mixtures has been brought into this chapter as well as classification examples for both substances and mixtures.

4.1.2. Scope

Annex I: ***4.1.1.3.1*** *Classification of substances and mixtures for environmental hazards requires the identification of the hazards they present to the aquatic environment. The aquatic environment is considered in terms of the aquatic organisms that live in the water, and the aquatic ecosystem of which they are part. The basis, therefore, of the identification of short-term (acute) and long-term (chronic) hazards is the aquatic toxicity of the substance or mixture, although this shall be modified by taking account of further information on the degradation and bioaccumulation behaviour, if appropriate.*

The classification scheme (CLP Annex I, part 4) has been developed with the objective of identifying those chemicals that present, through their intrinsic properties, a hazard to the aquatic environment covering the aquatic freshwater and marine ecosystems. For most substances, the majority of data available addresses this environmental compartment. The classification scheme is limited in scope in that it does not, as yet, include aquatic sediments, nor higher organisms at the top end of the aquatic food-chain, although these may to some extent be covered by the criteria selected.

Although limited in scope, it is widely accepted that this compartment is vulnerable, in that it is the receiving compartment for many harmful substances, and the organisms that live there can be very sensitive. It is also complex since any system that seeks to identify hazards to the environment must seek to define those effects in terms of wider effects on ecosystems, rather than on individuals within a species or population. For practical reasons, a limited set of specific properties has been selected through which the short-term (acute) and long-term (chronic) hazards can be best described: acute aquatic toxicity; chronic aquatic toxicity; lack of rapid degradability; and potential for or actual bioaccumulation. Relevant definitions for aquatic hazard classification of substances *i.e.* acute and/or chronic aquatic toxicity, availability and bioavailability to the aquatic environment are outlined in the CLP Regulation, Annex I, section 4.1.1.1. Some further guidance can be viewed in IR&CSA Chapter R.7b and Annex I of this Guidance. The rationale for the selection of these properties as the means to define the aquatic hazard will be described in more detail in the following sections of this guidance (e.g. see Annex I).

4.1.3. Classification of substances hazardous to the aquatic environment

4.1.3.1. Information applicable for classification of substances hazardous to the aquatic environment

4.1.3.1.1. Substance properties used for classification

Generally speaking, in deciding whether a substance should be classified, a search of appropriate databases and other sources of data should be made for at least the following substance properties: water solubility, octanol/water partition coefficient (log K_{ow}), acute aquatic toxicity ($L(E)C_{50}$), chronic aquatic toxicity (NOEC or equivalent EC_x[7]), degradation (evidence of rapid degradability, hydrolysis) and bioaccumulation (preferably bioconcentration factor in fish (BCF)). Other information might be considered on a case-by-case basis.

Although not used directly in the criteria, the water solubility and stability data are important since they are a valuable help in the data interpretation of the other properties. However, water solubility may be difficult to determine and is frequently recorded as simply being low, insoluble or less than the detection limit. This may create problems in interpreting aquatic toxicity and bioaccumulation studies (see also Annex III). Hydrolysis data (Test Methods Regulation (EC) No 440/2008; OECD TG 111) and information on the hydrolysis products as well as their behaviour in water might be helpful as well. As an example, for substances where the degradation half-life (DT_{50}) is less than 12 hours, environmental effects are likely to be attributed to the hydrolysis products rather than to the parent substance itself.

4.1.3.1.2. Information and data availability

Annex I: ***4.1.1.2.2*** *Preferably data shall be derived using the standardised test methods referred to in Article 8(3). In practice data from other standardised test methods such as national methods shall also be used where they are considered as equivalent. Where valid data are available from non-standard testing and from non-testing methods, these shall be considered in classification provided they fulfil the requirements specified in section 1 of Annex XI to Regulation (EC) No 1907/2006. In general, both freshwater and marine species toxicity data are considered suitable for use in classification provided the test methods used are equivalent. Where such data are not available classification shall be based on the best available data. See also part 1 of Annex I to Regulation (EC) No 1272/2008.*

The data used to classify a substance can be drawn from data required for other regulatory purposes as well as the relevant literature. A number of internationally recognised databases exist which can act as a good starting point. Such databases vary widely in quality and comprehensiveness, and it is unlikely that any one database will hold all the information necessary for a classification to be made. Some databases specialise in aquatic toxicity and others in environmental fate. Information can also be gathered from data submitted under plant protection products and/or biocidal products legislation.

Non-testing information

Information derived from (Q)SAR and read-across, grouping and categorisation can also be used, see also IR&CSA Chapter R.6 (see also section 1.4 of this Guidance).

Information sources

[7] If available, preference is given to EC_{10}, see OECD 2006.

Section R.3.4.1 of IR&CSA Chapter R.3 specifies a selection of freely available databases and databanks which might be consulted for classification purposes. All ECHA guidance documents are available on the Agency's website (https://echa.europa.eu/support/guidance).

Data can also be found through the eChemPortal, which is a global portal to information on chemical substances. The eChemPortal provides access to a number of databases, including the OECD HPV (Existing Chemicals Database) and the SIDS UNEP (Screening Information Dataset for High Volume Chemicals). The eChemPortal is currently hosted by the OECD: (https://www.echemportal.org/echemportal/)

Further guidance is given in Annex 0 to this document.

4.1.3.2. Evaluation of available information

4.1.3.2.1. General considerations

The term substance covers a wide range of chemicals (consult the *Guidance for identification and naming of substances under REACH and CLP*, Chapter 3) many of which pose challenges to a classification system based on rigid criteria. This section will thus provide some guidance on how these challenges can be dealt with based both on experience in use and clear scientific rationale.

The range of interpretational problems can be extensive and, as a result, such interpretation will always rely on the ability and expertise of the individuals responsible for classification. However, it is possible to identify some commonly occurring difficulties and provide guidance. Such difficulties can fall into a number of overlapping issues:

a. The difficulty in applying the current test procedures to some types of substances;

b. The difficulty in interpreting the data derived both from these 'difficult to test' substances (see section below) and from other substances;

c. The difficulty in interpretation of diverse datasets derived from a wide variety of sources (e.g. Weight of Evidence);

d. The difficulty of interpreting 'other' information

Regarding the use of test data, in general, only reliable information (*i.e.* with a Klimisch reliability score of 1 (reliable without restrictions) or 2 (reliable with restrictions)) should be used for classification purposes. However, good quality data may not always be available for all trophic levels. It will be necessary to consider data of lower quality for those trophic levels for which good quality data are not available. Consideration of such data, however, will also need to take into account the difficulties that may have affected the likelihood of achieving a valid result. For larger data sets, preference should be given to information with Klimisch score 1, while information with Klimisch score 2 can be used as supporting information. For more information on the Klimisch reliability scoring system, see IR&CSA Chapter R.4 section R.4.2.

4.1.3.2.2. Substances difficult to test

For many organic substances, the testing and interpretation of data presents no problems when applying both the relevant Test Methods Regulation (EC) No 440/2008 and/or OECD Test Guidelines and the classification criteria. There are a number of typical interpretational problems, however, that can be attributed to the properties of the substance being studied. These are commonly called 'difficult to test substances':

a. poorly soluble substances: these substances are difficult to test because they present problems in the preparation of a test solution, maintenance of test concentrations and verification of exposure during aquatic toxicity testing. In addition, many available data for such substances have been produced using 'solutions' in excess of the water solubility resulting in major interpretational problems in defining the true L(E)C_{50} or NOEC/EC_x for the purposes of classification. Interpretation of the partitioning behaviour can also be problematic where the poor solubility in water and octanol may be compounded by

insufficient sensitivity in the analytical method. Water solubility may be difficult to determine and is frequently recorded as simply being less than the detection limit, creating problems in interpreting both aquatic toxicity and bioaccumulation studies. In biodegradation studies, poor solubility may result in low bioavailability and thus lower than expected biodegradation rates. The specific test method or the choice of procedures used can thus be of key importance (see also Annex I.4.2);

b. unstable substances: such substances that degrade (or react) rapidly in the test system present both testing and interpretational problems. It will be necessary to determine whether the correct methodology in line with the guidance provided in section 4.1.3.3 has been used, whether it is the substance or the degradation/reaction product that has been tested, and whether the data produced is relevant to the classification of the parent substance (see also Annex I.4.3);

c. volatile substances: such substances that can clearly present testing problems when used in open systems should be evaluated to ensure adequate maintenance of exposure concentrations. Loss of test material during biodegradation testing is inevitable in certain methods and will lead to misinterpretation of the results (see also Annex I.4.1);

d. complex or multi-constituent[8] substances: such substances, for example, complex hydrocarbons, or other UVCB[9] substances, frequently cannot be dissolved into a homogeneous solution, and the multiple components make monitoring impossible. For organics, consideration therefore needs to be given to using the data derived from the testing of water-accommodated fractions (WAFs) for aquatic toxicity, and the use of such data in the classification scheme[10]. Biodegradation, bioaccumulation, partitioning behaviour and water solubility all present problems of interpretation, where each component of these complex or multi-constituent substances may behave differently (see also Annex I.4.5);

e. polymers: such substances frequently comprise a wide range of molecular masses, which individually might have different water solubilities. Special methods are available to determine the water soluble fraction and these data will need to be used in interpreting the test data against the classification criteria (see also Annex I.4.5);

f. inorganic compounds and metals: such substances, which can interact with the media, can produce a range of aquatic toxicities dependent on factors such as pH, water hardness etc. Difficult interpretational problems also arise from the testing of essential elements that are beneficial at certain levels. For metals and inorganic metal compounds, the concept of degradability as applied to organic compounds has limited or no meaning. Equally the use of bioaccumulation data should be treated with care (see also Annex IV);

g. surface-active substances: such substances can form emulsions in which the bioavailability is difficult to ascertain, even with careful preparation of solutions. Micelle formation can result in an overestimation of the bioavailable fraction even when 'solutions' are apparently formed. This presents significant problems of interpretation in each of the water solubility, partition coefficient, bioaccumulation and aquatic toxicity studies (see also Annex III.3.1);

[8] Further definitions are provided in the *Guidance for identification and naming of substances under REACH and CLP* (ECHA).

[9] UVCB means Substances of Unknown or Variable composition, Complex reaction products or Biological materials, see Chapter 4.3 of the *Guidance for identification and naming of substances under REACH and CLP*.

[10] Note that the toxicity is sometimes expressed as LL_{50}, related to the lethal loading level. This loading level from the WSF or WAF may be used directly in the classification criteria (see also Annex I.4.5 of this guidance document).

h. ionisable substances: such substances can change the extent of ionisation according to the level of counter ions in the media. Acids and bases, for example, will show radically different partitioning behaviour depending on the pH;

i. coloured substances: such substances can cause problems in the algal/aquatic plant testing because of the blocking of incident light;

j. impurities: some substances can contain impurities that can change in percentage and in chemical nature between production batches. Interpretational problems can arise where either or both the toxicity and water solubility of the impurities are greater than the parent substance, thus potentially influencing the toxicity data in a significant way. In general, the substance as manufactured including impurities should be tested and the classification should be based on these test results. To assess the sameness of two substances containing the same impurity in different amount see *Guidance for identification and naming of substances under REACH and CLP*, Chapter 5;

k. essential substances: some substances are essential to life, even though, like any substance, excessive concentrations can be harmful. This can lead to complex concentration/dose-response curves;

l. substances which can chelate or sequester essential elements, leading to the same problems of interpretation as in (k).

For further details see the OECD Guidance 23 Guidance Document on aqueous-phase aquatic toxicity testing of difficult test chemicals (OECD, 2019, http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2000)6/REV1&docLanguage=En) and also the IR&CSA Chapter R.7b, Appendix 7.8-1 and Annex I to the current Guidance Document.

4.1.3.2.3. Interpretation of data for aquatic toxicity, degradation and bioaccumulation

4.1.3.2.3.1. Aquatic toxicity

Annex I: ***4.1.2.7.1*** *Acute aquatic toxicity is normally determined using a fish 96 hour LC_{50}, a crustacea species 48 hour EC_{50} and/or an algal species 72 or 96 hour EC_{50}. These species cover a range of trophic levels and taxa and are considered as surrogate for all aquatic organisms. Data on other species (e.g. Lemna spp.) shall also be considered if the test methodology is suitable. The aquatic plant growth inhibition tests are normally considered as chronic tests but the EC_{50}s are treated as acute values for classification purposes (see note 2).*

Annex I: ***4.1.2.7.2*** *For determining chronic aquatic toxicity for classification purposes data generated according to the standardised test methods referred to in Article 8(3) shall be accepted, as well as results obtained from other validated and internationally accepted test methods. The NOECs or other equivalent EC_x (e.g. EC_{10}) shall be used.*

Fish, crustacea and algae or other aquatic plants are tested as surrogate species representing a range of trophic levels and taxa, and the test methods are highly standardised (see Annex I for further details). Valid data for short- and long-term tests on other species at the same trophic level shall also be considered, provided they are equivalent and suitable in terms of species relevance, testing conditions and test endpoints.

The purpose of classification is to characterise both the acute and long-term hazards in the aquatic environment. The acute and long-term hazards represent distinct types of hazard and should be determined independently.

The lowest available toxicity value(s) between and within the different trophic levels (fish, crustacea, algae/aquatic plants) will normally be used to define the appropriate hazard category(ies), although there may be circumstances where a weight of evidence approach is required (see section 4.1.3.2.4).

Care should be taken when classifying substances like ionisable organic chemicals or organo-metallic substances as the observed results may express different toxicities in freshwater and marine environments or when classifying poorly soluble substances (water solubility e.g. < 1 mg/L CLP Table 4.1.0 note 4), and substances with a specific mode of action inducing effects on reproduction or growth instead of lethality. In the latter cases, there is evidence that acute testing may not provide a true measure of the intrinsic toxicity.

Relevant descriptions of the type of acute and/or chronic aquatic toxicity tests have been outlined in detail in Annex I to the current Guidance and in IR&CSA Chapter R.7b, sections R.7.8.3-R.7.8.4. For classification and labelling purposes, tests using organisms outside the specified size (generally smaller) and/or tests with a differing test duration could be used if no other acceptable data are available.

There are some recently validated in vitro methods available for use as alternative data to determine acute and long-term hazards. Currently, in vitro tests cannot directly substitute in vivo data in terms of one for one replacement for classification purposes. However, in vitro data can already play a role as supporting evidence in a weight of evidence approach and there are ongoing efforts to develop and validate further in vitro methods which may add to our understanding of aquatic toxicity. Although the standard guideline in vivo methods remain the most informative for classification and labelling purposes, all available and relevant information on aquatic toxicity, including non-guideline methods, can be assessed on their own merits and carefully balanced in the overall weight of evidence.

4.1.3.2.3.2. Degradation

Annex I: ***4.1.2.9.1*** *Substances that rapidly degrade can be quickly removed from the environment. While effects of such substances can occur, particularly in the event of a spillage or accident, they are localised and of short duration. In the absence of rapid degradation in the environment a substance in the water has the potential to exert toxicity over a wide temporal and spatial scale.*

Annex I: ***4.1.2.9.2*** *One way of demonstrating rapid degradation utilises the biodegradation screening tests designed to determine whether an organic substance is "readily biodegradable". Where such data are not available, a BOD(5 days)/COD ratio ≥ 0,5 is considered as indicative of rapid degradation. Thus, a substance which passes this screening test is considered likely to biodegrade "rapidly" in the aquatic environment, and is thus unlikely to be persistent. However, a fail in the screening test does not necessarily mean that the substance will not degrade rapidly in the environment. Other evidence of rapid degradation in the environment may therefore also be considered and are of particular importance where the substances are inhibitory to microbial activity at the concentration levels used in standard testing. Thus, a further classification criterion is included which allows the use of data to show that the substance did actually degrade biotically or abiotically in the aquatic environment by > 70 % in 28 days. Thus, if degradation is demonstrated under environmentally realistic conditions, then the criterion of "rapid degradability" is met.*

The definition of degradation covers both biotic (biodegradation) and abiotic degradation processes. Data on degradation properties of a substance may be available from standardised tests, from other types of investigations, or they may be estimated from the structure of the molecules (see section 1.4). In Annex II.2 to this guidance a general overview of relevant definitions on how to use different (bio)degradability tests and guidance for the interpretation of test data in the context of classification and labelling is given. Additional information on (bio)degradation testing methods can be found in IR&CSA Chapter R.7b, section R.7.9. The OECD test methods 301A-F (C.4-A to F of the Test Methods Regulation 440/2008), OECD TG 310, or equivalent tests, are commonly used to determine 'ready biodegradability'. Some guidance on the use of (Q)SAR methods for degradability is presented in IR&CSA Chapter R.7b, section R.7.9.3.1.

The paragraphs below will focus on the guidance for using degradability data for classification & labelling under CLP. It should be noted that the guidance on degradability pertains primarily to individual substances. In the case of complex or multi-constituent substances, the proposed test approaches do not normally allow an unequivocal interpretation of the degradability of the individual components when the test material is the multi-constituent substance as a whole. Thus, results of biodegradability tests on complex or multi-constituent substances should be carefully evaluated before use for classification purposes is considered. See Annex II.3.1 for further details.

Annex I: ***4.1.2.9.3*** *Many degradation data are available in the form of degradation half-lives and these can be used in defining rapid degradation provided that ultimate biodegradation of the substance, i.e. full mineralisation, is achieved. Primary biodegradation does not normally suffice in the assessment of rapid degradability unless it can be demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment.*

Annex I: ***4.1.2.9.4*** *The criteria used reflect the fact that environmental degradation may be biotic or abiotic. Hydrolysis can be considered if the hydrolysis products do not fulfil the criteria for classification as hazardous to the aquatic environment.*

Annex I: ***4.1.2.9.5*** *Substances are considered rapidly degradable in the environment if one of the following criteria holds true:*

(a) if, in 28-day ready biodegradation studies, at least the following levels of degradation are achieved:

(i) tests based on dissolved organic carbon: 70 %;

(ii) tests based on oxygen depletion or carbon dioxide generation: 60 % of theoretical maximum.

These levels of biodegradation must be achieved within 10 days of the start of degradation which point is taken as the time when 10 % of the substance has been degraded; unless the substance is identified as an UVCB or as a complex, multi-constituent substance with structurally similar constituents. In this case, and where there is sufficient justification, the 10-day window condition may be waived and the pass level applied at 28 days, or

(b) if, in those cases where only BOD and COD data are available, when the ratio of BOD_5/COD is ≥ 0,5; or

(c) if other convincing scientific evidence is available to demonstrate that the substance can be degraded (biotically and/or abiotically) in the aquatic environment to a level > 70 % within a 28-day period.

The following decision scheme may be used as a general guidance to facilitate decisions in relation to rapid degradability in the aquatic environment and classification of chemicals hazardous to the aquatic environment.

A substance is considered to be **not** rapidly degradable **unless** at least one of the following is fulfilled:

a. The substance is demonstrated to be readily biodegradable in a 28-day test for ready biodegradability. The pass level of the test (70 % DOC removal or 60 % theoretical oxygen demand) must be achieved within 10 days from the onset of biodegradation, if it is possible to evaluate this according to the available test data (the 10-day window condition may be waived for complex multi-component substances with structurally similar constituents and the pass level applied at 28 days, as discussed in Annex II.2.1.2 to this document). If this is not possible, then the pass level should be evaluated within a 14-day time window if possible, or after the end of the test; or

b. The substance is demonstrated to be ultimately degraded in a surface water simulation test with a half-life of < 16 days (corresponding to a degradation of > 70 % within 28 days); or

c. The substance is demonstrated to be primarily degraded biotically or abiotically e.g. via hydrolysis, in the aquatic environment with a half-life < 16 days (corresponding to a degradation of > 70 % within 28 days), and it can be demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment.

When these preferred data types are not available rapid degradation may be demonstrated if one of the following criteria is justified:

a. The substance is demonstrated to be ultimately degraded in an aquatic sediment or soil simulation test with a half-life of < 16 days (corresponding to a degradation of > 70 % within 28 days); or

b. In those cases where only BOD_5 and COD data are available, the ratio of BOD_5/COD is greater than or equal to 0.5. The same criterion applies to ready biodegradability tests of a shorter duration than 28 days, if the half-life furthermore is < 7 days; or

c. A weight of evidence approach based on read-across provides convincing evidence that a given substance is rapidly degradable.

If none of the types of data mentioned above are available, the substance is considered as **not** rapidly degradable. This decision may be supported by fulfilment of at least one of the following criteria:

i. the substance is not inherently degradable in an inherent biodegradability test; or

ii. the substance is predicted to be slowly biodegradable by scientifically valid (Q)SARs, e.g. for the Biodegradation Probability Program, the score for rapid degradation (linear or non-linear model) < 0.5; or

iii. the substance is considered to be not rapidly degradable based on indirect evidence, such as knowledge from structurally similar substances; or

iv. no other data regarding degradability are available.

The percentage degradation reached after 28 days in ready biodegradability tests may be used directly for the assessment of 'rapid degradability' if no specific information on the time window is available or if the data were derived with the MITI I test (OECD TG 301C, 2006 or C.4-E of the Test Methods Regulation 440/2008). In the Closed Bottle test (OECD TG 301D, or C.4-F of the Test Methods Regulation 440/2008), a 14-day window may be used when measurements have not been made after 10 days. For some industrial chemicals that in terms of composition can be seen as multi-component substances, testing for 'ready biodegradability' can lead to interpretational problems (see Annex II to this guidance).

In all cases, it should be considered that IR&CSA Chapter R.7b, section R.7.9.4 states that "…ready biodegradability tests are intended for pure substances and are generally not applicable for complex compositions containing different types of constituents, like UVCBs". For a UVCB substance, observed biodegradation may indeed represent the biodegradation of only some constituents. In the same document, it is indicated that "it is sometimes relevant to examine the ready biodegradability of mixtures of structurally similar chemicals".

Selection of test systems

As regards section 4.1.2.9.5 (c) in Annex I to CLP ("other convincing scientific evidence available to demonstrate that the substance can be degraded in the aquatic environment to a level > 70 % within a 28-day period"), the evaluation of the fulfilment of this criterion should be conducted on a case-by-case basis by expert judgement. Test systems that can be used to demonstrate the occurrence of rapid degradability are listed in Annex II. This includes e.g. simulation tests under realistic conditions, mesocosms and field monitoring.

Inherent (OECD TG 302A and B, or C.9 and C.12 of the Test Methods Regulation 440/2008) and sewage treatment simulation (OECD TG 303, or C.10 of the Test Methods Regulation 440/2008) tests are not used in this context, due to the high levels of adapted biomass. Anaerobic degradation tests (OECD TG 311/ISO 11734 and analogous tests) are not suitable because of the specificity of the anaerobic compartments. Also the defined category of 'Enhanced Ready Biodegradation (Screening) Tests' in IR&CSA Chapter R.7b, section R.7.9 are not suitable for use in classification and labelling, as they are designed to promote biodegradation and are not as stringent as OECD TG 301-suite type tests. More specifically, section R.7.9.4.1 explicitly states that "a number of potential enhancements to the ready biodegradation test have been identified. These enhancements have been proposed for the determination of persistence in vPvB/PBT assessment only but are not to be used for Classification and Labelling and quantitative exposure and risk assessment".

Use of SARs and (Q)SARs

The estimation of degradation via SARs and/or (Q)SARs for hydrolysis and biodegradation is a rapidly developing field. The predictions from (Q)SAR models may be considered as contributing to a decision on ready biodegradability or rapid degradation for classification purposes. (Q)SAR models should be used with great care, taking into account the applicability domain and validation of the models. Current practice is to use the outcome of these biodegradation models to predict that a substance is not readily degradable, rather than *vice versa*. This is because models such as BIOWIN tend to predict non-ready biodegradability more certainly than ready biodegradability (IR&CSA Chapter R.7b, section R.7.9.4.1). However, (Q)SAR information can be used as a part of expert judgement and WoE practices, for example where very consistent measured and predicted data are available for a structurally analogous compound. More details can be found in IR&CSA Chapter R.7b, section R.7.9.4.1.

General interpretation problems and substances difficult to test

The UN GHS Annex 9 and OECD Guidance 23 (OECD 2019) discuss substances that are inherently difficult to test for biodegradability, and possible adjustments to overcome testing problems. Testing or interpretational problems may occur with e.g. complex multi-constituent substances, surface active agents, highly volatile or insoluble substances, substances that are toxic to microorganisms at normal test concentrations, and unstable molecules.

4.1.3.2.3.3. Bioaccumulation

Annex I: ***4.1.2.8.1*** *Bioaccumulation of substances within aquatic organisms can give rise to toxic effects over longer time scales even when actual water concentrations are low. For organic substances the potential for bioaccumulation shall normally be determined by using the octanol/water partition coefficient, usually reported as a log K_{ow}. The relationship between the log K_{ow} of an organic substance and its bioconcentration as measured by the bioconcentration factor (BCF) in fish has considerable scientific literature support. Using a cut-off value of log $K_{ow} \geq 4$ is intended to identify only those substances with a real potential to bioconcentrate. While this represents a potential to bioaccumulate, an experimentally determined BCF provides a better measure and shall be used in preference if available. A BCF in fish of* **≥** *500 is indicative of the potential to bioconcentrate for classification purposes. Some relationships can be observed between chronic toxicity and bioaccumulation potential, as toxicity is related to the body burden.*

The potential for bioaccumulation is an important criterion to determine whether a chemical substance is a potential hazard to the environment. Bioaccumulation of a substance into an organism is not a hazard in itself but should be considered in relation to potential long-term effects. Chemical concentration and accumulation may result in internal concentrations of a substance in an organism (body burden), which may or may not lead to toxic effects over long-term exposures. Further guidance on bioaccumulation is given in Annex III to this guidance. Bioaccumulation of metals is discussed in Annex IV.

Information on actual bioaccumulation of a substance may be available from standardised tests (e.g. Test Methods Regulation (EC) No 440/2008, OECD TG 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure) or information on the bioaccumulation potential, for organic substances, may be estimated from the structure of the molecule (see also IR&CSA Chapter R.7b, section R.7.10).

In general, the potential of an organic substance to bioconcentrate is primarily related to the lipophilicity of the substance. A surrogate measure of lipophilicity is the n-octanol/water partition coefficient (K_{ow}) which, for lipophilic non-ionised and non-surface active organic substances, undergoing minimal metabolism or biotransformation within the organism, is correlated with the bioconcentration factor. Therefore, K_{ow} is often used for estimating the bioconcentration of non-ionised organic substances, based on the empirical relationship between log BCF and log K_{ow}. For those organic substances, estimation methods are available for calculating the K_{ow}. Data on the bioconcentration properties of non-ionised organic substances may thus be:

1. Experimentally determined;
2. Estimated from experimentally determined K_{ow}; or
3. Estimated from K_{ow} values derived by use of (Q)SARs.

Experimentally derived BCF values of high quality are preferred for classification purposes. BCF results from poor or questionable quality studies should not be used for classification purposes if high quality data on log K_{ow} are available. If no BCF is available for fish species, high quality data on the BCF for some invertebrates (e.g. blue mussel, oyster and/or scallop) may be used as a worst case surrogate.

For non-ionised organic substances, experimentally derived high quality K_{ow} values are preferred. If no experimental data of high quality are available, validated Quantitative Structure Activity Relationships ((Q)SARs) for log K_{ow} may be used in the classification process. If data are available but not validated, expert judgement should be used. (Q)SAR BCF values can be used as part of a WoE approach, but it must be noted that high quality experimental BCF and Log k_{ow} values are always preferred and (Q)SAR BCFs are not considered a one for one substitute (See Annex III.2.2.2 for further details). BCF estimates based on *in vitro* OECD TGs 319A and B might be considered in a WoE approach for bioaccumulation provided that they fulfil relevant data quality requirements (IR&CSA Chapter R.7b, R.11.4.1.2.10). For ionised organic substances problems may occur, e.g. changes in pH which may significantly affect the water solubility and partition coefficient of the substance. Further guidance on how to deal with such difficulties in bioaccumulation testing is provided in the OECD Guidance 23 (OECD 2019).

4.1.3.2.4. Using weight of evidence in evaluations in the context of C&L

4.1.3.2.4.1. General aspects of weight of evidence

The weight of evidence approach is described in IR&CSA Chapter R.4, section R.4.4 as follows: 'The weight of evidence (WoE) approach is not a scientifically well-defined term or an agreed formalised concept. It involves assessing the relevance, reliability and adequacy of each piece of available information, holding the various pieces of information up against each other and reaching a conclusion on the hazard. This process always involves expert judgement. It is important to document and communicate how the evidence-based approach was used in a reliable, robust and transparent manner'.

Where there is only one experimental effect value per endpoint, classification and labelling decisions are relatively straightforward. However, this is often not the case when dealing with data-deficient substances or substances for which more than one valid value is available for a given endpoint. In both situations, available information needs to be evaluated carefully. Data deficiency may occur for substances for which there are no reliable data, or only experimental data which is irrelevant for classification and labelling. This might be the case for substances exempted from REACH such as polymers or substances manufactured in quantities < 1 tonne/annum.

The taxa chosen, fish, crustacea and aquatic plants, that represent the 'base-set' in most hazard profiles, represent a minimum dataset for a fully valid description of hazard. The lowest of the available toxicity values will normally be used to define the hazard category. Given the wide range of species in the environment, the three taxa tested can only be a rudimentary surrogate and the lowest value is considered to be a cautious approach for defining the hazard category. In doing so, it is recognised that the distribution of species sensitivity can be several orders of magnitude wide, and that there will thus be both more and less sensitive species in the environment. Therefore, when data are limited, the use of the most sensitive species tested gives a cautious but acceptable definition of the hazard.

There are some circumstances where it may not be appropriate to use the lowest toxicity value as the basis for classification. This will usually only arise where it is possible to define the sensitivity distribution with more accuracy than would normally be possible, such as when large datasets are available. Such large datasets should be evaluated with due caution. In case of multiple data points for the same effects endpoint, Guidance R.4 states that "Where there is more than one study for each endpoint, the greatest weight is attached to the studies that are the most relevant and reliable. Sound scientific judgement is an important principle in considering the adequacy, reliability, and relevancy of information and determining the key study".

Conversely, as CLP allows the use of expert judgment in employing non-testing information such as (Q)SARs, the classification of data-deficient substances could potentially be conducted in the absence of any experimental data.

In applying the WoE approach, the reliability of the information under evaluation needs to be taken into due account. Typically, this information originates from studies which have been ranked according to the Klimisch criteria. The scores assigned to the studies may serve as an indication of the 'weight' that the corresponding information could have in 'weighing the evidence'.

4.1.3.2.4.2. Guidance on WoE for data deficient substances

Either for those substances for which the standard data set of acute aquatic testing in fish, crustacea and algae/aquatic plants is not available or where there are data gaps, REACH introduces the concept of an 'Integrated Testing Strategy' (for further guidance see IR&CSA Chapter R.7b, Figure R.7.8-2). This outlines a stepwise approach on the use of test data and non-testing information, such as reliable (Q)SARs and *in vitro* testing. It outlines how the relevant information is collected and evaluated and in the final step, expert judgement is used to reach an overall assessment of the aquatic toxicity of the substance under evaluation, also taking into consideration metabolites, reaction products, and analogues.

For classification purposes, representative species should be chosen which cover a range of trophic levels and taxonomic groups, namely fish, crustacea and primary producers. Annex I to this document also provides guidance on the following where no experimental data are available: '(Q)SARs can be relied upon to provide predictions of acute toxicity to fish, crustacea (Daphnia and Mysid), and algae for non-electrolytes, non-electrophilic, and otherwise non-reactive substances. Care should be taken when evaluating the toxicity of poorly water soluble substances, where the quoted toxicity may be greater than the water solubility'.

4.1.3.2.4.3. Guidance on WoE for substances for which more than one valid piece of data is available for a given data element

The best quality data should be used as the fundamental basis for classification. Classification should preferably be based on primary data sources. It is essential that test conditions be clearly and completely articulated.

Where multiple studies for a taxonomic group are available, all studies that are assessed to have sufficient quality should be taken into consideration. The study showing the highest toxicity (e.g. the one with the lowest L(E)C_{50} or NOEC or EC_x) should normally be chosen as key study for aquatic hazard classification for that taxonomic group. However, in a WoE approach, a different weight may be given to studies irrespective the test results. For example: a judgement has to be

made on a case-by-case basis whether Klimisch 1 studies in a dataset are given more weight than Klimisch 2 studies or valid (Q)SAR data available for the same taxonomic group.

Lower quality information showing no or low toxicity should specifically be treated with care, especially where the quality assessment has revealed points of concern regarding methodology and reporting (e.g. maintenance of test concentrations). In addition it should be noted that substances which are difficult to test may yield apparent results that are not indicating the true toxicity. Expert judgement would also be needed for classification in these cases.

Assessment of data quality includes assessment of adequacy of the information for classification purposes and an assessment of both relevance and reliability. Details on the assessment of quality can be found in IR&CSA Chapter R.4.

Where more than one acceptable test is available for the same taxonomic group, the most sensitive (the one with the lowest L(E)C_{50} or NOEC/EC_{10}) is generally used for classification. However, this must be dealt with on a case-by-case basis. When larger data sets (four or more values) are available for the same species, the geometric mean of toxicity values may be used as the representative toxicity value for that species. In estimating a mean value, it is not advisable to combine tests of different species within a taxonomic group or in different life stages or tested under different conditions or duration. This implies that for substances where four or more ecotoxicity data on the same species and endpoint are available, the data could be grouped, and the geometric mean used as a representative toxicity value for that species.

In case of very large data sets meeting the criteria for applying the Species Sensitivity Distribution (SSD) approach (see IR&CSA Chapter R.10), statistical techniques (e.g. HC_5 derivation) can be considered to estimate the aquatic toxicity reference value for classification (equivalent to using the lowest EC_{50} or NOEC), in a weight of evidence approach.

4.1.3.2.4.4. Outliers

The WoE approach would also address potential outliers, since as a starting point, all data points for a specific trophic level/taxonomic group would be considered to come from the same sensitivity distribution. Only if a sufficiently large number of data were available for the same species and effects endpoint, appropriate statistical tests would be performed to confirm or disprove a particular value as an outlier.

The issue of possible 'outliers', which may exist, particularly in large data sets can be tackled according to a proposal in IR&CSA Chapter R.7b, section R.7.8.4.1.

4.1.3.2.4.5. Weight of evidence in degradation

Where multiple or conflicting datasets exist for a single chemical, the most reliable data should be selected first, and subsequently a 'weight of evidence' approach followed based on these data. This implies that if both positive (*i.e.* above the pass level) and negative results (below pass level) have been obtained for a substance in rapid degradability tests, then the data of the highest quality and the best documentation should be used for determining the rapid degradability of the substance. However, given the conservative nature of ready biodegradability tests positive results could be used irrespective of negative results when the scientific quality is good and the test conditions are well documented, *i.e.* the guideline criteria are fulfilled. See Annex II for further guidance.

4.1.3.2.4.6. Weight of evidence in bioaccumulation

When conflicting bioaccumulation data is available, see Annex III for further guidance.

4.1.3.3. Classification categories and criteria

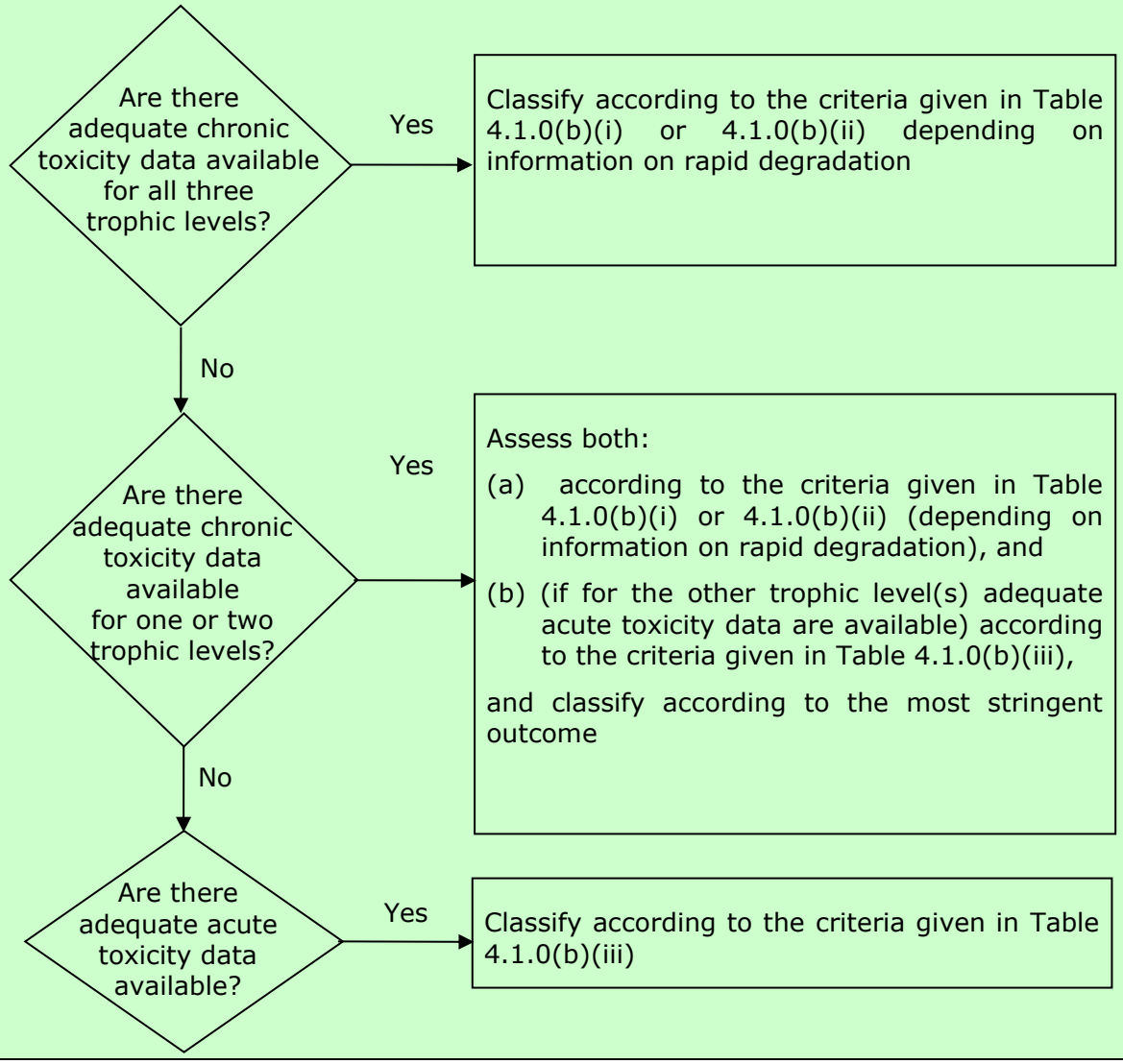
4.1.3.3.1. Outline of the core classification system

Annex I: ***4.1.2.2.*** *The core classification system for substances consists of one short-term (acute) hazard classification category and three long-term (chronic) hazard classification categories. The short-term (acute) and the long-term (chronic) hazard classification categories are applied independently.*

Annex I: ***4.1.2.3.*** *The criteria for classification of a substance in category Acute 1 are defined on the basis of acute aquatic toxicity data only (EC_{50} or LC_{50}). The criteria for classification of a substance into the categories Chronic 1 to 3 follow a tiered approach where the first step is to see if available information on chronic toxicity merits long-term (chronic) hazard classification. In absence of adequate chronic toxicity data, the subsequent step is to combine two types of information, i.e. acute aquatic toxicity data and environmental fate data (degradability and bioaccumulation data) (see Figure 4.1.1).*

Figure 4.1.1

Categories for substances long-term (chronic) hazardous to the aquatic environment



4.1.2.1. *The system for classification recognises that the intrinsic hazard to aquatic organisms is represented by both the acute and chronic toxicity of a substance. For the long-term (chronic) hazard separate hazard categories are defined representing a gradation in the level of hazard identified. The lowest of the available toxicity values between and within the different trophic levels (fish, crustacean, algae/aquatic plants) shall normally be used to define the appropriate hazard category(ies). There are circumstances, however, when a weight of evidence approach is appropriate.*

Chronic toxicity data, where available, are preferred for determining the long-term (chronic) hazard category. Where adequate chronic toxicity data exist for the three trophic levels and the lowest chronic toxicity value (that normally would define the appropriate hazard category) is below or equal to 1 mg/L, a long-term hazard classification is warranted. The actual category is also dependent on the information on rapid degradation. Chronic toxicity and the degradation properties of the substance should be considered when determining the potential hazard classification. Substances that do not rapidly degrade have a higher potential for longer term exposures and therefore should be classified in a more severe category than substances which are rapidly degradable.

Since the introduction of chronic aquatic toxicity criteria in ATP 2 to CLP (Table 4.1.0 of Annex I to CLP), chronic toxicity data has become widely available.

Chronic toxicity data (EC_x or NOEC), where available, should be used for long-term hazard classification. However, when assessing the adequacy of available chronic toxicity data, there may be some cases (for example, data poor substances) where the chronic toxicity data do not represent the species that is considered the most sensitive in available short-term toxicity tests. In such cases, the classification should be based on the toxicity data (acute or chronic) that gives the most strict classification (including the M-factor). In the absence of chronic toxicity data, suitable acute toxicity data can be used to determine long-term hazard classification in addition to acute hazard classification (Figure 4.1.1 and Table 4.1.0(b)(iii) of Annex I to CLP).

A review of the existing adequate and appropriate acute toxicity data and environmental fate data (degradability and bioaccumulation) is required for those trophic levels where adequate chronic toxicity data may be absent; to decide if a long-term hazard classification may be warranted.

While recognising that acute toxicity itself is not a sufficiently accurate predictor of chronic toxicity to be used solely and directly for establishing hazard, it is considered that, in combination with either a potential to bioaccumulate (*i.e.* experimentally determined BCF $\geq$ 500 or, if absent, the log $K_{ow} \geq 4$) or higher potential longer term exposure (i.e. lack of rapid degradation), it can be used as a suitable surrogate for long-term hazard classification purposes. Substances rapidly degrading that show acute toxicity with a significant degree of bioaccumulation will normally show chronic toxicity at a significantly lower concentration. Equally, substances that do not rapidly degrade have a higher potential for giving rise to longer term exposures which again may result in long-term toxicity being realised.

The hazard categories for acute and chronic aquatic toxicity and their related criteria are set out in CLP, Annex I, section 4.1, Table 4.1.0.

Annex I: *Table 4.1.0*
Classification categories for hazardous to the aquatic environment

(a) Short-term (acute) aquatic hazard	
Category Acute 1: *(Note 1)*	
96 hr LC_{50} (for fish)	*≤ 1 mg/l and/or*
48 hr EC_{50} (for crustacea)	*≤ 1 mg/l and/or*
72 or 96 hr ErC_{50} (for algae or other aquatic plants)	*≤ 1 mg/l. (Note 2)*
(b) Long-term (chronic) aquatic hazard	
(i) Non-rapidly degradable substances (Note 3) for which there are adequate chronic toxicity data available	
Category Chronic 1: *(Note 1)*	
Chronic NOEC or EC_x (for fish)	*≤ 0,1 mg/l and/or*
Chronic NOEC or EC_x (for crustacea)	*≤ 0,1 mg/l and/or*
Chronic NOEC or EC_x (for algae or other aquatic plants)	*≤ 0,1 mg/l.*
Category Chronic 2:	
Chronic NOEC or EC_x (for fish)	*≤ 1 mg/l and/or*
Chronic NOEC or EC_x (for crustacea)	*≤ 1 mg/l and/or*
Chronic NOEC or EC_x (for algae or other aquatic plants)	*≤ 1 mg/l.*
(ii) Rapidly degradable substances (Note 3) for which there are adequate chronic toxicity data available	
Category Chronic 1*: (Note 1)*	
Chronic NOEC or EC_x (for fish)	*≤ 0,01 mg/l and/or*
Chronic NOEC or EC_x (for crustacea)	*≤ 0,01 mg/l and/or*
Chronic NOEC or EC_x (for algae or other aquatic plants)	*≤ 0,01 mg/l*
Category Chronic 2:	
Chronic NOEC or EC_x (for fish)	*≤ 0,1 mg/l and/or*
Chronic NOEC or EC_x (for crustacea)	*≤ 0,1 mg/l and/or*
Chronic NOEC or EC_x (for algae or other aquatic plants)	*≤ 0,1 mg/l*
Category Chronic 3:	
Chronic NOEC or EC_x (for fish)	*≤ 1 mg/l and/or*
Chronic NOEC or EC_x (for crustacea)	*≤ 1 mg/l and/or*
Chronic NOEC or EC_x (for algae or other aquatic plants)	*≤ 1 mg/l.*
(iii) Substances for which adequate chronic toxicity data are not available	
Category Chronic 1*: (Note 1)*	
96 hr LC_{50} (for fish)	*≤ 1 mg/l and/or*
48 hr EC_{50} (for crustacea)	*≤ 1 mg/l and/or*
72 or 96 hr ErC_{50} (for algae or other aquatic plants)	*≤ 1 mg/l. (Note 2)*

and the substance is not rapidly degradable and/or the experimentally determined BCF ≥ 500 (or, if absent, the log K_{ow} ≥ 4). (Note 3).

***Category Chronic 2**:*

96 hr LC50 (for fish)	*> 1 to ≤ 10 mg/l and/or*
48 hr EC50 (for crustacea)	*> 1 to ≤ 10 mg/l and/or*
72 or 96 hr ErC50 (for algae or other aquatic plants)	*> 1 to ≤ 10 mg/l. (Note 2)*

and the substance is not rapidly degradable and/or the experimentally determined BCF ≥ 500 (or, if absent, the log K_{ow} ≥ 4). (Note 3).

***Category Chronic 3**:*

96 hr LC_{50} (for fish)	*> 10 to ≤ 100 mg/l and/or*
48 hr EC_{50} (for crustacea)	*> 10 to ≤ 100 mg/l and/or*
72 or 96 hr ErC_{50} (for algae or other aquatic plants)	*> 10 to ≤ 100 mg/l. (Note 2)*

and the substance is not rapidly degradable and/or the experimentally determined BCF ≥ 500 (or, if absent, the log K_{ow} ≥ 4). (Note 3).

Note 1: When classifying substances as Acute Category 1 and/or Chronic Category 1 it is necessary at the same time to indicate then appropriate M-factor(s) (see table 4.1.3).

Note 2: Classification shall be based on the ErC_{50} [= EC_{50} (growth rate)]. In circumstances where the basis of the EC_{50} is not specified or no ErC_{50} is recorded, classification shall be based on the lowest EC_{50} available.

Note 3: When no useful data on degradability are available, either experimentally determined or estimated data, the substance should be regarded as not rapidly degradable.

Classifications may also be made in cases where data are not available on all three trophic levels. In these cases, the classification may be subject to further information becoming available. In general, all the data available will need to be considered prior to assigning a classification. Where good quality data are available for a particular species or taxa, this should be used in preference to any lower quality data which might also be available for that species or taxa. Where good quality data are not available, lower quality data will need to be considered. In these circumstances, a judgement will need to be made regarding the true level of hazard. Consideration of such data, however, will also need to consider the difficulties that may have affected the likelihood of achieving a valid result. For example, the test details and experimental design may be critical to the assessment of the usability of some data, such as that from hydrolytically unstable chemicals, while less so for other chemicals. Such difficulties are described further in Annex I to this guidance.

Normally, the identification of hazard, and hence the classification will be based on information directly obtained from testing of the substance being considered. There are occasions, however, where this can create difficulties, or the outcomes do not conform to common sense. For example, some chemicals, although stable in the bottle, will react rapidly (or slowly) in water giving rise to degradation products that may have different properties. Where such degradation is rapid, the available test data will frequently define the hazard of the degradation products since it will be these that have been tested. These data may be used to classify the parent substance in the normal way. However, where degradation is slower, it may be possible to test the parent substance and thus generate hazard data in the normal manner. The subsequent degradation may then be considered in determining whether an acute or long-term hazard category should be based on parent or degradant data. There may be occasions when a substance tested may degrade to give rise to a more hazardous degradation product under environmental conditions. In these circumstances, the rate of generation of the more hazardous degradation product (i.e.,

quantity produced and time frame) should be considered to assess whether the classification should be based on data for the degradation product. OECD Guidance 23 (OECD 2019) provides useful information on this topic. Further information on unstable substances can be found in Annex I.4.1. There may also be instances where it may be necessary to consider data obtained from mixtures (e.g. formulated products) when assessing hazards to the aquatic environment. In such cases, it is important to understand the aquatic hazard presented by other constituents in order to conduct an accurate assessment of the substance being classified. In all cases, data on the substance being classified (including any relevant degradation products) is clearly preferred over data on mixtures.

4.1.3.3.2. The 'safety net'

Annex I: ***4.1.2.4*** *The system also introduces a "safety net" classification (referred to as Chronic 4) for use when the data available do not allow classification under the formal criteria for Acute 1 or Chronic 1 to 3 but there are nevertheless some grounds for concern (see example in Table 4.1.0).*

Annex I: *4.1.2.6. Table 4.1.0. continued*

'Safety net' classification

Chronic Category 4

Cases when data do not allow classification under the above criteria but there are nevertheless some grounds for concern. This includes, for example, poorly soluble substances for which no acute toxicity is recorded at levels up to the water solubility (note 4), and which are not rapidly degradable in accordance with section 4.1.2.9.5 and have an experimentally determined BCF ≥ 500 (or, if absent, a log K_{ow} ≥ 4), indicating a potential to bioaccumulate, which will be classified in this category unless other scientific evidence exists showing classification to be unnecessary. Such evidence includes chronic toxicity NOECs > water solubility or > 1 mg/l, or other evidence of rapid degradation in the environment than the ones provided by any of the methods listed in section 4.1.2.9.5.

Note 4: 'No acute toxicity' is taken to mean that the L(E)C_{50}(s) is/are above the water solubility. Also for poorly soluble substances, (water solubility < 1 mg/l), where there is evidence that the acute test does not provide a true measure of the intrinsic toxicity.

Category Chronic 4 is for example triggered for some poorly soluble substances, which are normally considered as those having a water solubility < 1 mg/L where no acute toxicity is expressed in toxicity tests performed at the solubility limit. If for such a substance, however, the BCF is ≥ 500, or if absent, the log K_{ow} is ≥ 4 (indicating a potential for bioaccumulation) and the substance is also not rapidly degradable, a safety net classification, Chronic 4 is assigned. For these types of substances the exposure duration in short-term toxicity tests may well be too short for a steady-state concentration of the substance to be reached in the test organisms. Thus, even though no acute toxicity has been measured in a short-term (acute) test, it remains a real possibility that such non-rapidly degradable and bioaccumulative substances may exert chronic effects, particularly since such low degradability may lead to an extended exposure period in the aquatic environment.

Another example is that Chronic 4 can also be applied where a concern can be suitably justified for a given species or trophic level (for example, a mechanistic basis for hazard or target organism(s) in the context of a PPP or biocide) and there is insufficient chronic toxicity data for the given species/trophic level to indicate that no classification is warranted. In such a case, the water solubility, rapid degradation, and/or bioaccumulation may strengthen the concern.

Alternatively, with a robust basis for concern they may not be such important elements of the assessment but this is a matter of expert judgement.

The precise definitions of the core elements of this system are described in detail in Annexes I-III to this guidance document. For metals and metal compounds (those relevant for assessment under Annex IV according to Annex IV.1), please see Annexes IV.5.2.2.2 and IV.5.3.2.2.

4.1.3.3.3. Setting an M-factor for highly toxic substances

4.1.2.5 *Substances with acute toxicities below 1 mg/l or chronic toxicities below 0,1 mg/l (if non-rapidly degradable) and 0,01 mg/l (if rapidly degradable) contribute as components of a mixture to the toxicity of the mixture even at a low concentration and shall normally be given increased weight in applying the summation of classification approach (see Note 1 of Table 4.1.0 and 4.1.3.5.5).*

When a substance is classified as category Acute 1 and/or category Chronic 1, (a) multiplying factor(s) (M-factor) has/have to be assigned (as described in Article 10 of CLP). Where appropriate, M-factors shall be set for acute and long-term hazard classification separately and are considered an integral part of the classification (Article 10 of CLP). This means that, for such classifications, there will normally be two M-factors (one for acute and one for long-term hazard) for one substance. It is important to also include the M-factor(s) in the SDS as other users in the supply chain might need it, e.g. for classification of mixtures containing that substance.

The M-factor itself should be derived using the table below (CLP, Annex I Table 4.1.3) and is dependent on the toxicity band of the substances. For example, a substance with an acute toxicity of 0.005 mg/L requires an acute M-factor of 100 to be assigned. Whereas a chronic toxicity of 0.005 mg/L requires a chronic M-factor of 10 to be assigned for a non-rapidly degradable substance and a chronic M-factor of 1 for a rapidly degradable substance.

Annex I: *Table 4.1.3*

Multiplying factors for highly toxic components of mixtures

Acute toxicity	*M factor*	*Chronic toxicity*	*M factor*	
$L(E)C_{50}$ value		*NOEC value*	*NRD[a] components*	*RD[b] components*
$0,1 < L(E)C_{50} \leq 1$	*1*	*$0,01 < NOEC \leq 0,1$*	*1*	*-*
$0,01 < L(E)C_{50} \leq 0,1$	*10*	*$0,001 < NOEC \leq 0,01$*	*10*	*1*
$0,001 < L(E)C_{50} \leq 0,01$	*100*	*$0,0001 < NOEC \leq 0,001$*	*100*	*10*
$0,0001 < L(E)C_{50} \leq 0,001$	*1000*	*$0,00001 < NOEC \leq 0,0001$*	*1000*	*100*
$0,00001 < L(E)C_{50} \leq 0,0001$	*10000*	*$0,000001 < NOEC \leq 0,00001$*	*10000*	*1000*
(continue in factor 10 intervals)		*(continue in factor 10 intervals)*		

[a] *Non-rapidly degradable.*

[b] *Rapidly degradable.*

The NOEC value in Table 4.1.3 (Annex I to CLP) refers to both NOEC and EC_x (toxicity values are in mg/L). The first two columns in Table 4.1.3 refer to the classification system in Table 4.1.0 (a) and (b)(iii), the last three columns refer to the respective classification system in Table 4.1.0 (b)(i & ii). In cases where chronic toxicity data are not available and Table 4.1.0 (b)(iii) is used for defining long-term aquatic hazard, the resulting M-factor derived for acute aquatic hazard classification is applied (Table 4.1.3, Acute toxicity column) to the long-term aquatic hazard classification, albeit stated separately.

For deriving the M-factors of metals and metal compounds (those relevant for assessment under Annex IV according to Annex IV.1), please see Annex IV.5.4.

4.1.3.4. Decision on classification: examples for substances

If the evaluation shows that the criteria are fulfilled, one category for acute aquatic hazard and/or one for long-term aquatic hazard should be assigned, as well as (an) M-factor(s) where applicable. For the labelling elements, such as hazard pictograms, signal words, hazard statements and precautionary statements, see section 4.1.6 of this guidance.

Further classification examples specific to metals and metal compounds are given in Annex IV to this guidance document.

The examples in this section are focussed on self-classification based on relevant data available. Mandatory use of harmonised classification for substances included in Table 3.1 of Annex VI, the use of information from the classification and labelling inventory and the use of the translation Table in Annex VII are not taken into account in these examples.

After data collection, classification starts with an evaluation of the adequacy of the data collected, assessment of the results, and concludes on the endpoints most relevant for environmental hazard classification. Where the assessment shows that criteria for environmental classification are fulfilled, an acute aquatic hazard and/or one category for long-term aquatic hazards should be determined. M-factor(s) should be assigned for classifications as Acute 1 and/or Chronic 1.

List of the examples of substance classification included in this section:

- Example A: Hydrophilic substance, straightforward classification based on acute and chronic toxicity data;
- Example B: Hydrophilic substance, straightforward classification based on acute data, no chronic toxicity data available;
- Example C: Moderately water soluble substance, straightforward classification based on acute data, chronic toxicity data available for two trophic levels; combined set of (Q)SAR data and experimental data;
- Example D: Substance with several toxicity data for one trophic level;
- Example E: "Safety net" classification category Chronic 4;
- Example F: Substance difficult to test, toxicity above level of water solubility.

Further classification examples specific to metals and metal compounds are given in Annex IV to this guidance.

The examples are presented using a logical format starting with a table listing for all relevant data elements: the information available, followed by an aquatic hazard assessment for each data element, a section showing the aquatic hazard classification, a section with the reasoning behind the conclusions, and finally a table presenting the applicable labelling elements.

Explanation of data elements used in the examples:

- Physico-chemical properties important for evaluation of aquatic hazards for the purpose of classification: Generally this consists of water solubility (mg/L) and log octanol/water partition coefficient (log K_{ow});
- Acute aquatic toxicity: Generally expressed in terms of LC_{50} or EC_{50} (mg/L);
- Long-term aquatic toxicity: Generally expressed in terms of NOEC or EC_x(mg/L);
- Degradation (evidence of rapid degradation): Generally expressed in terms of biotic or abiotic degradation of organic substances (or transformation of inorganic substances). In case of rapid primary degradation, information shall be given whether the degradation products can be classified as hazardous to the aquatic environment or not;
- Bioaccumulation: Generally expressed in terms of bioconcentration factor in fish.

Information on reliability is not taken into account in the examples below. For the purpose of the examples, the reliability score is assumed to be high (*e.g*. for experimental tests, Klimisch score 1 or 2) unless otherwise stated. Note that assigning a reliability score to studies is important - if a study is assessed as poorly reliable it is normally not usable for classification purposes.

Besides the conclusion from studies on relevant endpoints for classification, the following information is presented for each example in a separate column:

- Referral to applicable test method according to the EU Test Methods Regulation (EC) No 440/2008 or OECD test guideline or (Q)SAR model used;
- Some basic information on the test design (pH of the test media, renewal regime of test media (static, semi-static, flow-through));
- Use of measured or nominal test concentrations;
- Compliance of the experiment and reporting with OECD Good Laboratory Practice (GLP) rules;
- Specific information related to the relevant endpoints, as appropriate.

This information plays a crucial role when the adequacy of the data and the assessment of the study results are being evaluated for their applicability in the classification and labelling scheme. However, in these examples this information is included mainly to make the data more realistic.

4.1.3.4.1. Example A: Hydrophilic substance, straightforward classification based on acute and chronic toxicity data

DATA ELEMENTS		Value	Test method ((EC) No. 440/2008) or OECD guideline / remarks
Physico-chemical properties			
Water solubility:		1200 mg/L	A.6. / pH:7.0, non-GLP
Log octanol/water partition coefficient (log K_{ow}):		2.75	A.8. / pH:7.5, GLP
Acute aquatic toxicity			
Fish	*Oncorhynchus mykiss*:	12 mg/L (96 h LC_{50})	C.1. / static, non-GLP
	Lepomis macrochirus:	2.7 mg/L (96 h LC_{50})	C.1. / static, GLP
Crustacea	*Daphnia magna*:	18 mg/L (48 h EC_{50})	C.2. / static, non-GLP
Algae/aquatic plants			
	Scenedesmus subspicatus:	0.056 mg/L (96 h ErC_{50})	C.3. / static, GLP
	Lemna gibba:	0.031 mg/L (7 d ErC_{50})	C.26. / semi-static, GLP
Chronic aquatic toxicity			
Fish	*Danio rerio*:	1.2 mg/L (21 d NOEC)	OECD TG 210 / Early Life Stage toxicity test, flow-through, GLP
Crustacea	*Daphnia magna*:	1.1 mg/L (21 d NOEC)	C.20. / semi-static, GLP
Algae/aquatic plants			
	Scenedesmus subspicatus:	0.01 mg/L (96 h NOEC)	C.3. / static, GLP
Degradation (evidence of rapid degradation)			
Biotic degradation:		86 % in 28 days (10-day window fulfilled)	C.4-C / pH:7.5, GLP
Abiotic degradation, hydrolysis (half-life (d)):		No data	
Bioaccumulation			
Bioconcentration factor in fish (BCF):		No data	

Aquatic hazard assessment, conclusions and comments:

Physico-chemical properties:

- The substance is readily soluble. Log K_{ow} < 4, indicating low potential for bioaccumulation, which can be used in absence of BCF data.

Acute aquatic toxicity:

- The acute aquatic toxicity based on the lowest of the available toxicity values (i.e., algae/aquatic plants) is between 0.01 and 0.1 mg/L.

Long-term aquatic toxicity:

- The long-term aquatic toxicity based on the lowest of the available toxicity values (*i.e.*, algae/aquatic plants) is between 0.001 and 0.01 mg/L.

Degradation (evidence of rapid degradation):

- 70 % degradation in 28 days (10-d window fulfilled) (i.e., 86% in 28 days, based on dissolved organic carbon (DOC)) based on dissolved organic carbon (DOC) fulfils the criteria for rapid degradation.

Aquatic hazard classification and, where applicable, established M-factor(s):

Acute (short-term) aquatic hazard: category Acute 1, M-factor: 10.

Long-term aquatic hazard: category Chronic 1, M-factor: 1.

Reasoning:

Acute aquatic hazard: acute toxicity L(E)C_{50} ≤ 1 mg/L. M-factor based on L(E)C_{50} between 0.01 and 0.1 mg/L.

Long-term aquatic hazard:

The criteria for classification of a substance into the categories Chronic 1 to 3 follow a tiered approach where the first step is to see if adequate information on long-term toxicity is available allowing long-term hazard classification. In absence of adequate chronic toxicity data for some or all trophic levels, the subsequent step is to combine two types of information, i.e. acute aquatic toxicity data and environmental fate data (degradability and bioaccumulation data). For details see section 4.1.3.3 and Table 4.1.0.

- Adequate chronic toxicity data for all three trophic levels, long-term toxicity NOEC ≤ 0.01 mg/L, rapidly degradable. M-factor based on NOEC between 0.001 and 0.01 mg/L (rapidly degradable).

Labelling elements based on the classification:

Element	Code
GHS Pictogram	GHS09
Signal Word	Wng
Hazard Statement	H410[11]
Precautionary statement(s)	P273, P391, P501

[11] Note that in accordance with Article 27 of CLP the hazard statement H400 may be considered redundant and therefore not included on the label because hazard statement H410 also applies, see section 4.1.6 of this document.

4.1.3.4.2. Example B: Hydrophilic substance, straightforward classification based on acute data, no chronic toxicity data available

DATA ELEMENTS		Value	Test method ((EC) No. 440/2008) or OECD guideline / remarks
Physico-chemical properties			
Water solubility:		1200 mg/L	A.6. / pH:7.0, non-GLP
Log octanol/water partition coefficient (log K_{ow}):		2.75	A.8. / pH:7.5, GLP
Acute aquatic toxicity			
Fish	*Oncorhynchus mykiss*:	12 mg/L (96 h LC_{50})	C.1. / static, non-GLP
	Lepomis macrochirus:	2.7 mg/L (96 h LC_{50})	C.1. / static, GLP
Crustacea	*Daphnia magna*:	18 mg/L (48 h EC_{50})	C.2. / static, non-GLP
Algae/aquatic plants			
	Scenedesmus subspicatus:	0.056 mg/L (96 h ErC_{50})	C.3. / static, GLP
	Lemna gibba:	0.031 mg/L (7 d ErC_{50})	C.26. / semi-static, GLP
Chronic aquatic toxicity			
Fish:		No data	
Crustacea:		No data	
Algae/aquatic plants:		NOEC not reported	
Degradation (evidence of rapid degradation)			
Biotic degradation:		86 % in 28days (10-day window fulfilled)	C.4-C / pH:7.5, GLP
Abiotic degradation, hydrolysis (half-life (d)):		No data	
Bioaccumulation			
Bioconcentration factor in fish (BCF):		560 L/kg	C.13. / pH: 7.8, GLP, BCF (related to total radioactive residues because data for parent compound not available)

Aquatic hazard assessment, conclusions and comments:

Physico-chemical properties:

- The substance is readily soluble. Log K_{ow} < 4, indicating low potential for bioaccumulation, which can be used in absence of BCF data (see bioaccumulation assessment).

Acute aquatic toxicity:

- The acute aquatic toxicity based on the lowest of the available toxicity values (i.e., algae/aquatic plants) is between 0.01 and 0.1 mg/L.

Long-term aquatic toxicity:

- No adequate chronic toxicity data available for all three trophic levels.

Degradation (evidence of rapid degradation):

- 70 % degradation after 28 days (10d window fulfilled) (i.e. 86% in 28 days, based on dissolved organic carbon (DOC)) based on dissolved organic carbon (DOC) fulfils the criteria for rapid degradation.

Bioaccumulation:

- BCF > 500, hence high potential for bioaccumulation. BCF value overrules the use of log K_{ow} value which in this case is lower than the cut-off value of 4.

Aquatic hazard classification and, where applicable, established M-factor(s):

Acute aquatic hazard: category Acute 1, M-factor: 10.

Long–term aquatic hazard: category Chronic 1, M-factor: 10.

Reasoning:

Acute (short-term) aquatic hazard: acute toxicity L(E)C_{50} ≤ 1 mg/L. M-factor based on L(E)C_{50} between 0.01 and 0.1 mg/L.

Long-term aquatic hazard:

The criteria for classification of a substance into the categories Chronic 1 to 3 follow a tiered approach where the first step is to see if adequate information on long-term toxicity is available allowing long-term hazard classification. In absence of adequate chronic toxicity data for some or all trophic levels, the subsequent step is to combine two types of information, i.e. acute aquatic toxicity data and environmental fate data (degradability and bioaccumulation data). For details see section 4.1.3.3 and Table 4.1.0.

- No adequate chronic toxicity data available (for all three trophic levels);
- Lowest acute toxicity L(E)C_{50} ≤ 1 mg/L;
- Substance is rapidly degradable but the experimentally determined BCF > 500;
- Since the conclusion is based on Table 4.1.0 (b) (iii), therefore the M-factor is based on the acute toxicity between 0.01 and 0.1 mg/L. In this case, the same factor M applies for both acute and long-term hazard.

Labelling elements based on the classification:

Element	Code
GHS Pictogram	GHS09
Signal Word	Wng
Hazard Statement	H410[12]
Precautionary statement(s)	P273, P391, P501

[12] Note that in accordance with Article 27 of CLP the hazard statement H400 may be considered redundant and therefore not included on the label because hazard statement H410 also applies, see section 4.1.6 of this document.

4.1.3.4.3. Example C: Moderately water soluble substance, straightforward classification based on acute data, chronic toxicity data available for two trophic levels only; combined set of (Q)SAR data and experimental data

DATA ELEMENTS		Value	Test method ((EC) No. 440/2008) or OECD guideline / remarks
Physico-chemical properties			
Water solubility:		25 mg/L	A.6. / pH: 7.0, non-GLP
Log octanol/water partition coefficient (log K_{ow}):		5.75 3.9	A.8. / pH: 7.5, GLP (Q)SAR KOWINN, valid, non-GLP
Acute aquatic toxicity			
Fish	*Oncorhynchus mykiss*:	12.3 mg/L (96 h LC_{50})	C.1. / static, non-GLP
	Lepomis macrochirus:	22.5 mg/L (96 h LC_{50})	C.1. / static, GLP
Crustacea	*Daphnia magna*:	0.79 mg/L (48 h EC_{50})	C.2. / static, non-GLP
	Daphnia magna:	1.06 mg/L (48 h EC_{50})	(Q)SAR, ECOSAR, valid, non-GLP
Algae/aquatic plants			
	Scenedesmus subspicatus:	1.53 mg/L (96 h ErC_{50})	C.3. / static, GLP
Chronic aquatic toxicity			
Fish	*Oncorhynchus mykiss*:	0.56 mg/L (21 d NOEC)	OECD TG 210 / Early Life Stage toxicity test, flow-through, GLP
Crustacea:		No data	
Algae/aquatic plants			
	Scenedesmus subspicatus:	0.23 mg/L (96 h NOEC)	C.3. / static, GLP
Degradation (evidence of rapid degradation)			
Biotic degradation:		45 % in 28 days	C.4-C / pH: 7.5, GLP
Abiotic degradation, hydrolysis			
	(half-life (d)):	No data	
Bioaccumulation			
Bioconcentration factor in fish (BCF):		No data	

Aquatic hazard assessment, conclusions and comments:

Physico-chemical properties:

- The substance is moderately soluble. Log K_{ow} 5.75. Based on weight of evidence, valid K_{ow} estimated with (Q)SAR is overruled by valid GLP experimental data.

Note that use of experimental data and (Q)SAR data for estimation log K_{ow} should be carefully considered on a case by case basis. The validity of data may be dependent on the structure of the chemical. See Annex III.2.2 for more details on the use of log K_{ow} data and Annex III.3 for details on chemical classes that need special attention in this respect.

Acute aquatic toxicity:

- The acute aquatic toxicity based on the lowest of the available toxicity values is between 0.1 and 1 mg/L;
- For *Daphnia magna* two valid values are presented. A weight of evidence approach is applied in which the (Q)SAR data are outweighed by the valid experimental data. Hence, the lowest acute toxicity value of 0.79 mg/L is used for crustacea.

Long-term aquatic toxicity:

- Adequate chronic toxicity data available only for fish and algae/aquatic plants, not for crustacea;
- The chronic aquatic toxicity based on the lowest of the available toxicity values for fish and algae/aquatic plants is between 0.1 and 1 mg/L.

Since there is adequate chronic toxicity data available for two trophic levels, assess both:

a. according to the criteria given in Table 4.1.0(b)(i) or 4.1.0(b)(ii) (depending on information on rapid degradation), and

b. (if for the other trophic level(s) adequate acute toxicity data are available) according to the criteria given in Table 4.1.0(b)(iii),

and classify according to the most stringent outcome.

Degradation (evidence of rapid degradation):

- < 70 % degradation in 28 days based on dissolved organic carbon (DOC), does not fulfil the criteria for rapid degradation.

Bioaccumulation:

- Log K_{ow} 5.75, indicating a high potential for bioaccumulation, which can be used in the absence of BCF data.

Aquatic hazard classification and, where applicable, established M-factor(s):

Acute aquatic hazard: category Acute 1, M-factor: 1.

Long-term aquatic hazard: category Chronic 1, M-factor: 1.

Reasoning:

Acute (short-term) aquatic hazard: lowest acute aquatic toxicity L(E)C_{50} ≤ 1 mg/L. M-factor based on L(E)C_{50} between 0.1 and 1 mg/L.

Long-term aquatic hazard:

The criteria for classification of a substance into the categories Chronic 1 to 3 follow a tiered approach where the first step is to see if adequate information on long-term toxicity is available allowing long-term hazard classification. In absence of adequate chronic toxicity data for some or all trophic levels, the subsequent step is to combine two types of information, i.e. acute aquatic toxicity data and environmental fate data (degradability and bioaccumulation data). In this example the absence of long-term study for the species/trophic level (i.e. Daphnia/Crustacea) with the lowest acute toxicity value supports using the surrogate system. For details see section 4.1.3.3 and Table 4.1.0.

- Based on available chronic toxicity data (Table 4.1.0 (b)(i): lowest long-term aquatic toxicity NOEC ≤ 1 mg/L, not rapidly degradable, hence category Chronic 2;
- Surrogate approach (Table 4.1.0 (b)(iii): lowest acute aquatic toxicity L(E)C_{50} < 1 mg/L, not rapidly degradable (and log K_{ow}>4), hence category Chronic 1;
- Conclusion: category Chronic 1 applies following the most stringent outcome;
- Since the conclusion is based on the surrogate system (Table 4.1.0 (b) (iii)) the M-factor is based on the acute aquatic toxicity between 0.1 and 1 mg/L.

Labelling elements based on the classification:

Element	Code
GHS Pictogram	GHS09
Signal Word	Wng
Hazard Statement	H410[13]
Precautionary statement(s)	P273, P391, P501

[13] Note that in accordance with Article 27 of CLP the hazard statement H400 may be considered redundant and therefore not included on the label because hazard statement H410 also applies, see section 4.1.6 of this document.

4.1.3.4.4. Example D: Substance with several toxicity data for a trophic level

DATA ELEMENTS		Value	Test method ((EC) No. 440/2008) or OECD guideline / remarks
Physico-chemical properties			
Water solubility:		120 mg/L	A.6. / pH:7.0, non-GLP
Log octanol/water partition coefficient (log K_{ow}):		4.9	A.8. / pH:7.5, GLP
Acute aquatic toxicity			
Fish	*Lepomis macrochirus*:	108 mg/L (96 h LC_{50})	C.1. / static, GLP
Crustacea[14]	*Daphnia magna*:	40 mg/L (48 h EC_{50})	C.2. / static, GLP
	Procambarus clarkii:	0.12 mg/L (48 h EC_{50})	Method na. / static, GLP
	Asellus aquaticus:	0.4 mg/L (48 h EC_{50})	Method na. / static, non-GLP
	Mysidopsis bahia:	0.5 mg/L (48 h EC_{50})	Method na. / static, GLP
	Chironomus tentans:	0.8 mg/L (48 h EC_{50})	Method na. / static, GLP
Algae/aquatic plants *Pseudokirchneriella subcapitata:*		22 mg/L (96 h ErC_{50})	C.3. / static, GLP
Chronic aquatic toxicity			
Fish	*Pimephales promelas:*	1.1 mg/L (21 d NOEC)	OECD TG 210 / Early Life Stage toxicity test, flow-through, GLP, endpoint: growth
Crustacea	*Daphnia magna:*	1.2 mg/L (21 d NOEC)	C.20. / semi-static, GLP, endpoint: reproduction
Algae/aquatic plants *Pseudokirchneriella subcapitata:*		8.5 mg/L (96 h NOEC)	C.3. / static, GLP
Degradation (evidence of rapid degradation)			
Biotic degradation		No data	
Abiotic degradation, hydrolysis (half-life (d)):		No data	
Bioaccumulation			
Bioconcentration factor in fish (BCF):		No data	

[14] Some species in this trophic level may be representatives of other taxonomic groups than crustacea *e.g.* the non-biting midge *Chironomus tentans* is a representative of the subphylum Hexapoda (class Insecta).

Aquatic hazard assessment, conclusions and comments:

Physico-chemical properties:

- The substance is water soluble. log K_{ow} 4.9.

Acute aquatic toxicity:

- The acute aquatic toxicity (based on the lowest of the available toxicity values) is between 0.1 and 1 mg/L. The classification in this example should be based on the most sensitive species which is the crustacea *Procambarus clarkii*;

Note that in general for substances for which multiple toxicity data is available for a taxonomic group (in this case crustacea) on a case-by-case basis the toxicity data may be evaluated by weighting the evidence. If for example four or more acute LC_{50} values were available for the same fish species, then a geometric mean may be calculated (see section 4.1.3.2.4.3). In this specific example, acute toxicity data on five separate crustacean species is available and all – except one – are from GLP studies that are weighed equally in a weight of evidence approach. Accordingly, the lowest value is used for classification purposes.

Chronic aquatic toxicity:

- Adequate chronic toxicity data available only for fish and algae/aquatic plants. The chronic aquatic toxicity (based on the lowest of the two available toxicity values) is above 1 mg/L;
- For crustacea chronic toxicity data is available for *Daphnia magna*, which based upon the relatively large acute dataset is clearly the least sensitive of the species for which data is available. Hence, the chronic aquatic toxicity data set for aquatic invertebrates should be considered inadequate.

Degradation (evidence of rapid degradation):

- No data available for this substance. In such cases, the substance is considered as not rapidly degradable (see Table 4.1.0, Note 3).

Bioaccumulation:

- Log K_{ow} 4.9, indicating high potential for bioaccumulation, which can be used in the absence of BCF data.

Aquatic hazard classification and, where applicable, established M-factor(s):

Acute aquatic hazard: category Acute 1, M-Factor: 1.

Long-term aquatic hazard: category Chronic 1, M-Factor 1.

Reasoning:

Acute aquatic hazard: Acute aquatic toxicity L(E)C_{50} > 0.1 and ≤1 mg/L;

Long-term aquatic hazard:

The criteria for classification of a substance into the categories Chronic 1 to 3 follow a tiered approach where the first step is to see if adequate information on long-term toxicity is available allowing long-term hazard classification. In absence of adequate chronic toxicity data for some or all trophic levels, the subsequent step is to combine two types of information, i.e. acute aquatic toxicity data and environmental fate data (degradability and bioaccumulation data). For details see section 4.1.3.3 and Table 4.1.0.

- Adequate Chronic toxicity data available for two out of three trophic levels (fish and algae/aquatic plants), lowest NOEC above 1 mg/L. Conclusion for these two trophic levels:

NOEC-based system (Table 4.1.0 (b)(i): lowest long-term aquatic toxicity NOEC > 1 mg/L, hence not classified;

- Surrogate system (Table 4.1.0 (b)(iii)): lowest acute aquatic toxicity L(E)C_{50} < 1 mg/L (0.12 mg/L *Procambarus clarkii*), not rapidly degradable (and log K_{ow} > 4), hence category Chronic 1;
- Conclusion: category Chronic 1 applies following the most stringent outcome;
- Since the conclusion is based on the surrogate system (Table 4.1.0 (b) (iii)) the M-factor is based on the acute aquatic toxicity between 0.1 and 1 mg/L.

Labelling elements based on the classification:

Element	Code
GHS Pictogram	GHS09
Signal Word	Wng
Hazard Statement	H410[15]
Precautionary statement(s)	P273, P391, P501

[15] Note that in accordance with Article 27 of CLP the hazard statement H400 may be considered redundant and therefore not included on the label because hazard statement H410 also applies, see section 4.1.6 of this document.

4.1.3.4.5. Example E: 'Safety net' classification category Chronic 4

DATA ELEMENTS	Value	Test method ((EC) No. 440/2008) or OECD guideline / remarks
Physico-chemical properties		
Water solubility:	0.009 mg/L	A.6. / pH:7.0, non-GLP
Log octanol/water partition coefficient (log K_{ow}):	5.4	A.8. / pH:7.5, GLP
Acute aquatic toxicity		
Fish:	No data	
Crustacea *Daphnia magna*:	> 1 mg/L (48 h EC_{50})	C.2. / static, nominal concentration, non-GLP
Algae/aquatic plants:	No data	
Chronic aquatic toxicity		
Fish:	No data	
Crustacea:	No data	
Algae/aquatic plants:	No data	
Degradation (evidence of rapid degradation)		
Biotic degradation:	No data	
Abiotic degradation, hydrolysis (half-life (d)):	No data	
Bioaccumulation		
Bioconcentration factor in fish (BCF):	No data	

Aquatic hazard assessment, conclusions and comments:

Physico-chemical properties:

- The substance is poorly soluble. Log K_{ow} > 4, indicating high potential for bioaccumulation, which can be used in absence of BCF data.

Acute aquatic toxicity:

- Data poor substance. No acute toxicity recorded at levels up to the limit of water solubility.

Long-term aquatic toxicity:

- No adequate chronic toxicity data available for all three trophic levels.

Degradation (evidence of rapid degradation):

- The substance is considered not rapidly degradable by default in absence of measured data.

Bioaccumulation:

- Log K_{ow} 5.4, indicating high potential for bioaccumulation, which can be used in absence of BCF data.

Aquatic hazard classification and, where applicable, established M-factor(s):

Acute hazard: Not classified.

Long-term hazard: 'Safety net' classification category Chronic 4.

Reasoning:

Acute hazard: No acute aquatic toxicity recorded at levels up to the limit of water solubility;

Long-term hazard: No adequate chronic toxicity data available for all three trophic levels. Substance nevertheless of concern based on the following findings:

- Poorly soluble substance;
- No acute aquatic toxicity recorded at levels up to the limit of water solubility;
- Not rapidly degradable (by default in absence of measured data);
- High potential for bioaccumulation (in absence of BCF data, log K_{ow} > 4);
- No evidence on NOEC being above water solubility for all three trophic levels;
- No other evidence of rapid degradation in the environment.

Labelling elements based on the classification:

Element	Code
GHS Pictogram	-
Signal Word	-
Hazard Statement	H413
Precautionary statement(s)	P273, P501

4.1.3.4.6. Example F: Substance difficult to test, toxicity above level of water solubility

DATA ELEMENTS	Value	Test method ((EC) No. 440/2008) or OECD guideline / remarks
Physico-chemical properties		
Water solubility:	< 0.2 mg/L	A.6. / pH: 7.0, non-GLP
Log octanol/water partition coefficient (log K_{ow}):	No data	Not determined due to instability of the substance in water
Acute aquatic toxicity		
Fish *Oncorhynchus mykiss*:	12 mg/L (96 h LC_{50})	C.1. / static, nominal concentration, non-GLP
Crustacea *Daphnia magna:*	18 mg/L (48 h EC_{50})	C.2. / static, nominal concentration, non-GLP
Algae/aquatic plants *Pseudokirchneriella subcapitata:*	3.56 mg/L (96 h ErC_{50})	C.3. / static, nominal concentration, non-GLP
Chronic aquatic toxicity		
Fish:	No data	
Crustacea:	No data	
Algae/aquatic plants:	No data	
Degradation (evidence of rapid degradation)		
Biotic degradation:	No data	
Abiotic degradation, hydrolysis (half-life (d)):	< 0.5 days (longest half-life within pH 4-9)	C.7. / pH: 7.0, non-GLP
Bioaccumulation		
Bioconcentration factor in fish (BCF):	No data	

Aquatic hazard assessment, conclusions and comments:

Physico-chemical properties:

- The water solubility test is not considered to be valid (Klimisch 3) as the substance is known to rapidly hydrolyse and this was not considered in this study. Log K_{ow} not determined.

Acute aquatic toxicity:

- This data is based on initial measured concentrations in the suspension and the reported EC_{50} values are far above the water solubility (Klimisch score 3). Tests undertaken in a static regime which is inappropriate for a substance which rapidly hydrolyses (see also IR&CSA R.7b for guidance on how to test difficult substances);
- It is not clear whether the reported effects in the acute toxicity studies are due to physical effects of the undissolved substance particles in the test media on the test species or inherent toxicity of the substance.

Long-term aquatic toxicity:

- No adequate chronic toxicity data available for all three trophic levels.

Degradation (evidence of rapid degradation):

- In the assessment of rapid degradability hydrolysis can be considered if the hydrolysis products do not fulfil the criteria for classification as hazardous to the aquatic environment. In this example hydrolysis is sufficient to show a rapid degradability of the parent substance in the environment but no information is available about the breakdown product(s). More data on degradation of this/these compound(s) would be necessary;
- In absence of data to show a rapid degradation of the breakdown product(s) the parent substance is considered not rapidly degradable.

Bioaccumulation:

- Log K_{ow} could not be determined experimentally. The parent substance has a low potential for bioaccumulation due to hydrolytic instability.

Aquatic hazard classification and, where applicable, established M-factor(s):

Acute aquatic hazard: Not classified in absence of adequate data (data of poor quality).

Long-term aquatic hazard: category Chronic 4.

Reasoning:

Acute hazard (Table 4.1.0 (a)): No acute aquatic toxicity as no adequate acute data available;

Long-term hazard: No adequate chronic toxicity data available for all three trophic levels. Substance nevertheless of concern based on the following findings:

- Poorly soluble substance (< 0.2 mg/L);
- No acute aquatic toxicity recorded at levels up to the limit of water solubility;
- Not rapidly degradable (see section 4.1.3.2.3.2 of this guidance (CLP Annex I section 4.1.2.9.3);
- No evidence of NOEC being above water solubility for all three trophic levels.
- No information available on the hydrolysis products and hence dataset not decisive whether these fulfil the criteria for classification as hazardous to the aquatic environment based upon:
    - Toxicity;
    - Rapid degradability;
    - Bioaccumulation.

- In this case the safety net classification should be applied because of the large uncertainty on the fate and effects of the hydrolysis products.

Labelling elements based on the classification:

Element	Code
GHS Pictogram	-
Signal Word	-
Hazard Statement	H413
Precautionary statement(s)	P273, P501

4.1.4. Classification of mixtures hazardous to the aquatic environment

4.1.4.1. General considerations for classification of mixtures hazardous to the aquatic environment

Note that general principles for classification of mixtures under CLP are given in section 1.1.6.2 and section 1.6 of Part 1 of this guidance document series.

The basic principle of mixture classification under CLP is shown in the green box below and in Figure 4.1.2 which is also explained in the text below the box.

Annex I: *4.1.3.2* *The approach for classification of aquatic environmental hazards is tiered, and is dependent upon the type of information available for the mixture itself and for its components. Figure 4.1.2 outlines the process to be followed.*

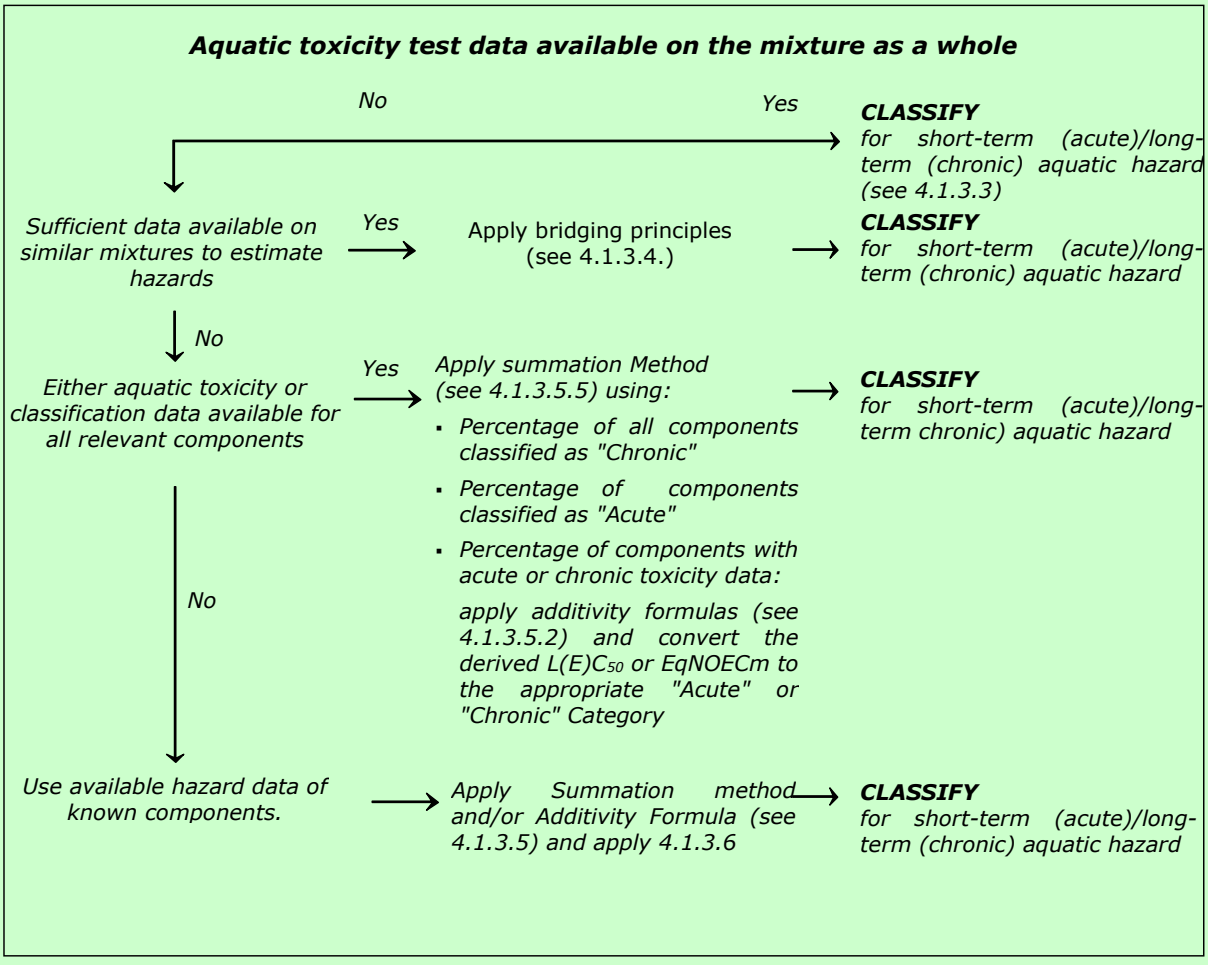
Elements of the tiered approach include:

classification based on tested mixtures;

classification based on bridging principles;

the use of "summation of classified components" and/or an "additivity formula".

Figure 4.1.2
Tiered approach to classification of mixtures for short-term (acute) and long-term (chronic) aquatic environmental hazards



Explanation of Figure 4.1.2:

- Horizontal arrow in first row: Where specific and valid test data are already available on the mixture, there is a general obligation to use these data on the mixture itself for classification purposes. Valid data must normally then be available on each of fish, crustacea and algae or other aquatic plants, unless a decision to classify in the most stringent category(ies) (Acute 1 and/or Chronic 1) can be made without a full dataset (see section 4.1.4.3 of this document).
- Horizontal arrows in second row: In other cases, sufficient data may be available on similar tested mixtures to estimate hazards using the bridging principles (see section 4.1.4.4 of this document).
- Horizontal arrows in third row: In general, however, where either aquatic toxicity or classification data are available for all relevant components of a mixture the aquatic hazard classification shall be made through the identification of the hazards of the respective components in a first step, and then in a second step through the summation of the quantities of these hazardous components, applying the summation method (see Section 4.1.4.5 of this document). When doing so:
    - The percentage of all components classified as Acute 1 and/or Chronic 1, 2, 3 & 4 is fed straight into the summation method (for relevant components see section 4.1.3.1 of Annex I to CLP);
    - For the percentage of the other components with acute or chronic toxicity data, the additivity formulas (see section 4.1.3.5.2 of Annex I to CLP) may be applied. The derived L(E)C_{50} or EqNOECm is converted to the appropriate "Acute" or "Chronic" Category and then, in a second step, fed into the summation method.[16]
- Horizontal arrows in fourth (last) row: Use available hazard data of known components.
    - This applies to mixtures containing unknown components and/or known components, for which neither toxicity data nor classifications are known. In these cases, apply the additional statement on the label and in the safety data sheet: "*Contains x % of components with unknown hazards to the aquatic environment*" (see the green box below). For classification based on the known part of the mixture, use the Summation Method and/or the Additivity Formula (see section 4.1.4.5 of this document).

Annex I: 4*.1.3.6.1* *In the event that no useable information on short-term (acute) and/or long-term (chronic) aquatic hazard is available for one or more relevant components, it is concluded that the mixture cannot be attributed to one or more definitive hazard category(ies). In this situation the mixture shall be classified based on the known components only, with the additional statement on the label and in the SDS that: "Contains x % of components with unknown hazards to the aquatic environment".*

4.1.4.2. Information requirements

Before a classification can be made, available information on toxicity of the mixture as a whole as well as all the available information on the composition of the mixture and the hazard category of relevant components (substances) should be gathered. Note that manufacturers, importers or downstream users are not requested by the CLP Regulation to generate new data for determining the aquatic hazard classification of the mixture. Rather the supplier should be contacted if it is

[16] As manufacturers and importers are obliged to classify all substances placed on the market within the EU, the summation method can usually be directly applied and the additivity formula will be of limited application.

considered that the information on the substance or mixture supplied is not sufficient for classification purposes.

Generally, therefore, the constituent substance classifications should be used as the basis for derivation of the correct hazard classification of the final mixture (see also section 1.6.4 of Part 1 of this guidance document series).

Article 11 of CLP refers to cut-off values. These values are the minimum concentrations for a substance to be taken into account for classification purposes. The substances meeting these criteria are relevant ingredients or relevant components. When a classified substance is present in a concentration above the generic cut-off value it contributes to the mixture classification even if it may not trigger classification of the mixture directly.

Annex I: ***4.1.3.1.*** *The classification system for mixtures covers all classification categories which are used for substances, i.e. categories Acute 1 and Chronic 1 to 4. In order to make use of all available data for purposes of classifying the aquatic environmental hazards of the mixture, the following is applied where appropriate:*

The "relevant components" of a mixture are those which are classified "Acute 1"or "Chronic 1" and present in a concentration of 0.1 % (w/w) or greater, and those which are classified "Chronic 2", "Chronic 3" or "Chronic 4" and present in a concentration of 1 % (w/w) or greater, unless there is a presumption (such as in the case of highly toxic components (see 4.1.3.5.5.5)) that a component present in a lower concentration can still be relevant for classifying the mixture for aquatic environmental hazards. Generally, for substances classified as "Acute 1" or "Chronic 1" the concentration to be taken into account is (0.1/M) %. (For explanation M-factor see 4.1.3.5.5.5).

For aquatic hazards the cut-off values are further addressed under section 1.1.2.2.2 (b) of Annex I to CLP. The calculation referred to in (b)(i) of that section, is found in section 4.1.3.1 of Annex I to CLP (see the green box above).

This signals that highly toxic components will need to be considered at lower levels than the generic cut-off values, and this applies to any substance to which an M-factor greater than 1 has been assigned (see section 4.1.4.5 of this document).

Note that generic concentration limits (GCLs) should be given in weight percentages except for certain gaseous mixtures where they may be best described in volume percentage, e.g. a single hazardous component in an inert diluent, e.g. nitrogen or helium.

When the information on the mixture has been gathered and validated, the following guidance should be followed depending on the type and level of information available.

4.1.4.3. Classification criteria for mixtures hazardous to the aquatic environment based on test data on the mixture as a whole

The testing of a mixture for aquatic toxicity is highly complex, both in terms of the conduct of the test, and in the interpretation of data from such testing. The different physico-chemical properties, such as water solubility, vapour pressure, and adsorption, make it almost impossible to prepare an exposure concentration that is characteristic of the mixture, while the multi-component analysis needed to verify such an exposure concentration is both complex and expensive.

Therefore, before any such new testing is conducted, alternative approaches such as the summation method, should be considered, particularly where testing would involve the use of vertebrate animals such as fish (see also section 1.1.6.2 of Part 1). Nevertheless, there are circumstances where test data may already be available and should then be examined to assess its relevance for the purposes of classification. Data which has been prepared for Regulatory use in compliance with standard guidelines, such as test data on plant protection or biocidal products,

may be considered as acceptable for classification. Where such valid test data, both acute and chronic, are available, they may be used in accordance with the general guidance below.

Annex I: ***4.1.3.3.1*** *When the mixture as a whole has been tested to determine its aquatic toxicity, this information can be used for classifying the mixture according to the criteria that have been agreed for substances. The classification is normally based on the data for fish, crustacea and algae/plants (see sections 4.1.2.7.1 and 4.1.2.7.2). When adequate acute or chronic toxicity data for the mixture as a whole are lacking, "bridging principles" or "summation method" should be applied (see sections 4.1.3.4 and 4.1.3.5).*

4.1.3.3.2 *The long-term (chronic) hazard classification of mixtures requires additional information on degradability and in certain cases bioaccumulation. Degradability and bioaccumulation tests for mixtures are not used as they are usually difficult to interpret, and such tests may be meaningful only for single substances.*

4.1.3.3.3 *Classification for category Acute 1*

(a) When there are adequate acute toxicity test data (LC_{50} or EC_{50}) available for the mixture as a whole showing $L(E)C_{50} \leq 1$ mg/l:

Classify mixture as Acute 1 in accordance with point (a) of Table 4.1.0.

(b) When there are acute toxicity test data ($LC_{50}(s)$ or $EC_{50}(s)$) available for the mixture as a whole showing $L(E)C_{50}(s) > 1$ mg/l for normally all trophic levels:

No need to classify for short-term (acute) hazard.

4.1.3.3.4 *Classification for categories Chronic 1, 2 and 3*

(a) When there are adequate chronic toxicity data (EC_x or NOEC) available for the mixture as a whole showing EC_x or NOEC of the tested mixture ≤ 1mg/l:

(i) Classify the mixture as Chronic 1, 2 or 3 in accordance with point (b)(ii) of Table 4.1.0. as rapidly degradable if the available information allows the conclusion that all relevant components of the mixture are rapidly degradable;

(ii) Classify the mixture as Chronic 1 or 2 in all other cases in accordance with point (b)(i) of Table 4.1.0. as non-rapidly degradable;

(b) When there are adequate chronic toxicity data (ECx or NOEC) available for the mixture as a whole showing ECx(s) or NOEC(s) of the tested mixture > 1 mg/l for normally all trophic levels:

No need to classify for long-term (chronic) hazard in categories Chronic 1, 2 or 3.

4.1.3.3.5 *Classification for category Chronic 4*

If there are nevertheless reasons for concern:

Classify the mixture as Chronic 4 (safety net classification) in accordance with Table 4.1.0.

Where a classification is made based on test data, valid data should normally be available on each of fish, crustacea and algae or other aquatic plants, unless a decision to classify in the most stringent category(ies) (Acute 1 and/or Chronic 1) can be made without a full dataset. To be valid, it would normally be necessary to show that the tested organism has been exposed to the toxic components of the mixture in proportion to the composition of the mixture, and that this exposure has been maintained for the duration of the test. If this cannot be accomplished the classification should be based on information on the individual components. It is insufficient to simply prepare a water-accommodated fraction (WAF) for testing.

When there is adequate toxicity test data available for the mixture as a whole, this may be simplified to two basic rules for each of acute and long-term hazard classification:

Classification for acute (short-term) aquatic hazard:

i. If the lowest valid acute/short-term L(E)C_{50} is ≤ 1 mg/L, classify as Acute 1.

ii. If valid acute/short-term test data are available on fish, crustacea and algae/aquatic plants (i.e. all three trophic levels), and all showing L(E)C_{50} > 1 mg/L, there is no need to classify for acute aquatic hazard.

Classification for long-term aquatic hazard:

i. If the lowest valid chronic toxicity test data (NOEC or EC_x) is ≤ 1 mg/L, classify as Chronic 1, 2 or 3, depending on the information on components degradability, e.g. if all components are known to be rapidly degradable.

ii. If valid chronic toxicity test data are available on fish, crustacea and algae/aquatic plants (i.e. all three trophic levels), and all showing NOEC or EC_x >1 mg/L, there is no need to classify for long-term aquatic hazard in Chronic 1, 2 or 3.

4.1.4.4. When experimental aquatic toxicity data are not available for the complete mixture: bridging principles

Annex I: ***4.1.3.4.1*** *Where the mixture itself has not been tested to determine its aquatic environmental hazard, but there are sufficient data on the individual components and similar tested mixtures to adequately characterise the hazards of the mixture, this data shall be used in accordance with the bridging rules set out in section 1.1.3. However, in relation to application of the bridging rule for dilution, sections 4.1.3.4.2 and 4.1.3.4.3 shall be used.*

4.1.3.4.2 *Dilution: if a mixture is formed by diluting another tested mixture or a substance classified for its aquatic environmental hazard with a diluent which has an equivalent or lower aquatic hazard classification than the least toxic original component and which is not expected to affect the aquatic hazards of other components, then the resulting mixture may be classified as equivalent to the original tested mixture or substance. Alternatively, the method explained in section 4.1.3.5 may be applied.*

4.1.3.4.3 *If a mixture is formed by diluting another classified mixture or substance with water or other totally non-toxic material, the toxicity of the mixture can be calculated from the original mixture or substance.*

For circumstances where no or inadequate test data are available on the mixture itself, the classification of a mixture may be determined based on sufficient data for individual components of the mixture and on another similar tested mixture by an appropriate application of any of the specified 'bridging principles'. The identified relevant information needs to be evaluated for the purpose of classification, by comparing it with the criteria in section 1.1.3 of Annex I to CLP. Those rules allow characterisation of the hazards of the mixture without performing tests on it, but rather by building on the available information on similar tested mixtures (see also Part 1, section 1.6.3.2 of this guidance document).

4.1.4.5. When hazard data (information on toxicity or classification) are available for all the components of the mixture

Annex I: ***4.1.3.5.1*** *The classification of a mixture is based on summation of the classification of its components. The percentage of components classified as "Acute" or "Chronic" is fed straight in to the summation method. Details of the summation method are described in 4.1.3.5.5.*

Where no or inadequate test data on the mixture itself is available and the bridging principles are not applicable, the classification of the mixture is based on information on the components. The information that will most usually be available to aid classification of a mixture will be the

classification applied to the individual components (substances). These data and any associated M-factor(s) are included in the safety data sheets (SDS) and also in the Classification and Labelling Inventory (C&L Inventory) established and maintained by the Agency in the form of a database [http://echa.europa.eu/information-on-chemicals/cl-inventory-database]. Where M-factors are not available for the harmonised classification or the SDS there is a general obligation under CLP Art. 10(2) to derive M-factors using available aquatic toxicity data. In cases where the aquatic hazard classification of a mixture will be made based on data on the components, it is therefore generally the summation of the quantities of the hazardous components that should be used to determine a specific hazard classification of the mixture.

Provided the classification data, in part or in total, and the % of these components in the mixture are known, a classification of the mixture can be made according to the summation method. The following text from CLP describes the application of this method.

Annex I*: 4.1.3.5.5* *Summation method*

4.1.3.5.5.1 *Rationale*

4.1.3.5.5.1.1 *In case of the substance classification categories Chronic 1 to Chronic 3, the underlying toxicity criteria differ by a factor of 10 in moving from one category to another. Substances with a classification in a high toxicity band therefore contribute to the classification of a mixture in a lower band. The calculation of these classification categories therefore needs to consider the contribution of any substance classified as Chronic 1, 2 or 3.*

4.1.3.5.5.2. *Classification procedure*

4.1.3.5.5.2.1 *In general a more severe classification for mixtures overrides a less severe classification, e.g. a classification with Chronic 1 overrides a classification with Chronic 2. As a consequence, in this example, the classification procedure is already completed if the result of the classification is Chronic 1. A more severe classification than Chronic 1 is not possible. Therefore it is not necessary to undergo the further classification procedure.*

4.1.3.5.5.3 *Classification for category Acute 1*

4.1.3.5.5.3.1 *First all components classified as Acute 1 are considered. If the sum of the concentrations (in %) of these components multiplied by their corresponding M-factors is greater than 25 % the whole mixture is classified as Acute 1.*

4.1.3.5.5.3.2 *The classification of mixtures for short-term (acute) hazards based on this summation of classified components is summarised in Table 4.1.1.*

Table 4.1.1
Classification of a mixture for short-term (acute) hazards, based on summation of classified components

Sum of components classified as:	*Mixture is classified as:*
Acute 1 × M (a) ≥ 25 %	*Acute 1*

(a) For explanation of the M-factor see 4.1.3.5.5.5

4.1.3.5.5.4 *Classification for the categories Chronic 1, 2, 3 and 4*

4.1.3.5.5.4.1 *First all components classified as Chronic 1 are considered. If the sum of the concentrations (in %) of these components multiplied by their corresponding M-factors is equal to or greater than 25 %, the mixture is classified as Chronic 1. If the result of the calculation is a classification of the mixture as Chronic 1, the classification procedure is completed.*

4.1.3.5.5.4.2 *In cases where the mixture is not classified as Chronic 1, classification of the mixture as Chronic 2 is considered. A mixture is classified as Chronic 2 if 10 times the sum of the concentrations (in %) of all components classified as Chronic 1 multiplied by their corresponding M-factors plus the sum of the concentrations (in %) of all components classified as Chronic 2 is equal to or greater than 25 %. If the result of the calculation is classification of the mixture as Chronic 2, the classification process is completed.*

4.1.3.5.5.4.3 *In cases where the mixture is not classified either as Chronic 1 or Chronic 2, classification of the mixture as Chronic 3 is considered. A mixture is classified as Chronic 3 if 100 times the sum of the concentrations (in %) of all components classified as Chronic 1 multiplied by their corresponding M-factors plus 10 times the sum of the concentrations (in %) of all components classified with Chronic 2 plus the sum of the concentrations (in %) of all components classified as Chronic 3 is ≥ 25 %.*

4.1.3.5.5.4.4 *If the mixture is still not classified in Chronic 1, 2 or 3, classification of the mixture as Chronic 4 shall be considered. A mixture is classified as Chronic 4 if the sum of the concentrations (in %) of components classified as Chronic 1, 2, 3 and 4 is equal to or greater than 25 %.*

4.1.3.5.5.4.5 *The classification of mixtures for long-term (chronic) hazards, based on this summation of the concentrations of classified components, is summarised in Table 4.1.2.*

Table 4.1.2
Classification of a mixture for long-term (chronic) hazards, based on summation of the concentrations of classified components

Sum of components classified as:	*Mixture is classified as:*
Chronic 1 × M (a) ≥ 25 %	*Chronic 1*
(M × 10 × Chronic 1) + Chronic 2 ≥ 25 %	*Chronic 2*
(M × 100 × Chronic 1) + (10 × Chronic 2) + Chronic 3 ≥ 25 %	*Chronic 3*
Chronic 1 + Chronic 2 + Chronic 3 + Chronic 4 ≥ 25 %	*Chronic 4*

(a) For explanation of the M-factor, see 4.1.3.5.5.5

4.1.3.5.5.1.2 *When a mixture contains components classified as Acute 1 or Chronic 1, attention must be paid to the fact that such components, when their acute toxicity is below 1 mg/l and/or chronic toxicity is below 0,1 mg/l (if non-rapidly degradable) and 0.01 mg/l (if rapidly degradable) contribute to the toxicity of the mixture even at a low concentration. Active ingredients in pesticides often possess such high aquatic toxicity but also some other substances like organometallic compounds. Under these circumstances the application of the normal generic concentration limits leads to an "under-classification" of the mixture. Therefore, multiplying factors shall be applied to account for highly toxic components, as described in section 4.1.3.5.5.5.*

For those components for which only toxicity data are available (i.e., no derived classification) the additivity formulas offer a way for estimating what the toxicity of a mixture would be if the individual substance toxicities could be 'added' to each other in a straightforward way. Thus it assumes a similar 'mode of action' for each component.

To make full use of this approach access to the whole aquatic toxicity dataset and the necessary knowledge to select the best and most appropriate data is required. Clearly, the best use would be to add up separately each of the fish toxicity data, the crustacea toxicity data and the algae/aquatic plants toxicity data to derive a specific toxicity value for each trophic level. The lowest of the toxicity values would normally be used to define the appropriate hazard category for the mixture. Indeed, if it is only possible to characterise part of the mixture in this way, that part can be assigned a hazard category (and an M-factor for categories Acute 1 and/or Chronic 1) and then, in a second step, be used in the summation method.

The use of the additivity formulae is limited to those circumstances where the substance hazard category is not known. The following text from CLP describes the application of the additivity formula.

Annex I: ***4.1.3.5.2*** *Mixtures can be made of a combination of both components that are classified (as Acute 1 and/or Chronic 1, 2, 3, 4) and others for which adequate toxicity test data is available. When adequate toxicity data are available for more than one component in the mixture, the combined toxicity of those components is calculated using the following additivity formulas (a) or (b), depending on the nature of the toxicity data:*

(a) Based on acute aquatic toxicity:

$$\frac{\sum \mathrm{Ci}}{\mathrm{L(E)C_{50m}}} = \sum_{\eta} \frac{\mathrm{Ci}}{\mathrm{L(E)C_{50i}}}$$

where:

C_i = concentration of component i (weight percentage);

$L(E)C_{50\,i}$ = (mg/l) LC_{50} or EC_{50} for component i;

η = number of components, and i is running from 1 to n;

$L(E)C_{50\,m}$ = L(E) C_{50} of the part of the mixture with test data;

The calculated toxicity may be used to assign to that portion of the mixture a short-term (acute) hazard category which is then subsequently used in applying the summation method;

(b) Based on chronic aquatic toxicity:

$$\frac{\sum Ci + \sum Cj}{EqNOEC_m} = \sum_{n} \frac{Ci}{NOECi} + \sum_{n} \frac{Cj}{0{,}1 \times NOECj}$$

where:

C_i = concentration of component i (weight percentage) covering the rapidly degradable components;

Cj = concentration of component j (weight percentage) covering the non- rapidly degradable components;

$NOEC_i$ = NOEC (or other recognized measures for chronic toxicity) for component i covering the rapidly degradable components, in mg/l;

NOECj = NOEC (or other recognized measures for chronic toxicity) for component j covering the non-rapidly degradable components, in mg/l;

n = number of components, and i and j are running from 1 to n;

$EqNOEC_m$ = Equivalent NOEC of the part of the mixture with test data;

The equivalent toxicity thus reflects the fact that non-rapidly degrading substances are classified one hazard category level more "severe" than rapidly degrading substances.

The calculated equivalent toxicity may be used to assign that portion of the mixture a long-term (chronic) hazard category, in accordance with the criteria for rapidly degradable

substances (point (b)(ii) of Table 4.1.0.), which is then subsequently used in applying the summation method.

4.1.3.5.3. *When applying the additivity formula for part of the mixture, it is preferable to calculate the toxicity of this part of the mixture using for each substance toxicity values that relate to the same taxonomic group (i.e. fish, crustacean, algae or equivalent) and then to use the highest toxicity (lowest value) obtained (i.e. use the most sensitive of the three taxonomic groups). However, when toxicity data for each component are not available in the same taxonomic group, the toxicity value of each component is selected in the same manner that toxicity values are selected for the classification of substances, i.e. the higher toxicity (from the most sensitive test organism) is used. The calculated acute and chronic toxicity is then used to assess whether this part of the mixture shall be classified as Acute 1 and/or Chronic 1, 2 or 3 using the same criteria described for substances.*

Note: the chronic additivity formula includes a factor of 10 correction for the non-rapidly degradable components to allow equivalent inclusion of not rapidly and rapidly degradable components. Therefore, toxicity values are to be treated as rapidly degradable when deriving a classification. That is, calculated equivalent toxicity values are compared with the criteria in Table 4.1.0 (b)(ii) and M-factors derived from the 'RD' column of Table 4.1.3. Note also that generic concentration limits (GCLs) should be given in weight percentages except for certain gaseous mixtures where they may be best described in volume percentage, e.g. a single hazardous component in an inert diluent, e.g. nitrogen or helium.

NOTICE: With the aquatic toxicity data at hand the ingredient substance classification and M-factor(s) could easily be gained by a direct comparison with the substance criteria, which then could be fed straight into the summation method. It will therefore usually not be necessary to use the additivity formulae.

4.1.4.6. When hazard data (information on toxicity or classification) are available for only some components of the mixture

This section is related to Figure 4.1.1 where one can decide to apply the summation method and/or the additivity formula (see section 4.1.3.5 of Annex I to CLP) and apply section 4.1.3.6 of Annex I to CLP.

Use available hazard data of known components.

- This applies to mixtures containing unknown components and/or known components, for which neither toxicity data nor classifications are known. In these cases, for labelling purposes consider the provisions of section 4.1.3.6 in Annex I to CLP. For classification based on the known part of the mixture, use the summation method and/or the additivity formula (see section 4.1.3.5 of Annex I to CLP).
- NOTE: If a mixture is classified in more than one way, the method yielding the most stringent result should be used.

4.1.4.7. Decision on classification: examples for mixtures

If the evaluation shows that the criteria are fulfilled, one category for acute aquatic hazard and/or one category for long-term aquatic hazards should be assigned. For the labelling elements, such as: hazard pictograms, signal words, hazard statements and precautionary statements, see section 4.1.6.

List of the examples on mixtures classification included in this section:

The classification system for mixtures is complex as different methods are available. Which method to use is dependent upon the type of information available.

- Example A: When classification data are available for some or all components of a mixture: straightforward application of the summation method.
- Example B1: When toxicity test data on the mixture as a whole are available for all three trophic levels: classification based on test data on the mixture.
- Example B2: When information on the classification of the components and test data on the mixture as a whole are available for some, but not all three trophic levels: classification based on the summation method.
- Example C: When no data are available on the mixture as a whole and its components, but test data are available on a similar tested mixture: use of the bridging principles – dilution with water.
- Example D: When only test data are available for some, but not all components of the mixture: use of the additivity formula and the summation method.

4.1.4.7.1. Example A: When classification data are available for some or all components of a mixture: straightforward application of the summation method

INFORMATION ON INGREDIENTS CLASSIFICATION AND CONCENTRATION					
	Acute aquatic hazard	**M**	**Long-term aquatic hazard**	**M**	**C (%)**
Astralamid	Acute 1	10	Chronic 1	10	1
Bastralamid	Acute 1	1	Chronic 2	-	3
Castralamid	Not classified	-	Chronic 2	-	10
Dastralamid	Not classified	-	Chronic 3	-	10
Estralamid	Not classified	-	Not classified	-	10
Festralamid	Not classified	-	Not classified	-	66

M = M-factor; C = Concentration

Aquatic hazard classification:

Acute aquatic hazard: Not classified.

Long-term aquatic hazard: Category Chronic 2

Reasoning:

- Valid test data on the mixture as a whole (for all three trophic levels) are not available.
- Valid test data on similar tested mixtures are not available, either, meaning that any bridging principle cannot be used.

Therefore, classification should be considered based on individual components using the summation method.

Acute aquatic hazard: Information on classification including associated M-factors and the % of the components in the mixture are available.

Classify for acute hazard if: $\sum$ (Acute 1 $\times$ M) $\geq$ 25%

Using the classification of the components of the mixture: (1 $\times$ 10) + (3 $\times$ 1) = 13 (which is < 25%). Hence, no classification for acute aquatic hazard.

Long-term aquatic hazard:

Step 1: Classify as Chronic 1 if: $\sum$ (Chronic 1 $\times$ M) $\geq$ 25% (if not, then go to Step 2).

Step 2: Classify as Chronic 2 if: $\sum$ (10 $\times$ Chronic 1 $\times$ M) + $\sum$ (Chronic 2) $\geq$ 25% (if not, then go to Step 3).

Step 3: Classify as Chronic 3 if: $\sum$ (100 $\times$ Chronic 1 $\times$ M) + $\sum$ (10 $\times$ Chronic 2) + $\sum$ (Chronic 3) $\geq$ 25% (if not, then go to Step 4).

Step 4: Classify as Chronic 4 if: $\sum$ (Chronic 1) + $\sum$ (Chronic 2) + $\sum$ (Chronic 3) + $\sum$ (Chronic 4) $\geq$ 25%

Using the classification of the components of the mixture:

Step 1: (1 × 10) = 10 (which is < 25% → Step 2).

Step 2: (10 × 1 × 10) + 3+10 = 113 (which is > 25%). Hence, classify as Category Chronic 2.

Labelling elements based on the classification:

Element	Resulting Labelling Elements (code)
GHS Pictogram	GHS09
Signal Word	-
Hazard Statement	H411
Precautionary statement(s)	P273, P391, P501

4.1.4.7.2. Example B1: When toxicity data on the mixture as a whole is available for all three trophic levels: classification based on test data for the mixture

INFORMATION ON INGREDIENTS CLASSIFICATION AND CONCENTRATION					
	Acute aquatic hazard	**M**	**Long-term aquatic hazard**	**M**	**C (%)**
Frusthrin	Acute 1	1	Chronic 1	1	40
Gladobrin	Acute 1	1	Chronic 3	-	60

M = M-factor; C = Concentration

Acute (short-term) aquatic toxicity	**Value**	**Test method ((EC) No. 440/2008) or OECD guideline / remarks**
Fish: Mixture (*Cyprinus carpio*)	19 mg/L (96 hr LC_{50})	C.1 / static, GLP
Crustacea: Mixture (*Daphnia magna*)	3.5 mg/L (48 hr EC_{50})	C.2 / static, GLP
Algae/aquatic plants: Mixture (*Scenedesmus subspicatus*)	15 mg/L (72 or 96 hr ErC_{50})	C.3 / static, GLP
Chronic (long-term) aquatic toxicity		
Fish: Mixture (*Cyprinus carpio*)	0.09 mg/L (12 d NOEC)	OECD 210 / Early Life Stage, flow through, GLP
Crustacea: Mixture (*Daphnia magna*)	0.05 mg/L (21 d NOEC)	C.20 / semi-static, GLP
Algae/aquatic plants: Mixture (*Scenedesmus subspicatus*)	1.5 mg/L (96 h NOEC)	C.3 / static, GLP

Aquatic hazard classification:

Acute aquatic hazard: Not classified.

Long-term aquatic hazard: Chronic 1.

Reasoning:

Acute aquatic hazard:

Valid test data for all the three trophic levels are available for the mixture as a whole, therefore no need to consider bridging principles or classification of individual components for acute hazard classification of the mixture. Test data showed that $L(E)C_{50}$ > 1 mg/L. Consequently - no classification for acute aquatic hazard.

Long-term aquatic hazard:

Valid test data for all the three trophic levels are available for the mixture as a whole, therefore no need to consider classification of individual components for long-term hazard classification of the mixture. Test data showed that NOEC < 0.1 mg/L. No information on rapid degradation. Hence, the mixture is considered being not rapidly degradable. The mixture is classified as category Chronic 1.

Labelling elements based on the classification:

Element	Resulting Labelling Elements (code)
GHS Pictogram	GHS09
Signal Word	Wng
Hazard Statement	H410
Precautionary statement(s)	P273, P391, P501

4.1.4.7.3. Example B2: When information on the classification of the components is available and toxicity data on the mixture as a whole is available for some, but not all three trophic levels: use of the summation method

INFORMATION ON COMPONENTS CLASSIFICATION AND CONCENTRATION					
	Acute aquatic hazard	**M**	**Long-term aquatic hazard**	**M**	**C (%)**
Frusthrin	Acute 1	1	Chronic 1	1	40
Gladobrin	Acute 1	1	Chronic 3	-	60

M = M-factor; C = Concentration

Acute (short-term) aquatic toxicity	**Value**	**Test method ((EC) No. 440/2008) or OECD guideline / remarks**
Algae/aquatic plants: Mixture (*Scenedesmus subspicatus*)	15 mg/L (72 or 96 hr ErC_{50})	C.3 / static, GLP
Chronic (long-term) aquatic toxicity		
Algae/aquatic plants: Mixture (*Scenedesmus subspicatus*)	1.5 mg/L (96 h NOEC)	C.3 / static, GLP

Aquatic hazard classification:

Acute aquatic hazard: Acute 1.

Long-term aquatic hazard: Chronic 1.

Reasoning:

- Valid test data on the mixture as a whole are available for one, but not for all the three trophic levels and we don't know if algae is clearly the most sensitive trophic level for the mixture.
- Neither is valid test data on similar tested mixtures available, meaning that the bridging principles could not be used.

Therefore, classification should for both acute hazard and long-term hazard be considered based on individual components using the summation method. Testing should not be conducted for the mixture for the remaining trophic levels.

Acute aquatic hazard:

Information on classification including associated M-factors and the % of the components in the mixture are available.

Classify for acute hazard if: $\sum$ (Acute 1 $\times$ M) $\geq$ 25%

Using the classification of the components of the mixture: (40 × 1) + (60 × 1) = 100 (which is ≥ 25%). Hence - category Acute 1.

Long-term aquatic hazard:

Information on classification including associated M-factors and the % of the components in the mixture are available.

Step 1: Classify as Chronic 1 if: ∑ (Chronic 1 × M) ≥ 25% (if not, then go to Step 2).

Using the classification of the components of the mixture:

Step 1: (40 × 1) = 40 (which is ≥ 25%). Hence - Category Chronic 1.

Labelling elements based on the classification:

Element	Resulting Labelling Elements (code)
GHS Pictogram	GHS09
Signal Word	Wng
Hazard Statement	H410[17]
Precautionary statement(s)	P273, P391, P501

[17] Note that in accordance with Article 27 of CLP hazard statement H400 may be considered redundant and therefore not included on the label because hazard statement H410 also applies, see section 4.1.6.

4.1.4.7.4. Example C: When no data are available on the mixture as a whole and its components, but test data are available on a similar tested mixture: use of the bridging principles – dilution with water

Test Species	Information / Data
Fish	No data available
Crustacea	No data available
Algae	No data available

A reference mixture has shown a LC_{50} of 0.5 mg/L and adequate NOECs in the range 0.07 to < 0.1 mg/L. Based on this data it has been classified as Category Acute 1 and Category Chronic 1.

Subsequently, this mixture has been diluted in water by factor of 10 and the newly diluted mixture shall now be classified.

Aquatic hazard classification:

Acute aquatic hazard: Not classified.

Long-term aquatic hazard: Category Chronic 2.

Reasoning:

The mixture is formed by diluting another classified mixture with water, the toxicity of the mixture can therefore be calculated from the original mixture. (see section 4.1.4.4 of this document and CLP Annex I, section 4.1.3.4.3.)

Acute aquatic hazard: LC_{50} = 5 mg/L (0.5x10). Hence - not classified.

Long-term aquatic hazard: Adequate NOECs in the range 0.7 to < 1 mg/L (0.07 x 10 and 0.1 x 10). Hence - category Chronic 2.

Labelling elements based on the classification:

Element	Resulting Labelling Elements (code)
GHS Pictogram	GHS09
Signal Word	-
Hazard Statement	H411
Precautionary statement(s)	P273, P391, P501

4.1.4.7.5. Example D: When test data are available for some, but not all components of the mixture: use of the additivity formula and of the summation method

INFORMATION ON COMPONENTS CLASSIFICATION AND CONCENTRATION					
	Acute aquatic hazard	**M**	**Long-term aquatic hazard**	**M**	**C (%)**
Component 1	-	-	-	-	50
Component 2	-	-	-	-	10
Component 3	-	-	-	-	10
Component 4	Not classified	-	Chronic 1	-	30

COMPONENT TOXICITY DATA				
Data elements		**Component 1 (50% of the mixture)**	**Component 2 (10% of the mixture)**	**Test method ((EC) No. 440/2008) or OECD guideline / remarks**
Physico-chemical properties				
Water Solubility:		200 mg/L	1000 mg/L	A.6 / pH: 7.0, non-GLP
Log octanol/water partition coefficient (log K_{ow}):		No data	No data	
Acute (short-term) aquatic toxicity				
Fish:	*Oncorhynchus mykiss*	No data	0.3 mg/L (96 hr LC_{50})	C.1 / static, GLP
Crustacea:	*Daphnia magna*	0.55 mg/L (48 hr EC_{50})	No data	C.2 / static, non-GLP
Algae/aquatic plants: *Scenedesmus subspicatus*		0.37 mg/L (72 hr E_rC_{50})	1.37 mg/L (72 hr E_rC_{50})	C.3 / static, GLP
Long-term aquatic toxicity				
Fish:	*Oncorhynchus mykiss*	0.07 mg/L (28 d NOEC)	1.3 mg/L (28 d NOEC)	OECD 210 / semi-static
Crustacea:	*Daphnia magna*	0.09 mg/L (21 d NOEC)	1.4 mg/L (21 d NOEC)	C.20 / semi-static, GLP

Algae/aquatic plants: *Scenedesmus subspicatus*	0.13 mg/L (72 hr NOEC)	0.53 mg/L (72 hr NOEC)	C.3 / static, GLP
Degradation (evidence of rapid degradation)			
Biotic degradation (% degradation in 28 days (or, if absent, half-life in water (d)):	No data	No data	
Abiotic degradation (Hydrolysis) (half-life (d)):	No data	No data	
Bioaccumulation			
Bioconcentration factor in fish (BCF):	No data	No data	

Long-term hazard classification is known for 30% of the mixture.

Test data is available for 60% of the mixture.

For 10% of the mixture no information is available.

Aquatic hazard classification:

Acute aquatic hazard: Category Acute 1.

Long-term aquatic hazard: Category Chronic 1.

Reasoning:

- Valid test data on the mixture as a whole (for all three trophic levels) are not available.
- Valid test data on similar tested mixtures are not available, either, meaning that any bridging principle cannot be used.

Therefore, classification should be considered based on individual components using the summation method.

NOTICE! In the case of the downstream user or importer not having the classification of all the components, further dialogue with the supplier may be necessary to obtain additional information. The suppliers in a supply chain shall cooperate to meet the requirements for classification, labelling and packaging (see Article 4(9) of CLP). This particular example, however, shows what could be done if the classification of some components in any case is not available (which, for example, could be the case when importing certain mixtures).

Acute aquatic hazard:

For component 1 the most sensitive species showed a L(E)C_{50} 0.37 mg/L. Thus, component 1, comprising 50% of the mixture, is classified as Acute 1; M-factor 1.

Subsequently used in the summation method, more than 25% of the mixture is classified as category Acute 1. Hence, the mixture is classified as Acute 1.

Alternatively: You can calculate the combined toxicity for components 1 and 2 applying the *Additivity Formula*[18]:

$L(E)C_{50m}$ = (50+10) / (50/0.37 mg/L + 10/0.3 mg/L) = 0.36 mg/L

Assign category Acute 1. This means that 60% of this mixture is classified as category Acute 1 and hence, subsequently used in the summation method, the whole mixture is classified as Acute 1.

Long-term aquatic hazard:

Assign hazard categories for each component for which there are adequate chronic toxicity data available:

	Relevant information	Category	C (%)
Component 1	0.07 mg/L (28 d NOEC Fish); No information on degradation. Hence, the substance is considered not rapidly degradable.	Assign Chronic 1, M-factor 1	50 %
Component 2	0.53 mg/L (72 hr NOEC Algae); No information on degradation. Hence, the substance is considered not rapidly degradable.	Assign Chronic 2	10%
Component 3	No data	-	10%
Component 4	Data for basis of classification not available.	Chronic 1	30 %

More than 25% of the mixture is classified as category Chronic 1 and thus, the mixture is classified as category Chronic 1.

Alternatively: You can apply the *Additivity Formula*[19] to calculate the combined toxicity for components 1 and 2 (60% of the mixture)

$EqNOEC_m$ = 60 / (50/(0.1 x 0.07) + 10/(0.1 x 1.3)) = 0.008 mg/L for fish

$EqNOEC_m$ = 60 / (50/(0.1 x 0.09)) + 10/(0.1 x 1.4)) = 0.011 mg/L for crustacea

$EqNOEC_m$ = 60 / (50/(0.1 x 0.13) + 10/(0.1 x 0.53)) = 0.015 mg/L for algae

The lowest calculated $EqNOEC_m$ is 0.008 mg/L for fish.

Following the not rapidly degradable correction applied by use of the additivity formula (i.e., a factor of 10 for non-rapidly degradable components), treat the values as those of a rapidly degradable substance by applying point (b) (ii) of Table 4.1.0 of Annex I to CLP. Subsequently, use the 'RD' column of Table 4.1.3. In conclusion, assign category Chronic 1, M-factor 1 to that part of the mixture.

[18] In many cases it is possible to use the summation method straight away by assigning hazard categories to single components of a mixture when data is available.

[19] See also section 4.1.4.6 of this guidance.

In addition component 4 of the mixture is classified as category Chronic 1 and comprises 30% of the mixture.

The long-term hazard category assigned to that part of the mixture the mixture is then subsequently used in applying the summation method:

Classify as Chronic 1 if: $\sum$ (Chronic 1 $\times$ M) $\geq$ 25%

$\sum (60 \times 1) + 10 = 70$

Thus, the mixture is classified as category Chronic 1.

Labelling elements based on the classification:

Element	Resulting Labelling Elements (code)
GHS Pictogram	GHS09
Signal Word	Wng
Hazard Statement	H410[20]
Precautionary statement(s)	P273, P391, P501

In the SDS and on the label it has to be stated: 'Contains 10% of components with unknown hazards to the aquatic environment'.

[20] Note that in accordance with Article 27 of CLP, the hazard statement H400 may be considered redundant and therefore not included on the label because hazard statement H410 also applies, see section 4.1.6 of this document.

4.1.5. Metal and metal compounds

4.1.2.10. *Inorganic compounds and metals*

4.1.2.10.1. *For inorganic compounds and metals, the concept of degradability as applied to organic compounds has limited or no meaning. Rather, such substances may be transformed by normal environmental processes to either increase or decrease the bioavailability of the toxic species. Equally the use of bioaccumulation data shall be treated with care([1]).*

4.1.2.10.1. *Poorly soluble inorganic compounds and metals may be acutely or chronically toxic in the aquatic environment depending on the intrinsic toxicity of the bioavailable inorganic species and the rate and amount of this species which enter solution. All evidence must be weighed in a classification decision. This would be especially true for metals showing borderline results in the Transformation/Dissolution Protocol.*

_____

([1]) Specific guidance has been issued by the European Chemicals Agency on how these data for such substances may be used in meeting the requirements of the classification criteria.

Annex IV provides the detailed guidance on the classification of metals and metal compounds.

The guidance on classification of alloys and complex metal containing materials is limited so far. More guidance is needed (see also Annex IV.5.5).

4.1.6. Hazard communication for hazards to the aquatic environment

A substance or mixture classified as hazardous and contained in packaging shall bear a label in accordance with the rules in Title III of CLP. The elements to be included in labels should be specified in accordance with the hazard pictograms, signal words, hazard statements and precautionary statements which form the core information of the CLP system. For general guidance on labelling see the *Introductory Guidance on the CLP Regulation (ECHA, 2019)* and also the *Guidance on Labelling and Packaging in accordance with Regulation (EC) No 1272/2008 (ECHA, 2019).*

Label elements shall be used for substances or mixtures meeting the criteria for classification in the hazard class *Hazardous to the Aquatic Environment* in accordance with Table 4.1.4 of Annex I to CLP.

Pictogram

The hazard pictogram shall satisfy the provisions of Annex V and Annex I, part 1.2 to the Regulation.

Symbol: *Environment*; Pictogram Code: *GHS09*

The pictogram GHS09 is required only for substances or mixtures classified as:

- Aquatic Acute 1 and/or
- Aquatic Chronic 1 or 2

Signal word

The label shall include the relevant signal word in accordance with the classification of the hazardous substance or mixture. The signal word relevant for the hazard class *Hazardous to the Aquatic Environment* is:

Warning

Signal Word Code: *Wng*

The signal word 'Warning' is required only for substances or mixtures classified as:

- Aquatic Acute 1 and/or
- Aquatic Chronic 1

Where the signal word 'Danger' is used on the label due to classification into another hazard class(es), the signal word 'Warning' shall not appear on the label.

Hazard statements

The label shall include the relevant hazard statements in accordance with the classification of the hazardous substance or mixture and shall be worded in accordance with Annex III to CLP.

The hazard statements (and the Hazard statement Codes) relevant for the hazard class *Hazardous to the Aquatic Environment* are:

- Very toxic to aquatic life (H400)
- Very toxic to aquatic life with long lasting effects (H410)
- Toxic to aquatic life with long lasting effects (H411)
- Harmful to aquatic life with long lasting effects (H412)
- May cause long lasting harmful effects to aquatic life (H413)

The hazard statement H400 is required only for substances or mixtures classified as:

- Aquatic Acute 1

The hazard statements H410 to H413 are respectively required for substances or mixtures classified as:

- Aquatic Chronic 1, 2, 3 or 4

Article 27 of CLP states that if a substance or mixture is classified within several hazard classes or differentiations of a hazard class, all hazard statements resulting from the classification shall appear on the label, unless there is evident duplication or redundancy.

This means that in line with Part 1 of Annex III to CLP, where the hazard statement H410 is used on the label due to classification in the long-term hazard category Chronic 1, the hazard statement H400 does not need to appear on the label. Furthermore, where a substance or a mixture is classified both in acute and long-term hazard categories, it is possible to use only hazard statement H410 on the label (see table 4.1 below).

Table 4.1 Hazard statement Codes relevant for the hazard class Hazardous to the Aquatic Environment

Aquatic hazard classification	Associated hazard statement	Associated hazard statement that could appear on the label
Acute 1	H400	H400
Acute 1 and Chronic 1	H400; H410	H410
Acute 1 and Chronic 2	H400; H411	H410
Acute 1 and Chronic 3	H400; H412	H410
Acute 1 and Chronic 4[21]	H400; H413	H410
Chronic 1	H410	H410
Chronic 2	H411	H411
Chronic 3	H412	H412
Chronic 4	H413	H413

Precautionary statements

In accordance with Articles 17 and 22 of CLP the label shall include the relevant precautionary statements. The precautionary statements that can in principle be used for the hazard class *Hazardous to the Aquatic Environment* according to CLP Annex I Table 4.1.4 are:

- Avoid release to the environment (P273)
- Collect spillage (P391)
- Dispose of contents/container to … (P501)

4.1.7. References

ECHA Guidance on information requirements and chemical safety assessment (version 4.0, June 2017), Chapter R7b

OECD 2019: Guidance Document on aqueous-phase aquatic toxicity testing of difficult test chemicals. *Series on Testing and Assessment Number 23 (Second Edition)* ENV/JM/MONO(2000)6/REV1 (http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2000)6/REV1&docLanguage=En) (referred here as OECD Guidance 23)OECD 2006: *Series on Testing and Assessment Number 54, Current approaches in the statistical analysis of ecotoxicity data: a guidance to application*. ENV/JM/MONO(2006)18

[21] Please note that this combined classification only applies for mixtures.

4.2 ENDOCRINE DISRUPTION FOR THE ENVIRONMENT

Section 4.2 was developed in close collaboration with EFSA.

Relationship with the ECHA/EFSA ED Guidance on assessing endocrine disrupting properties for biocidal products and plant protection products

The ECHA/EFSA ED Guidance on assessing endocrine disrupting (ED) properties (ECHA/EFSA, 2018), which expands on the '*Revised guidance document 150 on standardised test guidelines for evaluating chemicals for endocrine disruption*' (OECD GD 150; OECD, 2018a), was developed to assist applicants and assessors of the competent regulatory authorities in complying with their obligations to conclude on ED properties for biocidal products (BPs) and plant protection products (PPPs). More specifically, the ECHA/EFSA ED Guidance describes how to gather, evaluate and consider all relevant information for the assessment, apply a weight of evidence (WoE) approach and conduct a mode of action (MoA) analysis, in order to help in establishing whether the substance meets the criteria for approval under the BP[22] and PPP[23] Regulations. Therefore, the ECHA/EFSA ED Guidance remains the key guidance for scientific assessment of ED properties of BPs and PPPs.

In 2023, endocrine disruption was introduced into CLP as a hazard class with two categories. CLP covers classification of hazardous substances and mixtures across regulations and applies (among others) to industrial substances subject to the REACH Regulation[24], BPs and PPPs. Notably, CLP does not require the generation of new data for the purpose of CLP classification and, therefore, ED classification needs to be based on available data. Consequently, the format of the CLP guidance and that of the ECHA/EFSA ED Guidance are different owing to the regulatory framework. For hazard classification purposes this guidance on the application of the CLP criteria should be followed for all substances and mixtures.

Despite differences in the regulatory frameworks, it is important to note that the current ED criteria for BPs and PPPs are derived from the same basis as the ED hazards in Category 1 for human health (ED HH 1) or the environment (ED ENV 1) under the CLP criteria. While the format of this guidance on CLP and the ECHA/EFSA ED Guidance may differ due to the differences in scope of the applicable legislation, the guidance to conclude on ED hazards in Category 1 is largely equivalent and based on a similar scientific assessment in both documents.

Accordingly, active substances already concluded to meet the ED criteria under the BP[25] and PPP[26] procedures before the criteria in CLP became applicable, should under CLP be assigned to ED HH 1 or ED ENV 1. Similarly, substances identified as Substances of Very High Concern (SVHC) under REACH due to ED properties should also be assigned to ED HH 1 or ED ENV 1 under CLP.

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[22] Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products Text with EEA relevance. OJ L 167, 27.6.2012, p. 1–123. Available online: http://data.europa.eu/eli/reg/2012/528/oj

[23] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1–50. Available online: http://data.europa.eu/eli/reg/2009/1107/oj

[24] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. OJ L 396, 30.12.2006, p. 1–849. http://data.europa.eu/eli/reg/2006/1907/oj

[25] Commission Delegated Regulation (EU) 2017/2100 of 4 September 2017 setting out scientific criteria for the determination of endocrine-disrupting properties pursuant to Regulation (EU) No 528/2012 of the European Parliament and Council. OJ L 301, 17.11.2017, p. 1–5. Available online: http://data.europa.eu/eli/reg_del/2017/2100/oj

[26] Commission Regulation (EU) 2018/605 of 19 April 2018 setting out scientific criteria for the determination of endocrine-disrupting properties and amending Annex II to Regulation (EC) 1107/2009. OJ L 101, 20.4.2018, p. 33–36. Available online: http://data.europa.eu/eli/reg/2018/605/oj

4.2.1. Definitions and general considerations for endocrine disruption

The classification for ED ENV, similar to classification for ED HH, refers to a specific (*i.e.* endocrine) MoA which leads to an adverse effect(s). The classification criteria require evidence on three elements, *i.e.*, adverse effect(s), endocrine activity, and a biologically plausible link between the endocrine activity and the adverse effect(s) consistent with existing knowledge.

CLP, Annex I, section *4.2.1.1.* *For the purposes of section 4.2., the following definitions shall apply:*

(a) 'endocrine disruptor' means a substance or a mixture that alters one or more functions of the endocrine system and consequently causes adverse effects in an intact organism, its progeny, populations or subpopulations;

(b) 'endocrine disruption' means the alteration of one or more functions of the endocrine system caused by an endocrine disruptor;

(c) 'endocrine activity' means an interaction with the endocrine system that may result in a response of that system, of target organs or target tissues and that confers on a substance or mixture the potential to alter one or more functions of the endocrine system;

(d) 'adverse effect' means a change in morphology, physiology, growth, development, reproduction or lifespan of an organism, system, population or subpopulation that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences;

(e) 'biologically plausible link' means the correlation between an endocrine activity and an adverse effect, based on biological processes, where the correlation is consistent with existing scientific knowledge.

The definitions in CLP Annex I, section 4.2.1.1. are further explained below:

(a) The definition of '*endocrine disruptor*' (ED) in this guidance is based on the WHO/IPCS definition (WHO/IPCS,2002). It has been modified for the purposes of classification under CLP.

The definition uses the term "*intact organism"* which is understood to mean that the effect would occur *in vivo*, observable in a test animal system. However, it does not necessarily mean that an adverse effect has to be demonstrated in an intact test animal.

The 'endocrine system' in this context consists of hormone-producing tissues and their associated hormones that regulate the functioning of the organism;

(b) An ED may alter one or more functions of the endocrine system, *e.g.* hormonal synthesis, transport, signalling, regulation, or metabolism;

(c) A substance that has an '*endocrine activity*' has the potential to interact with and alter the function(s) of the endocrine system, target organs and tissues. This interaction may occur at any level in a biologically plausible sequence of events leading to an adverse effect;

(d) The definition of '*adverse effect*' is based on the WHO definition (WHO/IPCS, 2009). The definition of adversity is generic and not specific to the assessment of ED properties.

Current practices from other hazard classes for assessing adversity are applicable for deciding whether the observed effects are treatment-related and should be considered adverse;

(e) The '*biologically plausible link*' relies on an understanding of the fundamental biological processes involved and whether they are consistent with the sequence of the events proposed. The term '*correlation*' used in the definition means that endocrine activity and adverse effect(s) can be plausibly linked (connected) using existing knowledge as the most likely explanation for the observed effects; a causal relationship does not need to be proven.

In a MoA analysis, biological plausibility is considered to be the level of support for the links (connections) between the adjacent key events in the postulated MoA, *i.e.* the key event relationships (KERs); see section 4.2.2.3.4.

In addition, data with '*equivalent predictive capacity*' are defined as data obtained using alternative methods which can be used with a similar level of confidence as internationally recognised *in vivo* methods or human data to predict adversity or endocrine activity. Alternative methods do not need to be one-to-one replacements of an internationally recognised *in vivo* method, but can be *e.g.* a set of *in vitro* or *in silico* methods which together meet the requirement of equivalent predictive capacity, see sections 4.2.2.1.1 and 4.2.2.3.1.

CLP, Annex I, *Section 4.2.1.2.1.* *Substances and mixtures fulfilling the criteria of endocrine disruptors for the environment based on evidence referred to in Table 4.2.1 shall be considered to be known, presumed or suspected endocrine disruptors for the environment unless there is evidence conclusively demonstrating that the adverse effects identified are not relevant at the population or subpopulation level.*

More explicitly, substances or mixtures are classified as '*known or presumed*' or as '*suspected*' EDs for the environment if they may (or are suspected to) cause adverse effects in wildlife which have a consequence on the maintenance of the population or subpopulation[27], by altering the function of the endocrine system, *i.e.*, the substance has an endocrine MoA, in accordance with the criteria given in CLP, Annex I, section 4.2.2.1.

CLP, Annex I, section 4.2.1.2.2. *Evidence that is to be considered for classification of substances in accordance with other sections of this Annex may also be used for classification of substances as an endocrine disruptor for the environment where the criteria provided in this section are met.*

In other words, all relevant information for the determination of ED ENV is to be considered together. This includes information also considered in relation to the criteria for aquatic toxicity, information from other aquatic or non-aquatic species (*e.g.* birds, invertebrates) and information related to endocrine disruption for human health (see section 4.2.2.3.5 of this guidance).

[27] The term 'subpopulation' is mainly of relevance to humans where it indicates a subset of the population with distinguishing characteristics (for example children, when they are considered to be the most sensitive subpopulation). However, it may also be used in the environmental context to refer to a subset of the population within an ecosystem in a particular region or habitat with corresponding genetic clustering. The term population will be used in the subsequent sections of this guidance to mean population or subpopulation.

Classification as ED ENV is intended to indicate that a substance may cause an endocrine-related adverse effect. The sensitivity to such effects may depend on the life-stage investigated. Depending on the type of effect some life stages may be more sensitive than others.

In order to classify a substance as ED ENV, the adverse effects need to be relevant at the population level (see section 4.2.2.3.2 on population relevance).

It is sufficient that a substance meets the CLP ED criteria in one taxonomic group in order to conclude that it meets the CLP ED criteria for the environment.

The classification for ED ENV is independent of the classification of other hazard classes, including classification as ED HH. A substance may or may not be classified for ED ENV using the same evidence, irrespective of whether the substance is also classified for other hazard classes.

In addition, the classification of a substance as an ED ENV Category 1 or 2 (or no classification) is independent of the classification of the substance for ED HH 1, ED HH 2 or no classification. Therefore, a classification for ED ENV does not automatically translate into a classification for ED HH and *vice versa*. For example, a substance can be classified as ED ENV 2 or not classified, even if it is classified as ED HH 1.

The concept of ED "*potency*" is considered only in the context of setting specific concentration limits (SCL), see section 4.2.2.6. The CLP criteria for ED ENV do not specify any dose/concentration level above which the occurrence of an adverse effect is considered to be outside the criteria which lead to classification. In other words, even endocrine-related effects observed at high doses/concentrations (showing low potency) may still warrant classification.

The ED effect may be a threshold or a non-threshold effect, see the JRC report on `Thresholds for Endocrine Disrupters and Related Uncertainties´ (Munn and Goumenou, 2013). In the context of CLP, generic or specific concentration limits are required, see section 1.6.3.3.2. As a pragmatic solution to this requirement, SCLs must be set based on observed potency in available studies. When the ED adversity is observed already at very low doses/concentrations (high potency) or alternatively only at very high doses/concentrations (low potency), this guidance considers that potency can be regulated by setting a SCL which can be either lower, or, in exceptional cases, higher than the generic concentration limit (GCL). For setting an SCL, a careful assessment of doses or concentrations causing adversity is recommended for all substances.

ED modalities covered under CLP

The CLP criteria apply to all endocrine modalities. While the CLP criteria do not differentiate between different modalities, thus covering all endocrine-disrupting MoAs, it is acknowledged that this guidance mainly addresses the effects caused by estrogen, androgen, thyroid, and steroidogenic (EATS) modalities.

This is because the EATS modalities are the pathways for which there is currently the most knowledge available, *i.e.*, there is relatively good mechanistic understanding on how substance-induced perturbations may lead to adverse effects via an endocrine-disrupting MoA. In addition, there are at present only for the EATS modalities internationally accepted standard methods or test guidelines for *in vivo* (EATS) and *in vitro* (EAS) testing available where there is broad scientific agreement on the interpretation of the effects observed on the investigated parameters. Further information on EATS modalities can be found in section 4.2.2.3.1.

However, the general principles outlined in this guidance for evaluation of the data on the different criteria, WoE and decision on classification, are also applicable to non-EATS modalities. The existing knowledge for those modalities is not as advanced as that for the EATS modalities and future research is needed for a better understanding of non-EATS modalities. However, in some

cases it may be possible to reach a conclusion on the need to classify the substance based on a non-EATS MoA, for example, where scientific knowledge provides mechanistic information, that can be linked to adverse effects measured in standard tests. One example is related to effects on fecundity that could potentially occur also due to inhibition of retinoic acid. Other examples of non-EATS modalities can involve *e.g.* juvenile hormones, ecdysone or peroxisome proliferator-activated receptor-gamma (PPARγ) related endocrine disruption. It should be noted that ligands to some of these receptors (*e.g.* retinoids binding to the retinoic acid receptor, fatty acids binding to PPARγ) may not fit the conventional view of a hormone. Nonetheless, these ligands do fit the broad definition of a hormone as a substance, originating in one tissue and conveyed by the bloodstream to another tissue to exert physiological activity (OECD, 2012).

4.2.1.1. Taxa covered

Based on the current knowledge and understanding of the endocrine system as well as on the available testing methods, the current guidance, in line with the ECHA/EFSA ED Guidance, focuses on vertebrate organisms, mainly fish and amphibians. For other vertebrate taxa (besides mammals), like birds and reptiles, there are, currently, no standard methods which investigate endocrine specific endpoints. Similarly, due to the scarce knowledge on the endocrinology for invertebrates, this guidance does not specifically cover those organisms.

Nevertheless, the general principles outlined in this guidance for evaluation of the data on the different criteria, WoE and decision on classification, are also applicable to those organisms. Therefore, if available, information on invertebrates, birds and reptiles should be assessed and can be used to conclude on the need to classify the substance as ED ENV.

Data and effects on plants are not under the scope of this hazard class.

4.2.2. Classification of substances for endocrine disruption for the environment

4.2.2.1. Identification of hazard information

CLP itself does not set information requirements or require further testing of substances and mixtures for classification purposes (CLP Articles 5, 6 and 9) except for physical hazards (CLP Article 8 (2)). The assessment is based on the respective criteria and consideration of all available relevant information. All relevant information that addresses endocrine-related adverse effects and activities must be considered in a WoE approach; this includes guideline and research studies as well as alternative methods such as read-across and *in silico* predictions.

The main ways to gather all available information are by collecting studies and data from the registration dossiers, *e.g.* under REACH, BPR, PPPR, and conducting a literature search or a systematic literature review designed *inter alia* to avoid bias and capture as much as possible relevant scientific literature data. Further guidance is available in the ECHA/EFSA ED Guidance, section 3.2 and Appendix F. Also, previous regulatory assessments may serve as a starting point for additional literature search. Furthermore, information considered for other hazard classes may provide information relevant for ED ENV classification (see sections 3.6.2.1; 3.7.2.1; 3.9.2.1; 4.2.2.1).

Upon reviewing the literature, the information is deemed relevant when it investigates or brings information for the assessment of at least one of the three elements: *i.e.* endocrine activity, adverse effects or a biologically plausible link:

- Information on endocrine-related '*adverse effects'* for the environment is normally obtained from animal chronic studies. However, when available, non-animal methods or strategies (if providing an equivalent predictive capacity as animal data) may bring sufficient information on adversity for decision making on classification, particularly when supported by toxicokinetic data. Information on adversity may also be obtained using read-across or analogy, *e.g.* if the substances by analogy share a common MoA, or using read-across e.g. between substances with a common active metabolite/degradation product, or compounds with a different ratio of the same isomers, etc.

- Information on '*endocrine activity'* generally comes from *in vivo* or *in vitro* mechanistic studies. Information may also come from read-across, *in silico* models or omics approaches, if available. In addition, endocrine activity may also be inferred from observed adverse effects known to be mediated by endocrine activity, see '*EATS-mediated*' parameters in section 4.2.2.3.1.

- A *'biologically plausible link'* does not need to be demonstrated with substance specific data, but existing scientific knowledge can be used, *e.g.* textbooks and peer-reviewed scientific literature. Adverse Outcome Pathways (AOPs) can be helpful to establish biological plausibility, but they are not a prerequisite. Several AOPs related to endocrine disruption have been endorsed, see *e.g.* OECD Series on AOPs[28]. There are additional AOPs at various stages of development in the collaborative AOP-wiki (AOP-wiki)[29]. The presence of an AOP in the AOP-wiki does not necessarily indicate its relevance or reliability. Depending on the stage of development of the AOP in AOP-wiki, the amount of data needed to support biological plausibility may vary considerably. The validity of an AOP for a given substance should be considered using expert judgement.

4.2.2.1.1. Identification of animal data

Information considered for other hazard classes *e.g.* hazardous to aquatic environment, (section 4.1 of this guidance) as well as information relevant for ED HH (section 3.11 of this guidance) and information on birds, reptiles, or invertebrates may also provide information relevant for endocrine disruption for the environment.

All relevant information that addresses endocrine-related adverse effects and activities must be considered in a WoE approach; this includes guideline and research studies as well as alternative methods such as read-across and *in silico* predictions.

Animal studies to be considered for classification of substances as ED ENV are outlined in the OECD GD 150 '*Revised guidance document on standardised test guidelines for evaluating substances for endocrine disruption'*. This document provides widely accepted guidance on the interpretation of effects measured in relevant OECD test guidelines, and other standardised test methods, which may arise as a consequence of perturbations of the EATS modalities. It explains how these effects may be evaluated to support identification of EDs.

The OECD GD 150 includes the '*OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Substances*' (OECD CF; OECD, 2012) which lists the OECD test guidelines and standardised test methods available in 2018 that can be used to evaluate substances for endocrine disruption. It is not an exhaustive list and tests and assays other than those described in the list (*i.e.* other published or internationally recognised methods) may also be valuable for assessing substances for endocrine disruption and can also be used for classification if they are

[28] https://www.oecd-ilibrary.org/environment/oecd-series-on-adverse-outcome-pathways_2415170x

[29] aopwiki.org

relevant and considered predictive for wildlife. Research studies are an important source of information which must be considered in a WoE approach. New tests are continually being developed, which may provide useful information for classification. In particular, endpoints for non-EATS modalities are currently not well covered in the OECD test guidelines.

New approach methodologies (NAMs)

New approach methodologies (NAMs, *e.g. in vitro-, in silico- and omics*-methods; testing strategies; defined approaches, etc.) can be used to provide information about adverse effects or endocrine activity if they provide equivalent predictive capacity as animal data from internationally recognised *in vivo* methods or human data. OECD-validated NAMs or internationally recognised methods, if available, may be more relevant than non-validated methods. When the NAMs provide sufficient information on adverse effect(s) or endocrine activity, they can be used for classification purposes.

4.2.2.2. Classification criteria

The criteria for the classification of substances for ED ENV in CLP are given in the following table.

CLP, Annex I, *4.2.2.1. Hazard categories*

For the purpose of classification for endocrine disruption for the environment, substances shall be allocated to one of two categories.

Table 4.2.1

Hazard categories for endocrine disruptors for the environment

Categories	*Criteria*
CATEGORY 1	*Known or presumed endocrine disruptors for the environment* *The classification in Category 1 shall be largely based on evidence from at least one of the following:* *a) animal data;* *b) non-animal data providing an equivalent predictive capacity as data in point a.* *Such data shall provide evidence that the substance meets all the following criteria:* *(a) endocrine activity;* *(b) an adverse effect in an intact organism or its offspring or future generations;* *(c) a biologically plausible link between the endocrine activity and the adverse effect.* *However, where there is information that raises serious doubt about the relevance of the adverse effects identified at population or subpopulation level, classification in Category 2 may be more appropriate.*
CATEGORY 2	*Suspected endocrine disruptors for the environment*

	A substance shall be classified in Category 2 where all the following criteria are met: *(a) there is evidence of:* *i. an endocrine activity; and* *ii. an adverse effect in an intact organism or its offspring or future generations;* *(b) the evidence referred to in point (a) is not sufficiently convincing to classify the substance in Category 1;* *(c) there is evidence of a biologically plausible link between the endocrine activity and the adverse effect.*

The classification in Category 2 is also largely based on evidence from animal and non-animal data as described for Category 1. Where there is evidence conclusively demonstrating that the adverse effects are not relevant at the population level, the substance should not be considered an ED for the environment (see section 4.2.2.3.2).

4.2.2.2.1. Classification in the presence of other toxic effects

CLP, Annex I, section *4.2.2.2.2.* *Adverse effects that are solely non-specific consequences of other toxic effects shall not be considered for the identification of a substance as endocrine disruptor for the environment.*

'*Other toxic effects'* refers to (adverse) effect(s) other than the endocrine-related adverse effect(s). If a substance causes endocrine-related adverse effect(s) which occur as a consequence of other toxic effects, classification for ED ENV should be applied unless the effect(s) are demonstrated to be '*solely non-specific consequences of the other toxic effects'*. A '*non-specific consequence of the other toxic effects'* is understood as:

- an endocrine-related adverse effect that is conclusively demonstrated to occur secondary to excessive toxicity *i.e.* the co-occurring toxicity is so severe that the animals are suffering[30] or dying.

In principle, ED related adverse effect(s) seen only at very high dose/concentration levels in animal studies (for example doses/concentrations that induce severe suffering, excessive mortality) without being the consequence of an endocrine activity would not normally lead to classification. In all other cases, it needs to be demonstrated on a case-by-case assessment that ED related adverse effect(s) are solely a non-specific consequence of other toxic effects to be able to justify no classification.

Analyses of concentration/dose and temporal concordance between ED and other severe toxicity are important to assess if the endocrine system is out of balance solely due to a non-specific consequence of other toxic effects. However, the presence of other toxic effects or stress must not be used to dismiss classification unless it can be unequivocally demonstrated that the potentially endocrine-related adverse effects are solely non-specific consequences of other toxic effects. In reality, this may be difficult to demonstrate and therefore, dismissing the substance from ED classification (when there is evidence of endocrine activity, and adverse effect and a biologically plausible link between the two) may in many cases be done only when the other co-occurring toxic effects are so severe that the animals are suffering or dying. In this respect, it is

[30] Examples of suffering include lethargy, observations that the animals stay on the bottom of the tank, or lie motionless.

important to evaluate carefully the life stages affected by mortality or suffering. If, for example, post-hatch mortality is observed in the F1 generation of a multi-generation study, this should not call into question potential ED-related effects in the parent F0 generation, in the absence of mortality or suffering in adults of the F0 generation.

To consider an ED-related adverse effect solely as a non-specific consequence of other toxic effects, there must be evidence for a biologically plausible sequence of events demonstrating that it is solely other toxic effects that causes the adverse effect, and which also excludes the endocrine MoA as the most likely cause for the observed adverse effect(s). Therefore, in such a case, data is needed to demonstrate the non-ED MoA induced by other toxic effects and the assessment is best done by a comparative MoA assessment. For further guidance on how to conduct a comparative MoA analysis, see *e.g.* Meek *et al.* 2014a and 2014b.

When assessing the potential influence of co-occurring other toxic effects to the concurrent endocrine-related adverse effect(s) in animals, the empirical support needs to be evaluated carefully. In particular, it may be helpful to assess the different types of effects observed and evaluate the temporal and dose/concentration-concordance between the potential mechanisms and the different types of effects observed. It must be noted that in the case of non-mammalian data, the empirical support will be mainly based on the evaluation of the dose/concentration-response relationship due to the available data set not often allowing for the evaluation of the temporal concordance and consistency among species (often only studies on a single species are available).

To consider that the potential endocrine-related effect(s) are a consequence of the other toxic effects, the other (potentially excessive) toxic effect(s) should precede the endocrine-related effect(s) in time (the other toxic effects should precede or co-occur with the endocrine-related effects) or should occur at lower or same dose/concentration levels as endocrine–related effects. An evaluation of the appropriateness of the dose/concentration spacing may also help to assess if the effects are solely the non-specific consequence of other toxic effects.

However, these cases should be evaluated on a case-by-case basis taking into consideration aspects such as the dose/concentration-response in the endocrine-related adverse effects, the severity of the other toxic effects observed and whether the potential endocrine-related effects are only observed at the same doses/concentrations where other toxic effects are observed. Aspects such as analogy with other substances, the overall (eco) toxicological data package suggesting a specific non-endocrine MoA etc, may be considered to substantiate that the potential endocrine-related adverse effects are most likely the non-specific consequence of other toxic effects.

Considering the complexity of the endocrine system, the conclusion that a certain adverse effect is a non-specific consequence of other toxic effects needs to be assessed carefully and on a case-by-case basis, considering the full picture and pattern of effects.

4.2.2.2.2. Relevant concentrations for classification

The interpretation of adverse effects observed at certain concentrations or at certain levels of toxicity should not be confused with the top dose/concentration to be used in animal studies. The former pertains to the evaluation of existing data, while the latter refers to the selection of the doses/concentrations when performing a study.

Test guidelines specify the approach to determine the highest test dose/concentration to be tested. The top dose/concentration selected for the ecotoxicological studies should provide information on substance toxicity at an exposure to the tested agent that should be compatible with animal survival and permits data interpretation in the context of the use of the study. In ecotoxicology, this can be assessed by using the concept of the maximum tolerated concentration

(MTC). For tests on aquatic organisms, the maximum concentration defined through a range finding test or from other toxicity data, or the limit concentration as defined in the relevant OECD guidelines, should be considered to establish this value.

The MTC should not be confused with a demarcation or a concentration, above which the results are not relevant for classification purposes or with a justification that the effect is '*solely a non-specific consequence of other toxic effects'*. Although a MTC is aimed at when performing an ecotoxicological study to investigate the endocrine-related adverse effect of a substance, endocrine-related adverse effects are not relevant for classification only when they are the non-specific consequence of other toxic effects (see section 4.2.2.2.1).

There are no generic dose/concentration or toxicity levels that can be used as universal demarcation limits for such effects.

4.2.2.3. Evaluation of hazard information

Appropriate classification for endocrine disruption will always depend on an integrated assessment of all relevant available data using a WoE approach. This includes positive and negative data from all relevant sources of information, see section 4.2.2.1. Datasets should be analysed using WoE and expert judgment and the combined, weighted outcome compared with the CLP criteria.

4.2.2.3.1. Evaluation of data on adverse effect(s)

Data on adverse effect(s) are considered similarly to the respective sections of this guidance on the hazard to the aquatic compartment. All parameters related to effects on reproduction (*e.g.* fertility, fecundity, etc.) in the case of EAS modalities, on development/growth (hindlimb length, developmental stage, time to metamorphosis) for the T modality, and behavioural effects that are considered to be population relevant, must be considered in the assessment of adversity (see Tables 15 and 16 of ECHA/EFSA ED Guidance). It should be highlighted that some individual parameters may not be considered adverse in isolation. In any case, the conclusion on adversity relies on a combination of parameters and the observation of a pattern of effects across studies. Information on other toxic effects must also be considered in the assessment of adverse effect(s).

OECD GD 150 provides guidance on how to interpret parameters normally investigated in (eco)toxicity studies, also applied in ECHA/EFSA ED Guidance. OECD GD 150 differentiates between:

- '*EATS-mediated*' parameters, which are parameters measured *in vivo* that contribute to the evaluation of adversity, while at the same time (due to the nature of the effect and the existing knowledge as described in OECD GD 150, section B2) they are also considered indicative of an EATS MoA and therefore (in the absence of other explanations) also infer an underlying *in vivo* mechanism. This group includes the parameters mainly labelled in OECD GD 150 as 'endpoints for estrogen-mediated activity', 'endpoints for androgen-mediated activity', 'endpoints for thyroid-related activity' and/or 'endpoints for steroidogenesis-related activity'. Examples of these parameters for environment are sex ratio and some changes in gonad histology[31];

- '*Sensitive to, but not diagnostic of, EATS*' parameters measured *in vivo* that contribute to the evaluation of adverse effect(s). Due to the nature of the effect and the existing knowledge, these effects cannot be considered diagnostic on their own of any of the EATS

[31] More detailed guidance on specific gonad histopathology examination in fish is given in OECD (2010a).

modalities. Nevertheless, in the absence of more diagnostic parameters, these effects can indicate an endocrine MoA and be relevant for classification, if they are accompanied with evidence of endocrine activity and the biologically plausible link between the endocrine activity and the observed adverse effect. Examples of these parameters for the environment are fecundity, growth, hatching success, behaviour (*e.g.* stickleback nesting, courtship, mating, aggressiveness).

All parameters reported in OECD GD 150 are considered relevant to support ED-related adverse effects. They are mainly derived from guideline studies, *i.e.* standardised test methods validated for regulatory decision making (*e.g.* EU test methods/OECD test guidelines or United States Environmental Protection Agency (US EPA)/Food and Drug Administration (FDA) test guidelines).

In addition to results from guideline studies, results from well-performed and reported studies other than those listed in OECD GD 150 may also include '*EATS-mediated*', '*Sensitive to, but not diagnostic of, EATS*' or '*non-EATS*' parameters which may provide relevant information. Therefore, the data used to classify a substance can be drawn from standard studies or other scientific data, *e.g.* peer reviewed literature studies, Q(SAR) data, internationally recognised databases of *in vitro* high-throughput chemical screening. All relevant data needs to be evaluated carefully in a WoE approach (see Section 4.2.2.3.5).

In case NAMs provide data with equivalent predictive capacity as animal data, they can be used to provide sufficient data for adverse effect(s) for classification.

Furthermore, read-across or analogy can also be used to provide information about adversity, *e.g.* if the substances share a common MoA or induce similar adverse effects. When using data from another substance, potential differences in toxicokinetics and toxicodynamics should be considered.

For further details see ECHA/EFSA ED Guidance - Tables 15 and 16 are useful as they show the assignment of '*EATS-mediated*' parameters, and '*Sensitive to, but not diagnostic of, EATS*' parameters from the most common test guidelines, see also Table B.1 in OECD GD 150.

4.2.2.3.2. Population relevance

CLP, Annex 1, section: ***4.2.1.2.1.*** *Substances and mixtures fulfilling the criteria of endocrine disruptors for the environment based on evidence referred to in Table 4.2.1 shall be considered to be known, presumed or suspected endocrine disruptors for the environment unless there is evidence conclusively demonstrating that the adverse effects identified are not relevant at the population or subpopulation level.*

CLP, Annex 1, section: ***4.2.2.1****. Where there is evidence conclusively demonstrating that the adverse effects identified are not relevant at the population or subpopulation level, the substance shall not be considered an endocrine disruptor for the environment.*

The criteria stipulate that substances and mixtures fulfilling the criteria must be considered as ED ENV unless there is evidence conclusively demonstrating that the adverse effects identified are not relevant at the population level. The criteria also stipulate that only when there is evidence conclusively demonstrating that the adverse effects are not relevant at the population level, the substance must not be considered an ED ENV.

In applying the WoE approach, the assessment of the scientific evidence must consider if the adverse effects identified may impact the maintenance of wildlife populations in terms of abundance/biomass and in terms of structure. This consideration is in line with the general level of protection in ecotoxicology where the entity to be protected is the population of wildlife. If data from multiple species are available, the population relevance of the observed adverse effect should be assessed taxon by taxon.

When assessing the effects observed in the available (eco)toxicological studies, relevant parameters for the effects on wildlife are those parameters expected to show adverse effects on the population in the environment. Effects on growth (body weight and length), development and reproduction (such as fecundity, fertility, sex ratio, hatching success and offspring survival) in single species in laboratory studies are generally regarded relevant for the maintenance of the wild population (European Commission, 2011; Marty *et al.*, 2017). Effects observed in toxicity studies conducted in the laboratory, in some circumstances, may be even more severe in the field where animals need also to cope with additional stressors, *e.g.* predation, food availability, etc. Therefore, when effects are observed in those parameters the relevance at the level of population is inferred unless the contrary is proven.

Behavioural changes and impaired ability to cope with additional stress are factors implicitly covered by the definition of adverse effect(s), since they could affect development and reproductive performance, and hence impact the population stability. Therefore, they should be considered relevant at the population level (Agerstrand *et al.*, 2020). However, it is acknowledged that current standard tests are not specifically designed to capture all behavioural effects (European Commission, 2011) and the ability to cope with stressors.

Effects in reproductive organs (*e.g.* gonads) are considered as population relevant since they are expected to have a direct impact on reproduction. On the other hand, effects in non-reproductive organs, can be considered as relevant at the population level on a case-by-case basis, *e.g.* when accompanied by a pattern of effects which are considered to be all related to the same MoA. If effects in non-reproductive organs are the only effects available in the data package for the substance, and apical effects were not investigated, the population relevance of those effects cannot be excluded.

When evaluating mammalian data to reach a conclusion on the classification for the environment, further consideration is needed to evaluate whether some ED-related adverse effects observed in mammals can be considered adverse for mammals as wildlife species at the level of population. Regarding adverse effects in mammalian species, it has to be noted that the entity to be protected in mammalian toxicology is the individual organism, while for wild mammals the entity to be protected is the population. This means that, although to conclude on wild mammals the same dataset is used as the one used to conclude on human health, each effect and parameter must be considered from a different perspective, *i.e.* relevance of the effect observed for wild mammal populations. Therefore, in the evaluation of the ED potential in mammals, the assessment for human health may consider changes at organ level as adverse, but these may or may not impact the maintenance of the population. Thus, it is recommended that effects at organ level should be considered in a WoE approach together with any other effects observed which could be related to the same pattern of effects.

It should be noted that effects observed in rodents are of high concern for wildlife species with a natural low reproductive output, including top predators and other mammals (including endangered species), as negative effects on reproduction have an even higher potential for causing long term negative effects at the population level for such taxa.

Effects on growth, development and reproduction should generally be regarded relevant for the maintenance of the wild population if they are statistically significant compared to the controls. However, the lack of statistical significance should not be the only reason for concluding a lack of

treatment-related effect. Biological relevance[32] should also be considered when assessing the presence/absence of treatment related effects, particularly in situations where an effect is observed which may not necessarily be statistically significant. This, for example, could happen in the case of screening studies which are known to have a limited statistical power. Besides the two aspects mentioned above (statistical significance and biological relevance), the overall dataset should be carefully considered to understand whether a pattern of effects is observed. If a pattern of effects is observed, changes observed below a certain magnitude, which in isolation would not be considered relevant, could still contribute to the assessment of adversity, if considered to be part of the pattern identified.

When evaluating mammalian data to reach a conclusion on the classification on wild mammals, although the same dataset is used as the one used to conclude on human health, as indicated above, each effect and parameter must be considered from a different perspective. Therefore, in the evaluation of the ED potential in mammals, the assessment for human health may consider as adverse changes observed with very low incidence but considered severe enough to establish the adverse effect(s) (*e.g.* tumours). Those effects should be regarded as of population relevance unless it can be argued that the effects are not relevant for wild animal populations, *e.g.* as they are not expected to occur at a high enough prevalence in a population to impact population survival/maintenance.

Future developments in the field of effect and population models may be considered as valuable tools in better understanding the population relevance of the observed adverse effects. The advancement of novel techniques such as AOPs and population modelling may facilitate improved understanding of the connection between disruptions in endocrine systems at the lower levels of biological organization and their repercussions at the population level.

Specific considerations related to the thyroid modality - mammals

As explained above, when evaluating mammalian data to reach a conclusion on the classification for the environment, further consideration is needed to evaluate whether some ED-related adverse effects observed in mammals can be considered adverse for mammals as wildlife species at the level of population. In the case of the thyroid modality, as for the other modalities, the thyroid endpoints are not looked at in isolation, but the whole data package is evaluated holistically. In particular, organ level endpoints should be assessed using a WoE approach considering apical endpoints in the dataset. The level of effect (as to population relevance) and the type/directionality of the effect (as to whether this matches the proposed MoA) should be specifically addressed. Therefore, to reach a conclusion on the need to classify the substance, it may be necessary to reconsider the mammalian data package to further understand whether there are other effects which may be due to the same ED MoA and can further support the conclusion on population relevance. For example, thyroid histopathological findings observed in rats are relevant at population level if observed together with impairment of growth/development and/or reproduction or with support of other data in a WoE approach.

However, if the data package does not contain information on other effects potentially related to the same MoA because those were not investigated, it cannot be excluded that thyroid histopathological findings observed in the rats are of population relevance.

Specific considerations related to the thyroid modality - non-mammalian organisms

[32] Guidance on biological relevance can be found in the Scientific opinion of the EFSA Scientific Committee on "Statistical Significance and Biological Relevance", EFSA Journal 2011: 9(9): 2372 and in the Guidance on the assessment of the biological relevance of data in scientific assessments, EFSA Journal 2017. Doi: 10.2903/j.efsa.2017.4970

In the case of amphibians, normally apical endpoints are investigated together with thyroid histopathology. Therefore, in such situation changes in thyroid histopathology are considered adverse at the population level when observed together with effects on development (*e.g.* accelerated or asynchronous). Population relevance can be excluded only if thyroid histopathological changes are observed and development was investigated, but no concomitant effects were observed, provided that the power of the test is sufficient to elicit an effect. This is because thyroid histopathology often exhibits compensation to thyroid insufficiency (Marty *et al*., 2017). In rare cases the thyroid histology may be the only endpoint assessed, and therefore case-by-case consideration should be made to come to a conclusion on the relevance of that effect at the population level. Nevertheless, changes in development in amphibians, even if observed in the absence of investigation of thyroid histopathology, are considered population relevant effects.

Several potential endpoints for disruption of the Hypothalamic-Pituitary-Thyroid (HPT) axis in fish have been described in the scientific literature, such as thyroid histopathology, thyroid hormone levels, gene expression, swim bladder development and inflation, neurodevelopment, eye development, behaviour. There are also several AOPs under development where histopathology of the thyroid is linked to adverse and population relevant effects also in fish, *e.g.* eye development (AOP-wiki 363) and swim bladder inflation (AOP-wiki 156, 158, 159). Although it is acknowledged that those endpoints are currently not included in standard fish tests, future developments in this field may provide a better understanding of the population relevance of the observed adverse effects.

CLP, Annex 1, section *4.2.2.1*. *(Table 4.2.1) However, where there is information that raises serious doubt about the relevance of the adverse effects identified at population or subpopulation level, classification in Category 2 may be more appropriate.*

According to the criteria, classification as Category 2 may be more appropriate when effects are observed, either in mammalian or non-mammalian species, but there are serious doubts that those effects would be relevant at the population level, *i.e.* that the observed effects would impede the maintenance of the population. This conclusion needs to be taken with caution using a WoE approach.

4.2.2.3.3. Evaluation of endocrine activity

In terms of endocrine activity, the OECD GD 150 differentiates between:

- *In vitro* mechanistic – parameters measured *in vitro*, that provide information on the mechanism through which a substance could be considered endocrine active (*e.g.* by binding to and activating a receptor or interfering with specific enzymes in endocrine pathways);

- *In vivo* mechanistic – parameters measured in vivo that provide information on endocrine activity that are usually not considered adverse per se. Changes in sex hormone levels are generally considered *in vivo* mechanistic. An example of these parameters for environment is vitellogenin (VTG).

  As described in section 4.2.2.3.1 above, '*EATS-mediated*' parameters are also considered indicative of an EATS MoA and thus, (in the absence of other explanations) also infer an underlying *in vivo* mechanism;

- *In silico* approaches as described in section 4.2.2.3.3.2 also inform on endocrine activity. The applicability domain of the models should be considered.

4.2.2.3.3.1. *In vitro* data

Currently, there are only a few standardised *in vitro* assays based on non-mammalian receptors and/or enzymes. However, since the endocrine system is known to be conserved across vertebrates, *in vitro* assays with mammalian cells can be used in a WoE approach to give indications on possible MIEs or interference with a certain pathway also for non-mammalian species, see further information in section 3.11.2.3.2.1. Moreover, the OECD GD 150 clearly indicates that: "*The in vitro screens in question (although at present based largely on mammalian receptors and/or enzymes) are generally capable of providing information applicable to both humans and vertebrate wildlife (OECD, 2010b). Such extrapolation of in vitro information is generally qualitative (...)*".

The *in vitro* tests, when used in isolation, lack the complexity of an intact organism. Single assays often identify if a substance is capable of binding to a receptor or interfering with a pathway. Particular attention should be given to *in vitro* data and the considerations of absorption, distribution, metabolism, excretion (ADME) properties which may not be covered by current *in vitro* test guidelines *e.g.* those measuring protein binding or disruption of endocrine pathways. Therefore, when interpreting the results of *in vitro* tests, the possible lack of a metabolising capacity or competence of the system, as well as the possible lack of consideration of other ADME properties, should be considered. To partly overcome this limitation, metabolism may be addressed by adding (part of the) metabolising systems to the test system, or test data on metabolites of the substance could be directly used. Results from a battery of *in vitro* tests for substances that are not metabolised *in vivo* may in some cases be conclusive on endocrine activity. Similarly, data may be conclusive if both the parent substance and the metabolites are tested. Therefore, all mechanistic information should be considered together to reach a conclusion on endocrine activity.

Most of the current available *in vitro* assays focus on specific interactions of substances with cellular components, such as nuclear hormone receptors or enzymes in specific pathways (*e.g.* aromatase). However not all endocrine related adverse effects are mediated through a direct action on these molecules. Additionally, compounds might act via more than one mechanism and some pathways, that might be potentially causing an ED adverse effect *in vivo*, might not be covered by the currently available *in vitro* assays. Overall, no single test can be expected to detect all types of endocrine activity. To partially overcome this limitation, several *in vitro* tests investigating different points of perturbation of endocrine pathways should be assessed together. However, the eventual ED effect *in vivo* might be a consequence of disturbance of several pathways simultaneously, some of which might not be covered by available *in vitro* tests.

The capacity of organisms to compensate for a certain level of changes in hormonal regulation may not yet be assessable in an *in vitro* system. Furthermore, the applicability domain as well as overall validity and reliability of *in vitro* tests must be considered. A negative single *in vitro* result alone cannot be used to exclude endocrine activity.

Therefore, conclusions on the endocrine activity of a substance can only be drawn in the context of what the respective *in vitro* assays were developed to evaluate (*e.g.* receptor binding, enzyme inhibition) taking into consideration any identified limitations.

4.2.2.3.3.2. *In silico* data

In silico predictions may be used as supporting information for endocrine modalities within a WoE approach. The different types of *in silico* prediction methods can be grouped as: molecular modelling of receptor interactions, (Q)SAR modelling and other events, and profilers based on structural alerts and decision trees; for further details see section 4.1 of the ECHA/EFSA ED Guidance. QSAR predictions may also support read-across.

The evidence from *in silico* predictions is strengthened if the same result is obtained with independent *in silico* models. Whenever *in silico* methods are used, the general provisions outlined in ECHA Guidance on IRs & CSA, Chapter R.6: QSARs and grouping of chemicals (ECHA, 2008) and *'(Q)SAR Assessment Framework'* (OECD, 2023) should be followed. Attention should be paid to the interpretation of results to understand the specific basis and scope of the prediction for each endocrine pathway, considering the performance and the applicability domain of each *in silico* predictive model when drawing conclusions.

4.2.2.3.3.3. *In vivo* data

In vivo studies also provide information on endocrine activity. '*EATS-mediated*' adverse effects infer an underlying *in vivo* mechanism which should be used for the identification of the endocrine activity; see section 4.2.2.3.1. The OECD GD 150 also lists assays providing *in vivo* mechanistic information. However, *in vivo* mechanistic data have some limitations, and the applicability domain should be carefully assessed. For further details, see ECHA/EFSA ED Guidance.

4.2.2.3.4. Mode of action analysis and evaluation of biological plausibility

CLP, Annex I, Section *4.2.1.1.* *"biologically plausible link" means the correlation between an endocrine activity and an adverse effect, based on biological processes, where the correlation is consistent with existing scientific knowledge.*

Guidance on how to postulate and conclude on MoA(s), assess the biological plausibility of a link between endocrine activity and adverse effects as well as to identify which further information could help to clarify the postulated MoA(s), is provided in section 3.5 of the ECHA/EFSA ED Guidance.

When potential endocrine-related adverse effect(s) and endocrine activity are identified, the link between the two must be established and justified based on biological plausibility, according to the CLP ED criteria. To conclude on the biological plausibility of the link, it may not be necessary to have demonstrated the whole sequence of events leading to the adverse effect. Existing knowledge from, *e.g.* endocrinology and/or (eco)toxicology, may be sufficient to conclude on the biological plausibility of the link between adverse effects and the endocrine activity.

Biological plausibility may be demonstrated by conducting a MoA analysis, which must be determined in the light of current scientific knowledge using all available relevant information in a WoE approach. For classification purposes, knowledge and demonstration of the full MoA is not a requirement. The MoA analysis should aim at establishing biological plausibility based on the consistency and coherence of the responses obtained on measured parameters with a postulated MoA.

The level of information required for a MoA analysis varies depending on which parameters are adversely affected.

For example, '*EATS-mediated*' adversity is considered indicative of an EATS MoA and, thus, also infers an underlying *in vivo* mechanism (in the absence of other explanations). In such cases, the analysis of the biological plausibility may draw conclusions from the broader scientific knowledge. Therefore, less information would be required for a MoA analysis and without recourse to a detailed MoA analysis compared to adversity based on other parameters, *i.e.,* the MoA analysis can be very simple. This is because there is a biologically plausible link between the adverse effect and endocrine activity in an EATS modality which is the most likely explanation of the effects observed. Therefore, in the absence of other explanations, *i.e.* an alternative MoA considered as a more likely explanation, an ED MoA can be considered plausible.

This contrasts with adversity based on '*sensitive to but not diagnostic of EATS*' and '*non-EATS mediated*' parameters where more evidence is needed to support that a substance affects one or more KEs in the postulated MoA. In this case, the conclusion will depend on the degree of evidence provided for the key events (KEs) in the postulated MoA.

As with all assessments, a consistent pattern of effects strengthens the empirical support for KEs of the postulated MoA. The final WoE conclusion must consider all available data.

Mode of action analysis

A MoA can be described as a series of biological events, *i.e.*, KEs that lead to a specific adverse effect. The first KE in the series is referred to as the molecular initiating event (MIE), see Figure 4-2.1.

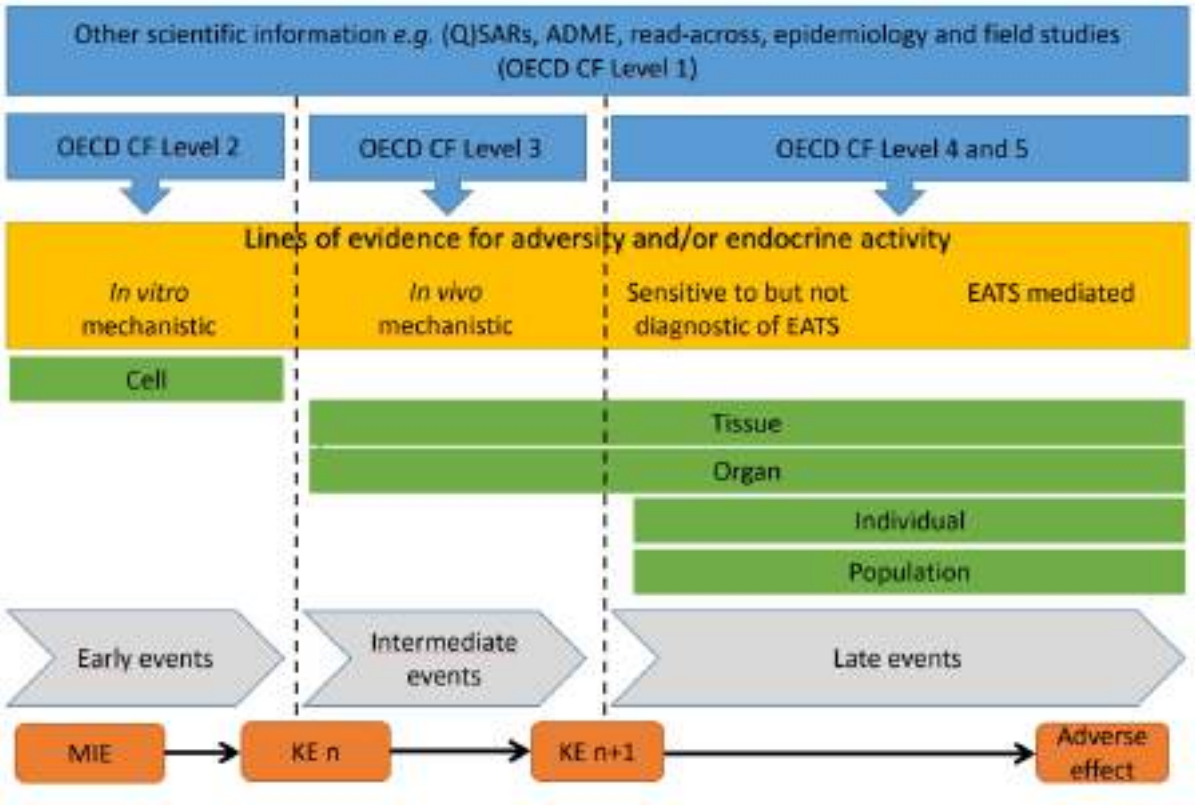
This guidance uses AOP terminology for the MoA analysis. However, this does not imply that the AOP approach must be used for the MoA analysis.

An endocrine MoA means that the adverse effect is mediated through an alteration of one or more functions of the endocrine system, *e.g.* hormonal synthesis, transport, signalling, regulation, or metabolism, *i.e.*, it is not only mediated via hormone-receptor interactions. Normally, an endocrine MoA contains some earlier KEs (which provide mechanistic information at the molecular or cellular level concerning endocrine activity) and some later KEs (which provide information at the organ or system level, including the adverse effect).

This sequence includes at least one endocrine-mediated KE which may or may not also be adverse (see ECHA/EFSA ED Guidance); *e.g.* the MIE does not need to be known or endocrine related. KEs are events that are considered essential to the induction of the (eco)toxicological response as outlined in the postulated MoA and they can represent either endocrine activity or adversity. KEs are empirically observable and measurable steps and can be placed at different levels of biological organisation (at cell, tissue, organ, and individual or population level); see Figure 4.2-1. To support the plausibility of a KE, there needs to be experimental data in which the event is characterised and consistently measured or existing knowledge on which basis the event is understood. KEs are interconnected and this linkage is termed a key event relationship (KER).

Figure 4.2.1 Scheme illustrating how the evidence can be organised to support the postulated mode of action. The arrows linking KEs represent the KE relationships. It should be noted that

the borders between the different OECD CF levels are not absolute in terms of parameters measured and in their contribution to the WoE.



KE: key event; MIE: molecular initiating event.

CLP, Annex I, section *4.2.2.3.3.* *Using a weight of evidence determination, the link between the endocrine activity and the adverse effects shall be established based on biological plausibility, which shall be determined in light of available scientific knowledge. The biologically plausible link does not need to be demonstrated with substance specific data.*

Evaluation of the biological plausible link between an endocrine activity and an adverse effect

The first step in assessing biological plausibility is to gather information from scientific literature / existing knowledge on possible endocrine-related MoAs that are related to the types of adverse effects and endocrine activity observed for the substance or related substances subject to classification (see further details in section 3.11.2.1 of Part 3). The evidence available for the substance subject to classification must be assessed against the hypothesis for MoA with its KEs to be able to conclude on a biologically plausible link between the observed endocrine activity and adverse effect(s).

The conclusion on biological plausibility is based on whether the included KERs are consistent with the general knowledge of biology and the evidence available for the substance affecting one or more KEs. The analysis of the biological plausibility for the KER refers only to the broader knowledge of the biology, physiology, endocrinology and toxicology involved. In a postulated MoA, the KERs need to be consistent with the current understanding of biology, physiology, endocrinology and toxicology.

Existing adverse outcome pathways (AOPs) and MoAs can be used as a starting point for the postulated MoA against which the evidence can be systematically organised. Evidence on adverse effect(s) and endocrine activity, assessed for dose and temporal concordance, can provide empirical support to the KEs.

Several AOPs related to endocrine disruption have been established and endorsed, *e.g.* OECD Series on AOPs and EFSA PPR Panel, 2023. There are also numerous AOPs under development in the AOP-wiki, or published in the literature. The amount of empirical support needed to establish the KERs varies depending on how well developed the AOP in question is. In cases where the MoA is based on a robust[33] or OECD endorsed AOP, the biological plausibility of the KERs does not need to be demonstrated with experimental data. However, existing data on adversity and endocrine activity should be used to provide the empirical support needed to establish that the postulated MoA is plausible. Lack of a robust or OECD endorsed AOP should not be considered negatively in cases where there is convincing evidence for a biologically plausible link between observed endocrine activity and adversity.

The assessment should, when possible, include consideration of the modified Bradford Hill criteria, *i.e.*, essentiality, dose/incidence and temporal concordance, specificity, consistency and analogy (see further definition in Table 4.2.1. and ECHA/EFSA, 2018). In particular, dose/incidence and temporal concordance are valuable to support or disprove the plausibility of the KERs and should always be assessed. For example, a MIE is expected to occur below or at doses/concentrations where a downstream KE or an adverse outcome is observed. Similarly, early KEs should occur before or at the same time as the adverse outcome. However, since substance specific information on all the Bradford Hill criteria is rarely available, the absence of evidence to demonstrate these individual factors should not be used to exclude classification as an ED if the overall evidence supports a plausible link to an ED MoA.

It must be also noted that in the case of non-mammalian data, the empirical support will be mainly based on the evaluation of the dose/concentration-response relationship due to the available data set not often allowing for the evaluation of the temporal concordance and consistency among species (often only studies on a single species are available).

It is recognised that there may be cases where the biological relationship between two KEs may be very well established:

- When adverse effects are '*EATS-mediated*'*.* These parameters provide evidence for adversity, while at the same time (due to the nature of the effect and existing knowledge as described in the OECD GD 150) they are also considered indicative of an EATS MoA and thus (in the absence of other explanations) also infer an underlying *in vivo* mechanism. Where both adversity and endocrine activity data are provided by the same study, it may be possible to conclude on the biological plausibility of the link without recourse to a detailed MoA analysis;

- When the MoA analysis is based on a robust or endorsed AOP, *e.g.* OECD Series on Adverse Outcome Pathways[34]. In this situation, the biological plausibility is provided by the documentation for the KERs in the AOP used, *e.g.* OECD series on AOP No. 4 links aromatase inhibition to reproductive dysfunction in fish.

However, for adverse effect(s) based on '*Sensitive to, but not diagnostic, of EATS*', the evidence that the adverse effects are (exclusively) caused by an endocrine MoA is not as strong as for adversity based on *'EATS-mediated'* parameters. Therefore, postulated MoA and its biological

[33] Robust in this context means AOPs that have a broad acceptance in scientific literature.

[34] OECD Series on Adverse Outcome Pathways | OECD iLibrary (oecd-ilibrary.org)

plausibility would need to be supported by a more detailed MoA analysis. For example, a decrease in fecundity in fish together with a reduction in VTG concentration in females could be considered caused by an endocrine MoA, *i.e.* AR agonism or aromatase inhibition, if it were supported by mechanistic data such as those described in the endorsed OECD AOP 4 or 9[35], respectively.

Similarly, for adverse effect(s) based on non-EATS modalities (*i.e.*, adversity resulting from impairment of endocrine modalities other than E, A, T or S), the evidence that the adverse effect(s) are caused by an endocrine MoA needs to be substantiated with a more extensive MoA analysis than for '*EATS-mediated*' adverse effects; unless the biologically plausible link is based on existing scientific knowledge, *e.g.* a robust or OECD endorsed AOP.

A substance may have one or more MoAs, which can be endocrine or non-endocrine. The potential of a substance to elicit more than one MoA can lead to difficulties in concluding on the biological plausibility. If there are indications that a substance may act via multiple MoAs, the evaluation should first focus on the MoA for which the most convincing evidence is available. The number of potential MoAs to be considered will vary on a case-by-case basis.

Furthermore, there may be more than one MoA with similar effects; hence, it may be necessary to undertake an analysis for more than one postulated MoA for a particular adverse effect. There may be also situations where a pattern, which includes 'EATS mediated' adverse effects, has been identified. However, due to the complexity and cross-talk within the endocrine system, it may not be possible to identify the specific modality.

In such cases, a biological plausible link should be considered established for an 'EATS-mediated' MoA and classification as Category 1 or 2 may be warranted depending on the strength of evidence.

Comparative MoA analysis

To consider an ED-related adverse effect as a specific consequence of another non-endocrine MoA*,* there must be evidence for a biologically plausible sequence of events that excludes an endocrine MoA as the most likely explanation for the observed adverse effect(s). To demonstrate this, MoA data is needed on the alternative MoA and the assessment is best done by a comparative MoA assessment. It should be noted that it may be difficult to demonstrate that the effects are solely non-endocrine related because standard studies generally do not provide mechanistic information. Therefore, further mechanistic studies may be needed. An additional complication is that substances may have more than one MoA, including an ED MoA. In this situation, the ED MoA should still be considered for classification. For further guidance on how to conduct a comparative MoA analysis, see ECHA/EFSA ED Guidance.

Table 4.2.1. Explanations of the terms: analogy, essentiality, consistency, dose and incidence concordance, MoA, specificity and temporal concordance (adapted and modified from Bradford Hill criteria).

Term	Explanation
Analogy	A consistent observation across (related) substances having a well-defined MoA.

[35] AOP 4 (OECD series, endorsed): Aromatase Inhibition leading to Reproductive Dysfunction (in Fish); AOP 9 (OECD Series, endorsed): Androgen receptor agonism leading to reproduction dysfunction (in repeat-spawning fish)

Essentiality	Essentiality is one of the elements that should be considered (when data are available) when performing the WoE analysis using the Bradford Hill considerations. In the context of the MoA/AOP frameworks, essentiality refers to key events. For determining essentiality, it should be demonstrated whether downstream KEs and/or the adverse effect is prevented/decreased if an upstream event is experimentally blocked. It is generally assessed based on direct experimental evidence of the absence/reduction of downstream KEs when an upstream KE is blocked or diminished (*e.g.* in null animal models or reversibility studies).
Consistency	Consistency is the pattern of effects across species/ organs/test systems that are expected based on the postulated MoA/AOP. In developing a MoA, consistency also refers to the repeatability of the KEs in the postulated MoA in different studies. Consistent observation of the same KE(s) in several studies with different study designs increases the support.
Dose and incidence concordance	Dose and incidence concordance are elements valuable for the evaluation of the empirical support. In a MoA/AOP context, dose and incidence concordance are verified when the key events are observed at doses or incidences below or similar to those associated with the adverse effect (or key events downstream).
Mode of Action	A biologically plausible sequence of key events at different levels of biological organisation, starting with the exposure to a substance and leading to an observed (adverse) effect.
Specificity	Specificity should be understood as the extent to which the MoA for the adverse effect is likely to be endocrine-related, *i.e.* whether an adverse effect is a consequence of the hypothesised endocrine MoA, and not a result of other non-endocrine mediated MoA, including the result of excessive other toxic effects.
Temporal concordance	Temporal concordance increases the empirical support of the biologically plausible link. This is done by evaluating whether key events within the MoA are observed in the hypothesised order.

4.2.2.3.5. Weight of evidence and expert judgement

According to the CLP ED criteria, WoE and expert judgement must be applied when concluding on the CLP ED criteria (CLP Article 9 in conjunction with CLP, Annex I, sections 1.1.1. and 4.2.2.1.); see guidance on WoE in sections 1.4 of this guidance.

CLP, Annex I, *section 4.2.2.3.1.* *Classification as an endocrine disruptor for the environment is made on the basis of an assessment of the total weight of evidence using expert judgment (see section 1.1.1.). This means that all available information that bears on the determination of endocrine disruption for the environment is considered together, such as:*

(a) in vivo studies or other studies (e.g. in vitro, in silico studies) predictive of adverse effects, endocrine activity or biologically plausible link in animals;
(b) data from analogue substances using structure-activity relationships (SAR),

(c) evaluation of substances chemically related to the substance under study may also be included (grouping, read-across), particularly when information on the substance is scarce;
(d) any additional relevant and acceptable scientific data.

A WoE determination means that all available, relevant information bearing on the determination of hazard is considered together, including:

(a) relevant animal data; the results of suitable *in vitro* tests; and relevant *in silico* predictions;
(b) information from the application of the Category approach (grouping, read-across); (Q)SARs etc.;
(c) peer-reviewed published studies; and
(d) any additional data, for example information used for the evaluation of the substance as an ED for human health, including physico-chemical parameters and information on known metabolites or degradation products should be considered where relevant.

Formation of a metabolite with endocrine activity or adversity indicates that exposure to the substance might result in endocrine-related adverse effects. Therefore, endocrine activity or adversity observed with the metabolite must be considered in the classification of the parent substance. If data are available, quantity and stability of the metabolite(s) formed should be considered (*e.g.* if the metabolite is stable for a period long enough to exhibit toxicological properties or if it is an intermediate which is rapidly changed to other metabolites). Even if a substance has been tested as negative for ED, it may be in certain instances classified in Category 1 or 2 based on the formation of metabolites with ED properties.

Considering the similarity of metabolization across vertebrates (Auer *et al*., 2017), if a metabolite is formed in any vertebrate species, it is assumed by default that this metabolite is also formed in other vertebrates unless demonstrated otherwise.

If a substance degrades (biotically or abiotically) in the environment and the degradation (or transformation or breakdown) product shows endocrine activity and/or adverse effect(s), this should be taken into account in the assessment of classification for the parent substance.

CLP, Annex I, section *4.2.2.3.2.* *In applying the weight of evidence determination and expert judgement, the assessment of the scientific evidence referred to in section 4.2.2.3.1 shall, in particular, consider all of the following factors:*

(a) both positive and negative results;
(b) the relevance of the study design for the assessment of adverse effects and its relevance at the population or subpopulation level, and for the assessment of the endocrine activity;
(c) the adverse effects on reproduction, growth/development, and other relevant adverse effects which are likely to impact on populations or subpopulations;
(d) the quality and consistency of the data, considering the pattern and coherence of the results within and between studies of a similar design and across different species;
(e) the route of exposure, toxicokinetic and metabolism studies;
(f) the concept of the limit dose (concentration), and international guidelines on maximum recommended doses (concentrations) and for assessing confounding effects of excessive toxicity;
(g) where available, adequate, reliable and representative field or monitoring data or results from population models.

The WoE approach for identifying EDs should involve transparent assessment and consideration of all available data based on factors such as relevance, quality, and consistency, see CLP, Annex I, section 1.1.1.3.

The quality and consistency of the data should be given appropriate weight. Both positive and negative results should be assembled in a single WoE determination, separated for endocrine activity and adversity; see CLP, Annex I, section 1.1.1.3 and section 1.4 in this guidance).

Although the quality, reliability, relevance and applicability domain of a study per se affects the weight given to the study, there are also several other, "external" factors that may influence the WoE assessment, as mentioned above in the green boxes. Information on toxicokinetics, (*e.g.* sex differences, accumulation in tissues, information on major metabolites), physicochemical properties (*e.g.* vapour pressure, solubility and unspecific binding in *in vitro* test systems), read-across/analogy and availability of substance specific data may have influence on how much weight each piece of information can be given. In general, substance specific information is given more weight than other data unless there are reasons not to do so. For example, read-across or analogy can sometimes provide stronger evidence for classification than the substance-specific data.

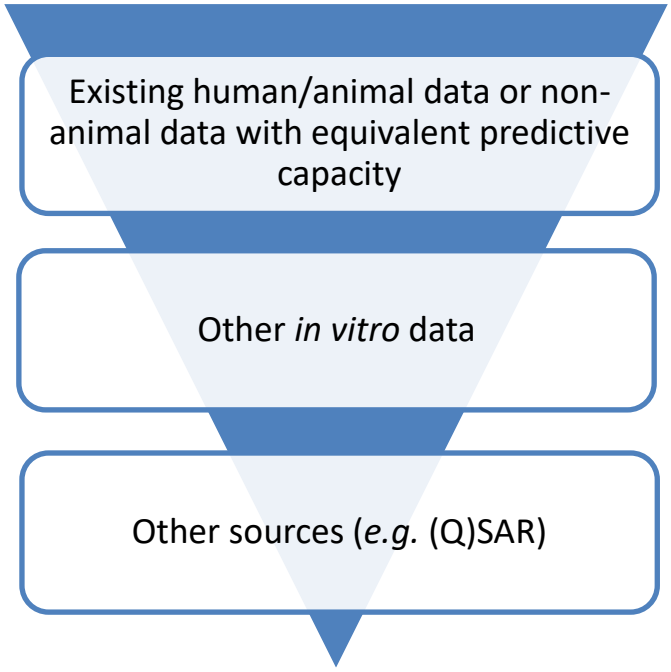
The assessment must weigh all evidence and be performed on a case-by-case basis using expert judgement. A single positive study can, however, be sufficient for classification.

CLP, Annex I, section 4.2.2.2.1 *Classification shall be made on the basis of the appropriate criteria outlined above, and a weight of evidence determination of each of the criteria (see section 4.2.2.3) and an overall weight of evidence determination (see section 1.1.1).*

WoE for endocrine disruption must be conducted independently for adverse effect(s) and for endocrine activity. Thereafter, the overall WoE for all these elements together must be conducted in the MoA analysis, also including the conclusion on the biologically plausible link.

Figure 4.2-2 provides an illustration of the relative weight of different types of data. In the case of conflicting results, a decision on the weight to be assigned to the different types of data has to be made. It needs to be noted that the relative weights indicated in Figure 4.2-2 assume comparable quality of the data. WoE considerations need to take into account, on a case-by-case basis, the quality, consistency, nature, severity, relevance and applicability domain of the different types of data available. The figure illustrates a decreasing weight of the information from top to bottom.

Figure 4.2.2 Simplified illustration of the relative weight of the available information with similar or comparable quality



When contradicting data of comparable quality and predictive capacities assessing similar endpoints belongs to different 'hierarchical levels', the following considerations should be included in the WoE approach:

- When there are relevant positive data which belong to a higher level in the hierarchy than the available negative data, more weight should normally be given to the positive data.

- When the negative data belong to a higher level in the hierarchy than the positive data, more weight should normally be given to the negative data, and a careful evaluation of the reasoning should be conducted considering differences in dose/concentration levels used, species differences, differences in the quality and reliability of data etc. Furthermore, there may be cases where the mechanism investigated at the lower level of the hierarchy (*e.g. in vitro*) is not covered by the investigations at the higher level of the hierarchy (*e.g. in vivo*), or *e.g.* there may be lack of sensitivity in a well conducted *in vivo* study. In such cases negative data at the higher level should not be given higher weight than the positive data at the lower level of the hierarchy.

- In all of the above cases, it is important to assess the full data set and a scientifically justified explanation should be provided. In general, positive results that are relevant for classification should not be overruled by negative findings without a scientifically sound and transparent explanation based on the analysis of biological plausibility. All existing evidence should be systematically organised against existing AOPs or known MoAs.

Field or monitoring studies can also contribute to the WoE, for more information see in section 3.2 of the ECHA/EFSA ED Guidance.

4.2.2.3.6. Use of evidence considered for classification as endocrine disruptor for human health when assessing classification as endocrine disruptor for the environment

CLP, Annex I, section *4.2.2.3.4*. *Using a weight of evidence determination, evidence considered for the classification of a substance as an endocrine disruptor for human health referred to in section 3.11. shall be considered when assessing the classification of the substance as an endocrine disruptor for the environment under section 4.2.*

Because of the high level of conservation of the endocrine system across taxonomic groups, the conclusion on the classification as ED for the environment makes use of all available data in mammalian and non-mammalian species in a holistic approach. The Revised Guidance Document 150 states that: "*Cross-species extrapolations should be considered during data assessment. Endocrine systems with respect to hormone structure, receptors, synthesis pathways, hormonal axes and degradation pathways are well conserved across vertebrate taxa especially in the case of estrogen, androgen and thyroid hormones and steroidogenesis.*" And: "*When interpreting data for endocrine assessment, this conservation should be borne in mind as results from tests using human in vitro or non-human mammalian (in vitro and in vivo) systems may be highly relevant for vertebrate wildlife species and vice versa. In addition, results from non-human mammalian studies are also highly relevant for mammalian wildlife species.*"

Furthermore, the ECHA/EFSA ED Guidance (2018) specifies that the same database can be used to conclude on the ED properties for human health and the environment: "*The information needed to assess ED properties for humans and non-target organisms may overlap. Mammalian data are always relevant for ED assessment on non-target organisms. Furthermore, there may be information on non-target organisms that could be relevant also for the ED assessment for humans.*" and "*[...] it is recommended to strive for a conclusion on the ED properties with regard to humans and in parallel, using the same database, to strive for a conclusion on mammals as non-target organisms.*"

Current advances within the development of AOP networks demonstrate that some molecular initiating events and key events are linked to a broad range of adverse outcomes in different species across toxicology and ecotoxicology (for EDs typically humans, rodents, fish and amphibians). By using well-developed AOP networks, cross-species information could be utilised in the evaluation of environmental endocrine disruption to a much higher degree than previously done for environmental ED assessment, see *e.g.* Haigis *et al.*, 2023 and Figure 3.11 in this Guidance on the AOP network for thyroid effects.

Therefore, effects on mammals can also give information on endocrine disruption in non-mammalian vertebrates and data on mammals and other taxa should be considered together in a holistic approach as part of the available evidence for reaching a conclusion on the need to classify the substance. See also population relevance (section 4.2.2.3.2 of this guidance).

4.2.2.4. Decision on classification

Substances are classified as EDs for the environment in Category 1 or 2 when there is sufficient evidence that the three empirical elements (a) adverse effect(s) (b) endocrine activity and (c) the biologically plausible link as indicated in CLP, Annex I: Table 4.2.1 (for details see section 4.2.2.2 of this guidance) are met. If one of the three elements is not met, classification of the substance is not warranted.

To be able to meet the classification criteria, it is highly important to understand the biologically plausible link between endocrine activity and observed adverse effect(s) that is relevant at the population level (see more information on population relevance under section 4.2.2.3.2).

Where there is evidence conclusively demonstrating that the adverse effects are not relevant at the population level, no classification is warranted. If there are serious doubts based on all available information about the relevance of the adverse effects at the population level, this should be taken into account in the classification, and Category 2 classification should be considered.

The allocation of the substance to Category 1 or 2 or no classification depends on the strength and consistency of the available evidence, *i.e.* on how convincing the evidence for criteria (a) and (b) is as well as whether a plausible link between the two can be established. Allocation to Category 1 is warranted when the evidence for adverse effect(s) and endocrine activity is sufficiently convincing considering all available relevant data in the WoE on the substance. Sufficiently convincing evidence for Category 1 may also be based on appropriate and robust read-across or analogy or grouping, when those approaches are sufficiently justified for that substance and a biologically plausible link is established. Also, evidence on a certain pattern of adverse effect(s) observed, which is generally known to be linked to a certain type of endocrine activity (*i.e.* '*EATS-mediated*'), can lead to Category 1 classification.

When the evidence for either adverse effect(s) or endocrine activity or both is not sufficiently convincing to place the substance in Category 1, then Category 2 or no classification may be warranted. This may be caused by issues related to reliability, dosing/concentration settings, parameters covered, life-stage investigated or exposure duration, serious doubts on the relevance at the level of population, incidence of the effects, divergencies between results in different studies if not explainable by differences in study design (*i.e.* lack of consistency), inconsistent pattern of effects, etc., or when chance, bias or confounding factors cannot be ruled out with reasonable confidence.

For example, if there are serious concerns regarding the study design or conduct or the interpretation of existing information, or if there is insufficient information available to make a conclusion on Category 1, or if the adverse effect is considered to be not sufficiently convincing for Category 1 (*e.g.* if a broad range of relevant ED related endpoints are investigated in well-conducted reliable studies, and the ED related effect(s) is observed with low incidence), classification for Category 2 or no classification may be more appropriate.

Evidence on essentiality, consistency, analogy, specificity as well as empirical support for dose-temporal concordance may affect the strength of evidence. In cases where two different MoAs, one endocrine and one non-endocrine could explain the same adverse effect, the WoE of both MoAs should be assessed in a comparative analysis (see section 3.5 of the ECHA/EFSA ED Guidance). However, when the endocrine MoA is the most likely, even in the presence of an alternative non-endocrine MoA, the ED MoA should be used for classification.

Regarding the reliability of studies, it should be noted that some parameters may be reliably investigated although the study may not be considered fully reliable as regards all parameters due to specific deficiencies which do not affect all the investigated /observed effects. Therefore, reliability should always be assessed with care, and the overall study reliability scores do not necessarily indicate how much weight can be given for a subset of investigations and results in the study in an overall WoE assessment. This applies to the assessment of all types of studies but particularly non-guideline and non-GLP studies.

Sufficient evidence for the empirical element (c) (a biologically plausible link between endocrine activity and the adverse effect) to classify a substance in Category 1 or Category 2 can be based on *e.g.*:

- understanding of the key event relationships (KER) based on broad acceptance, *e.g.* in scientific literature or in an endorsed AOP, see OECD Series on AOPs[36]), *i.e.* the postulated endocrine MoA and the KEs need to be consistent with the current understanding of physiology, endocrinology and (eco)toxicology by addressing structural and/or functional relationships between KEs;

- if the KER is plausible based on analogy with accepted biological relationships even when scientific understanding is not completely established;

- when there are dose and time concordance between early KEs and later KEs. It should be noted that evidence to show this is rarely available and lack of evidence on these parameters should not lead to a lower- or non-classification;

- existing knowledge on endocrinology / toxicology may be sufficient to assess the biological plausibility (*e.g.* if MoA is mainly established and empirically supported based on EATS or other less explored endocrine function mediated parameters).

- when adverse effects are '*EATS-mediated*'. These parameters provide evidence for adversity, while at the same time (due to the nature of the effect and existing knowledge as described in the OECD GD 150) they are also considered indicative of an EATS MoA and thus (in the absence of other explanations) also infer an underlying *in vivo* mechanism. Because both data on adversity and endocrine activity are provided by the same study, it may be possible to reach a conclusion on the biological plausibility of the link without recourse to a detailed MoA analysis.

In general, EATS mediated adverse effects can directly trigger ED ENV 1, whereas for adverse effects '*sensitive to, but not diagnostic of, EATS*' effects and '*non-EATS mediated*' adverse effects, an ED MoA must be demonstrated in more detail for a classification in ED ENV 1. Such effects could also potentially lead to an ED ENV 2 classification (see parameters in Tables 15 and 16 of ECHA/EFSA ED Guidance). It should be highlighted that some individual parameters described in Tables 15 and 16 may not be considered sufficient in isolation for covering the element of adversity. In such cases, the conclusion on classification relies on a combination of parameters and the observation of a pattern of effects.

The following scenarios can be identified.

If **adverse effect(s) are based on '*EATS-mediated parameter(s)*'**, the pattern of adverse effect(s) observed provide evidence for both adverse effect(s), endocrine activity and the biologically plausible link. Therefore, classification ED ENV 1; EUH430 or ED ENV 2 EUH431 is warranted depending on the strength of the available evidence even without specific mechanistic information or identification of the specific MoA, unless demonstrated not to be ED in a MoA analysis (with a fully developed non-ED MoA) supported by sufficient data. Consideration should be given to the existence of a pattern of effect and a WoE assessment should always be conducted to put any adverse effects into context.

If **adverse effect(s) are based on '*Sensitive to, but not diagnostic of, EATS parameters*'**, or **'non-EATS mediated parameters'**, different classification outcomes are possible depending on:

i. the strength of the evidence for the three elements in CLP, Annex I, section 4.2.2.1;

ii. whether '*(non-) EATS-mediated*' parameters have been extensively or partially investigated

[36] https://www.oecd-ilibrary.org/environment/oecd-series-on-adverse-outcome-pathways_2415170x

and found positive or negative;

iii. the available information on whether other types of endocrine activity, including activity not already inferred by '*(non-) EATS-mediated*' parameters, is available; and

iv. the WoE.

Classification may also be warranted in cases when there is evidence that the elements indicated in CLP, Annex I, section 4.2.2.2 *i.e.* (a) endocrine activity, (b) adverse effect(s), (c) plausible link are met, however there is not enough information to postulate a detailed MoA due to the lack of thorough mechanistic information. This is for example the case when a pattern of adverse effects has been identified which, based on current knowledge, is concluded to be related to endocrine disruption (adverse effects which are considered EATS mediated or '*sensitive to, but not diagnostic of, EATS*' or '*non-EATS mediated*'), but due to the complexity and crosstalk of the endocrine system, it is difficult to identify the specific modality. In this situation, classification as ED ENV 1; EUH430 or ED ENV *2*; EUH431 may be justified based on the strength of the evidence.

The substance should not be classified, for example, when:

- no adverse effect(s) are observed. This includes adaptive responses that are demonstrated not to be ecotoxicologically relevant, *i.e.* not adverse per se or not leading to adverse effects); or
- adverse effect(s) are not relevant at the population level; or
- no endocrine activity is observed or cannot be inferred from the adversity; or
- no biologically plausible link can be established, *i.e.* adverse effects are observed which cannot be linked to the observed endocrine activity using existing knowledge; or
- adverse effect(s) are solely a non-specific consequence of other toxic effects (see CLP, Annex I, section 4.2.2.2.2.) *i.e.* observed adverse effects are a consequence of excessive other toxic effects; or
- a non-endocrine MoA, as a result of a comparative MoA analysis, has been demonstrated to be the most likely explanation of the observed adverse effect(s).

A distinction may need to be made between whether the data are sufficient to conclude on classification for ED or whether some important data are lacking or inconclusive and therefore the outcome of "no classification" is due to lack of data or inconclusive data for the modalities assessed.

To summarise, for Category 2, the situation may be also that Category 1 classification cannot be concluded due to inconclusive data but the currently available data better supports Category 2 classification.

Ultimately, a WoE approach and expert judgement is needed to decide on the appropriate Category.

4.2.2.4.1. Specific considerations related to the thyroid modality with respect to decision on classification

As mentioned in section 3.11.2.3.1 of this guidance, the thyroid system is highly conserved across vertebrates, therefore, indications of interference with thyroid function or thyroid hormone signalling in one species may well lead to similar affects in others, including in wildlife species such as amphibians.

The classification of a substance as ED ENV can, in some situations, already be reached considering the available evidence on the thyroid modality from mammals if that evidence allows to classify the substance as ED HH.

This is the case when the adverse effect(s) observed in mammals leading to the classification for HH are considered to be population relevant (for example if (neuro)developmental effects in mammals are observed). In such case, classification for ED ENV is also warranted (see section 4.2.2.3.2 on population relevance).

If adverse effect(s) observed in mammals, taking into account the whole data package in a WoE approach, are considered not relevant at the population level, classification for environment is still warranted when there is other information specific for the environment indicating adversity unless the effects are shown not to be population relevant. The allocation to Category 1 or 2 will depend on the type of evidence available and on the strength of that evidence.

In case there is no evidence from mammals, or the substance is not classified for ED HH for the thyroid modality, classification as Category 1 is warranted if there is at least one *in vivo* long-term test in a non-mammalian species showing evidence of adverse effects relevant at the population level, or non-animal data providing an equivalent predictive capacity to the *in vivo* data. When the *in vivo* information is available only at the screening level, classification in either Category 1 or Category 2 should be considered on a case-by-case basis, depending on whether it is positive for adverse effect(s) or only for endocrine activity. If only mechanistic information is available and positive, due to the absence of evidence on adverse effect(s), no classification is warranted.

4.2.2.5. Classification of substances and mixtures containing ED constituents/components

In analogy to the approach used for CMR substances, from a compositional and a regulatory point of view the situation for substances containing ED constituents, additives or impurities is the same as for mixtures containing components classified for these hazard classes. For this reason, the classification procedure for ED endpoints that is foreseen by CLP for mixtures containing ED components, is considered applicable also to substances containing ED constituents, additives or impurities (see sections 4.2.3.1 and 4.2.3.2 of this guidance).

As discussed in section 4.2.3.2 below, mixtures containing components classified as EDs must be normally classified using only the relevant available information for the individual substances in the mixture. Further, in cases where the available test data on the mixture itself demonstrate positive ED effects which have not been identified from the information on the individual substances, those data must also be taken into account.

When testing a diluted substance, such as mixtures or substances containing ED constituents, it should be noted that tests may fail to detect ED effects due to low doses/concentrations of the constituents. Consequently, there is an increased risk that ED hazards would not be detected, *i.e.* dilution might compromise the threshold of detection for ED hazards. Therefore, negative test data on mixtures containing components with these hazards must not be accepted.

According to Article 10(1), generic and specific concentration limits (GCLs and SCLs) are similarly assigned to substances in other substances and substances in mixtures. A GCL will apply to EDs unless the data justifies setting an SCL.

4.2.2.6. Setting of specific concentration limits

CLP, Article 10(1) *Specific concentration limits and generic concentration limits are limits assigned to a substance indicating a threshold at or above which the presence of that substance in another substance or in a mixture as an identified impurity, additive or individual constituent leads to the classification of the substance or mixture as hazardous.*

Specific concentration limits shall be set by the manufacturer, importer or downstream user where adequate and reliable scientific information shows that the hazard of a substance is evident when the substance is present at a level below the concentrations set for any hazard class in Part 2 of Annex I or below the generic concentration limits set for any hazard class in Parts 3, 4 and 5 of Annex I.

In exceptional circumstances specific concentration limits may be set by the manufacturer, importer or downstream user where he has adequate, reliable and conclusive scientific information that a hazard of a substance classified as hazardous is not evident at a level above the concentrations set for the relevant hazard class in Part 2 of Annex I or above the generic concentration limits set for the relevant hazard class in Parts 3, 4 and 5 of that Annex.

The concept of applying the SCL is described in section 1.5 of this guidance. The information on GCL of a mixture classified as ED for the environment is described in section 4.2.3.1.

To align the protection levels for EDs for human health and the environment the SCLs for ED effects for the most potent substances need to be derived. As explained in section 4.2.1, the concept of ED "potency" is considered only in the context of setting specific concentration limits.

4.2.2.6.1. Procedure

In general, the SCLs for ED properties are set based on the potency of the adverse effect, which is a pragmatic approach used in EU legislation to further inform the downstream user or supplier on the presence of a hazardous substance in a mixture. However, it should be noted that for some endocrine disruption endpoints, potency may vary. As a pragmatic solution, specific concentration limits must be set based on observed potency in available studies. When data allows to set an SCL, the SCLs for ED must be set following the procedure described below instead of using the GCL. The way of setting the SCL for ED for the environment will depend on the source of data used to classify a substance for this hazard class.

The ED effect level (*e.g.* EC10, NOEC, LOEC or DNEL from any relevant studies[37] where adverse effect(s) are observed with sufficient confidence) for adverse endpoints can be considered for setting the SCLs (see section 4.2.2.3.1 of this guidance), but the CLP criteria for ED ENV do not specify any concentrations above which the production of an adverse effect is considered to be outside the criteria which lead to classification, provided that the used concentrations are still within the recommendations for test concentrations set by the corresponding OECD test guidelines.

When the ED ENV classification is based on the mammalian data used for the ED HH classification and there is no relevant non-mammalian information, derivation of the SCLs should be calculated according to the same principles as described in section 3.11.2.6.

[37] SCL may also be calculated based on effect levels derived from the screening studies. It has to be noted however that such provisional NOECs, EC_{10}, etc. can be higher than the effect values derived in the definitive studies.

However, when the ED ENV classification is based on information on non-mammalian organisms the following scenarios for the derivation of concentration limits are possible.

a. When the adverse effect used for the ED ENV classification comes from the non-mammalian toxicity study from which the EC_{10} or NOEC value[38] for the specific ED parameters indicating adverse effects can be derived and is below 0.1 mg/L, the SCL should be calculated as presented in Table 1 below:

   i. For substances with EC_{10} or NOEC ≤ 0.00001 mg/L, the SCL that is 100-fold lower than GCL should be considered on a case-by-case basis. This is introduced to cover extremely potent ED substances.

   ii. For substances with 0.00001 mg/L < EC_{10} or NOEC ≤ 0.001 mg/L, the SCL should be 10-fold lower than a default GCL.

   iii. For substances with 0.001 mg/L < EC_{10} or NOEC ≤0.1 mg/L, the GCL as presented in the CLP, Annex I, Table 4.2.2 should be applied.

b. When the adverse effect used for ED ENV classification comes from the non-mammalian toxicity study from which the EC_{10} or NOEC value is above 0.1 mg/L, the GCL as indicated in the CLP, Annex 1, Table 4.2.2. should be used.

Table 4.2.2 SCL derivation based on non-mammalian data

Potency	Effect leading to adverse effect(s) (Non-mammalian study) [mg/L][a, b]	SCL (Cat1)	SCL (Cat2)
Very high potency (see bullet point a.i. above)	EC_{10} or NOEC ≤ 0.00001	GCL/100 = 0.001%	GCL/100 = 0.01%
High potency (see bullet point a.ii. above)	0.00001 < EC_{10} or NOEC ≤ 0.001	GCL/10 = 0.01%	GCL/10 = 0.1%
Medium potency (see bullet point a.iii. above)	0.001 < EC_{10} or NOEC ≤ 0.1	no SCL derived, GCL =0.1%	no SCL derived, GCL =1%
Low potency (see bullet point b. above)	EC_{10} or NOEC > 0.1 mg/L	no SCL derived, GCL =0.1%	no SCL derived, GCL =1%

[a] *When the adverse effect used for ED ENV classification would come from the non-aquatic non-mammalian toxicity study where the results are expressed in mg/kg (e.g. bird reproduction studies), the SCLs should be calculated based on the same principles as described in section 3.11.2.6, particularly following a method similar to 3.7.2 above.*

[b] *If a NOEC value is not available, the LOEC may be used to calculate the SCL, however, when calculating the SCL it should be taken into account that the NOEC value would be lower than the LOEC.*

[38] If available, EC10 is preferred over NOEC, see section 4.1.3.1.1

In exceptional cases a higher SCL than the GCL can also be set for EDs. A higher SCL should only be set where there are adequate, reliable, and conclusive scientific information that a hazard of a substance classified as hazardous is clearly above the level of the GCL.

When there are several types of effects and ways to calculate SCLs, the lowest SCL should be selected for the classification. Only one SCL can be set for ED ENV.

When the calculated SCL or GCL is not considered protective enough (*e.g.* due to a non-threshold MoA), the SCL corresponding to the extreme potency group may be set by default, unless an even lower SCL is justified. Therefore, the assessment of dose-response related information together with setting SCLs should be conducted with caution.

4.2.3. Classification of mixtures for endocrine disruption for environment

4.2.3.1. Classification criteria for mixtures

Endocrine disruption classification of mixtures is based on the presence of an ingredient classified for endocrine disruption (see CLP Article 6(3) and CLP, Annex I, section 4.2.3). Only in case there is data available for the mixture itself which demonstrate effects not apparent from the ingredients, might this data be used for classification. In other words, data on tested mixtures can be used only when it demonstrates classification for ED ENV, in line with CLP, Annex I, section 4.2.3.2.1. *i.e.*, not for "no classification". If such data is not available for the mixture itself, data on a similar mixture can be used in accordance with the bridging principle; see CLP, Annex I, section 1.1.3. Furthermore, it should be noted that various test guidelines have not been validated for mixtures and therefore, it is questionable if these tests may provide adequate results.

From a compositional and an (eco)toxicological point of view, the situation for substances containing ED constituents, additives or impurities is the same as for mixtures containing components classified for these endpoints. For this reason, the classification procedure for mixtures containing ED components is considered applicable also to substances containing ED constituents, additives, or impurities (see sections 1.1.6.1, and 3.11.3.1.1 to 3.11.3.2 of this guidance).

CLP, Annex I, section: ***4.2.3.1.1.*** *A mixture shall be classified as an endocrine disruptor for the environment where at least one component has been classified as a Category 1 or Category 2 endocrine disruptor for the environment and is present at or above the appropriate generic concentration limit as shown in Table 4.2.2 for Category 1 and Category 2, respectively.*

As such, each component in a mixture classified as an ED is compared separately to their respective generic or specific concentration limit to conclude on the classification of the mixture, unless the additivity principle applies.

The additivity concept may have to be applied for EDs (see also section 1.6.3.4.3 of this guidance). For a given effect, the SCL, if available, needs to be taken into consideration when applying the additivity concept - this will include potency considerations. Exposure to EDs with both similar and dissimilar modes of action can lead to combination effects if they impact the same physiological process(es), or have the same target organs for toxicity. If one single classified substance is present in the mixture above the generic or specific concentration limit, the mixture must be classified for that hazard. If the mixture contains two or more substances each below the generic or specific concentration limits, the mixture will not be classified, unless the additivity concept applies. For endocrine disruption, it is reasonable to assume additivity for substances

with a similar or related mechanism or MoA or adverse outcome (*e.g.* exposure to a combination of anti-androgenic, estrogenic and steroidogenic or even thyroid disrupting substances can lead to additivity), unless there are specific reasons not to do so.

The mechanism does not need to be the same. Similar to most of the HH hazard classes, the same adverse outcome between substances can already suggest additivity.

It is important in the assessment of potential additivity to consider if constituents with the same biological targets have different effects or mechanism behind the effects (*e.g.* they may have agonistic or antagonistic activity or even partial activity at the same receptor). In this case a careful assessment is needed since also dissimilar modes of action can cause the same adverse outcomes in an additive manner.

CLP, Annex I*: Table 4.2.2*

Generic concentration limits of components of a mixture classified as endocrine disruptor for the environment that trigger classification of the mixture

Component classified as:	*Generic concentration limits triggering classification of a mixture as:*	
	Category 1 endocrine disruptor for the environment	*Category 2 endocrine disruptor for the environment*
Category 1 endocrine disruptor for the environment	*≥ 0,1 %*	
Category 2 endocrine disruptor for the environment		*≥ 1 %* [Note 1]

Note: The concentration limits in this Table shall apply to solids and liquids (w/w units) as well as gases (v/v units).

Note 1: If a Category 2 endocrine disruptor for the environment is present in the mixture as an ingredient at a concentration ≥ 0,1 % a SDS shall be available for the mixture upon request.

4.2.3.1.1. When data are available for the individual ingredients

CLP, Annex I, section *4.2.3.1.1.* *A mixture shall be classified as an endocrine disruptor for the environment where at least one component has been classified as a Category 1 or Category 2 endocrine disruptor for the environment and is present at or above the appropriate generic concentration limit as shown in Table 4.2.2 for Category 1 and Category 2, respectively.*

Additivity can be considered on a case-by-case basis, particularly when the data suggests the same/related endocrine MoA or mechanism or adverse outcome for different ingredients of the mixture. It should be noted that when based on available data the SCLs could be derived on individual ingredients, which must be used instead of GCLs in accordance with CLP Article 10.

4.2.3.1.2. When data are available for the complete mixture

CLP, Annex I, section *4.2.3.2.1.* *Classification of mixtures shall be based on the available test data for the individual components of the mixture using concentration limits for the components classified as endocrine disruptor for the environment. On a case-by-case basis, test data on the mixture as a whole may be used for classification when demonstrating endocrine disruption for the environment that has not been established from the evaluation based on the individual components. In such cases, the test results for the mixture as a whole must be shown to be conclusive taking into account dose (concentration) and other factors such as duration, observations, sensitivity and statistical analysis of the test systems. Adequate documentation supporting the classification shall be retained and made available for review upon request.*

4.2.3.1.3. When data are not available for the complete mixture: bridging principles

CLP, Annex I, section *4.2.3.3.1.* *Where the mixture itself has not been tested to determine its endocrine disruption for the environment, but there are sufficient data on the individual components and similar tested mixtures (subject to paragraph 4.2.3.2.1.) to adequately characterise the hazards of the mixture, those data shall be used in accordance with the applicable bridging principles set out in Section 1.1.3.*

Bridging Principles will only be used on a case-by-case basis (see section 1.6.3.2 of this guidance). Data on similar tested mixtures can be used only when it demonstrates classification for endocrine disruption for environment, in line with CLP, Annex 1, section 4.2.3.2.1. *i.e.* not for "no classification". Note that the following bridging principles are not applicable to this hazard class:

- concentration of highly hazardous mixtures
- interpolation within one hazard Category

(see CLP, Annex I, sections 1.1.3.3 and 1.1.3.4)

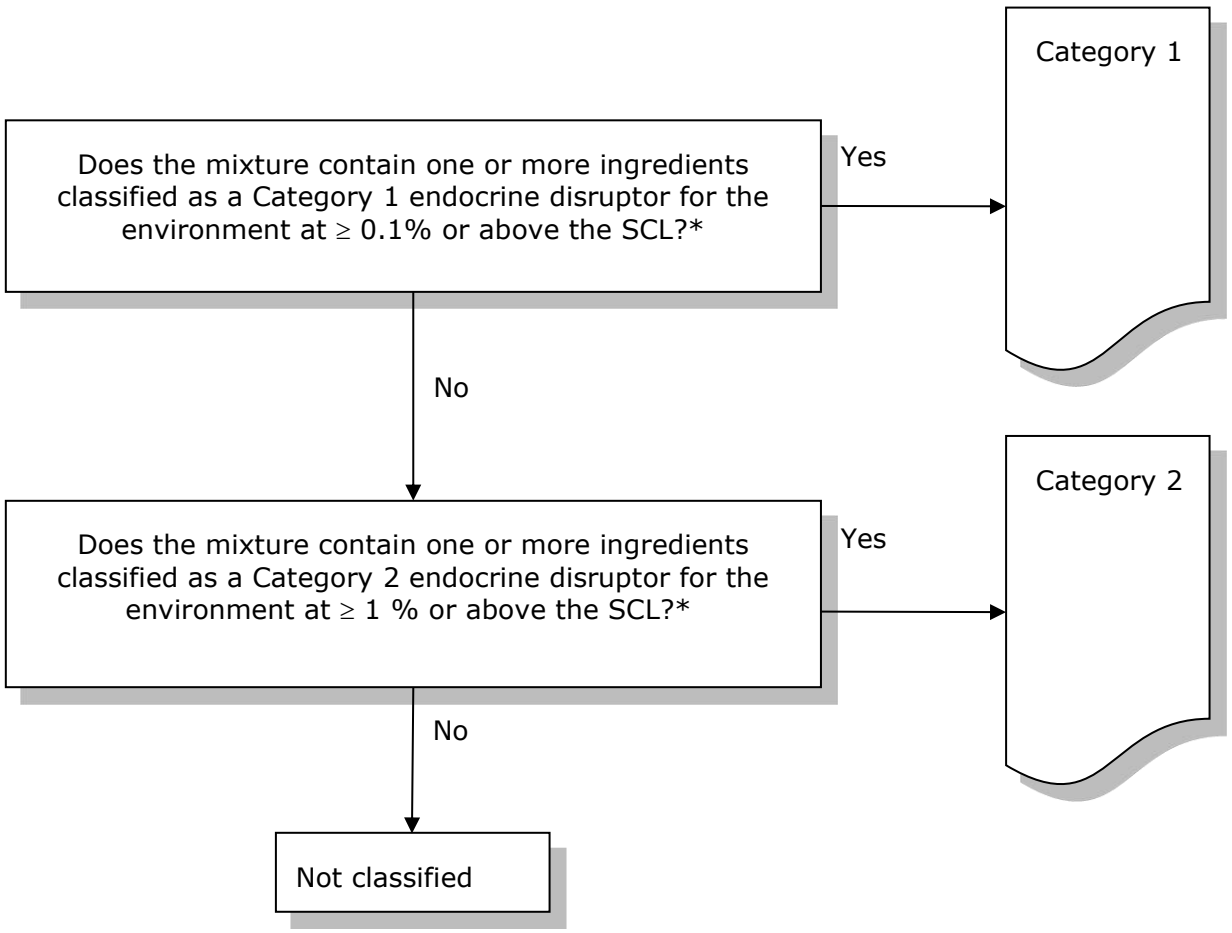
4.2.3.2. Decision logic for classification of mixtures

The decision logic for classification of mixtures in Figure 4.2-4 and Figure 4.2-5 is provided here as additional guidance. The person responsible for classification should study the criteria before and during use of the decision logic presented below.

<u>Classification of mixtures for endocrine disruption for environment</u>

Classification based on individual ingredients of the mixture

Figure 4.2.3 Decision logic for classification of mixtures based on individual ingredients of the mixture

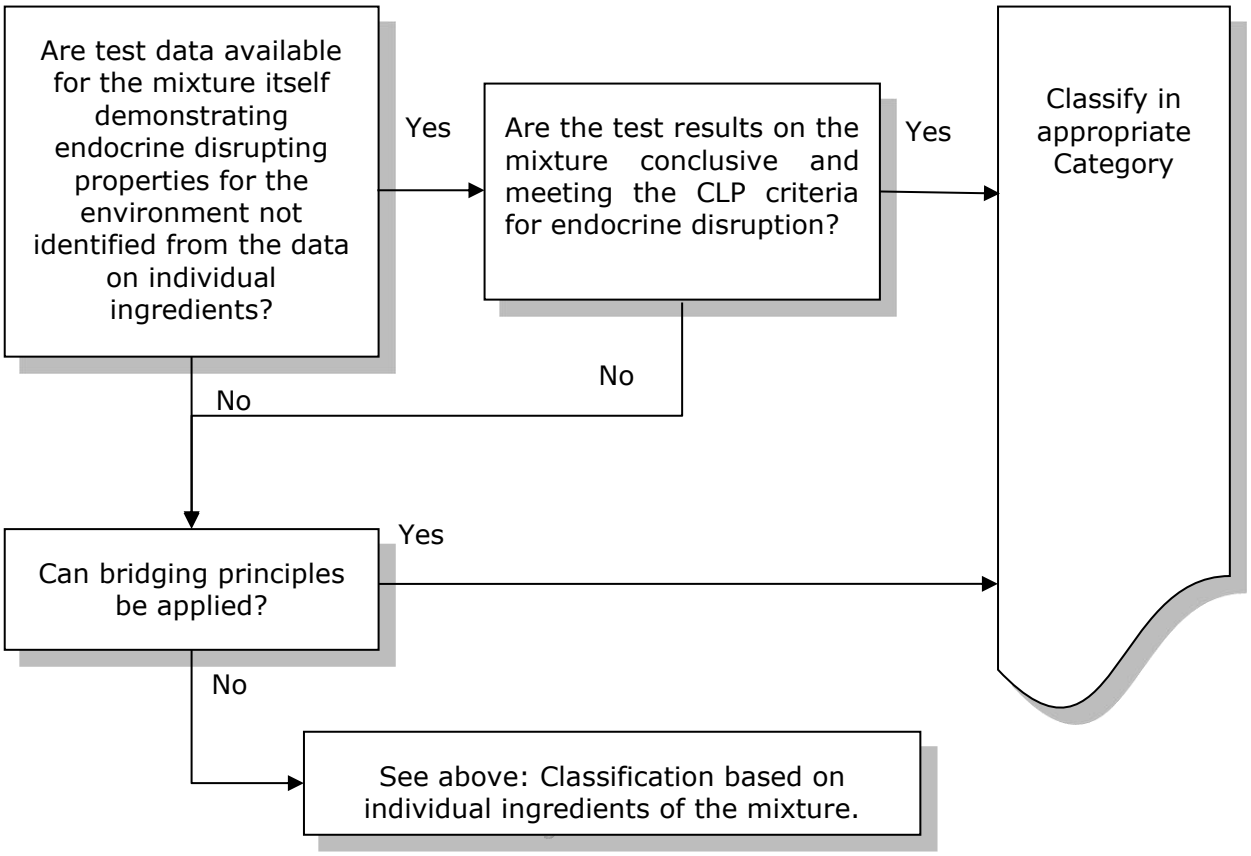


*Applicability of additivity approach should also be considered.

Modified classification when the test data on the mixture itself supports more stringent classification than evaluation based on individual ingredients

Test data on mixtures may be used for classification when demonstrating effects that have not been established from the evaluation based on the individual ingredients; CLP Article 6(3) and CLP, Annex I, section 4.2.3.2.1.

Figure 4.2.4 Decision logic for classification of mixtures when the test data on the mixture itself supports more stringent classification than evaluation based on individual ingredients



4.2.4. Hazard communication in the form of labelling for endocrine disruption for environment

4.2.4.1. Pictograms, signal words, hazard statements and precautionary statements

Classification	*Category 1*	*Category 2*
GHS Pictograms		
Signal Word	*Danger*	*Warning*
Hazard Statement	*EUH430: May cause endocrine disruption in the environment*	*EUH431: Suspected of causing endocrine disruption in the environment*
Precautionary Statement Prevention	*P201* *P202*	*P201* *P202*

	P273	*P273*
Precautionary Statement Response	*P391*	*P391*
Precautionary Statement Storage	*P405*	*P405*
Precautionary Statement Disposal	*P501*	*P501*

A pictogram is currently not applicable for the new hazard classes, as explained in recital (17) of Regulation 2023/707, and may be introduced at a later stage if adopted in the context of the UN GHS. When included in GHS, but not yet implemented in CLP, it is strongly recommended to be used.

The wording of the Precautionary Statements is found in CLP, Annex IV, Part 2.

Further explanations on the precautionary statements can be found in Annex IV of CLP.

4.2.4.2. Additional labelling provisions

There are no additional labelling provisions for substances and mixtures classified as EDs in CLP. However, there may be provisions laid out in other regulations such as REACH which need to be considered, when relevant.

4.2.5. Examples

The substances in the examples are fictitious. They do not represent real cases and are not to pre-empt the classification assessment in concrete cases. These examples are meant to illustrate what type of data may lead to classification for ED and to show how an assessment according to this guidance could potentially be approached. Only the ED-related information leading to classification or supporting classification is included in the examples, but not the whole data set or a detailed description of the effects, or a full WoE analysis. All the endocrine-related effects reported for the different examples leading to classification are considered adverse *i.e.* statistically significant compared to the control and biologically relevant. The reliability reported for the studies is according to Klimisch score. The test concentrations in the examples are considered acceptable unless stated otherwise. The decision on classification is influenced by the strength of the overall evidence available and should be decided on a case-by-case basis.

List of examples:

EXAMPLES ED ENV 1 (SEE SECTION 4.2.5.1)

Example 1: Classification as ED ENV 1 of a substance already classified as Repr. 1B and ED HH 1. There are no data available in fish or other wildlife organisms, therefore classification is solely based on data on mammals showing adverse effect(s) at population level. The example is focused on EAS modalities (SCL is the same as calculated for ED HH classification).

Example 2: Classification as ED ENV 1 based on fish data. The example is focused on EAS modalities (SCL calculation: GCL to be applied as no SCL is derived) for a data-rich substance.

Example 3: Classification as ED ENV 1 based on fish data. The example is focused on EAS modalities (SCL calculation: GCL to be applied as no SCL derived) for a data-poor substance.

4.2.5.1. Examples ED ENV 1

4.2.5.1.1. Example 1 - ED ENV 1 (EAS modalities)

Available information in mammals and conclusion for classification as ED HH 1.

See information in example 1 in section 3.11.5.1.1 of this guidance.

Available information for environment:

There is no aquatic *in vivo* long-term data for fish and other aquatic vertebrates.

The assessment for the environment is based on the mammalian data used for the human health assessment.

There is no additional mechanistic information available which was not already considered with regard to human health.

Assessment:

Adverse effect(s):

The adverse effects on uterus and ovarian weight, and oestrous cycle are considered '*EAS mediated*'. The effect on age at first oestrus is an '*EAS mediated*' parameter and provides clear evidence of an endocrine MoA. This is further supported by the observed effects on corpora lutea and litter size that are considered '*sensitive to, but not diagnostic of, EAS*' parameters, indicating a wider pattern of effects likely to be EAS mediated. All effects are observed in the absence of other toxic effects. The pattern of effects identified is considered relevant at the level of population for wild mammals.

Endocrine activity:

In the absence of additional information specific to the environment, the assessment with regard to human health is fully applicable for environment. As explained in section 3.11.5.1.1., there is a positive uterothrophic assay indicating estrogenic activity, further supported by QSAR predictions and ER binding capacity.

Biologically plausible link:

There is evidence of a biologically plausible link because the parameters measured *in vivo* that contribute to the evaluation of adverse effect(s) at population level at the same time provide evidence for specific EAS modes of action. Due to the nature of the effect and the existing knowledge on mammalian reproductive endocrinology, these adverse effects are considered diagnostic of an EAS MoA and thus (in the absence of other explanations) also infer an underlying *in vivo* mechanism.

Conclusion:

The substance caused significant effects on fertility (such as reduction in number of corpora lutea, reduced number of implantation sites, reduced litter size) in reproductive toxicity studies leading to a reduced number of offspring.

As effects on growth, development and reproduction in single species are generally regarded relevant for the maintenance of wild populations, the observed effects on reproduction and pubertal development in rats are relevant for mammalian populations in the environment (wild mammals).

Therefore, it is concluded that the substance meets the CLP criteria for ED ENV 1.

SCL calculation:

The ED classification is derived based on the mammalian data, therefore the SCL as calculated for the ED HH classification should be used. For details on calculation of SCL see HH example 1, section 3.11.5.1.1 of this guidance. According to mammalian data no SCL needs to be set for this substance.

4.2.5.1.2. Example 2 - ED ENV 1 (EAS modalities)

Available information:

The substance was concluded not to meet the CLP criteria as ED HH due to the absence of a pattern of '*EATS-mediated*' adversity.

In vivo information:

- Fish full lifecycle test conducted with sheepshead minnow *Cyprinodon variegatus* (FFLCT, OPPTS 850.1500, reliability 1, 100 days exposure, measured test concentrations: 0, 0.016, 0.038, 0.068, 0.15, 0.29, 0.55 mg/L):
    - No effects on hatching success or survival of F0.
    - Effects on hatching success in F1 generation observed, but at concentrations where reproduction was severely decreased and thus this information in the F1 is likely to reflect the quality of eggs produced.
    - No effects on weight and length of larvae of F0.
    - Reproduction (fecundity) significantly reduced at 0.15, 0.29 and 0.55 mg/L (NOEC = 0.068 mg/l (mean measured)).
    - F1 hatching success significantly reduced at 0.29 and 0.55 mg/L.
    - F1 28-day post-hatch survival significantly reduced at 0.55 mg/L.
    - Gonad histopathology not assessed.

- Fish full lifecycle test conducted with fathead minnow *Pimephales promelas* (FFLCT, OPPTS 850.1500, reliability 1, 256 days exposure, test concentrations: 0, 0.0078, 0.022, 0.063, 0.188 and 0.558 mg/L) with inclusion of all the parameters foreseen to be investigated in the OECD TG 240:
    - No effects on hatching success or fertility of F1 or F2 generations.
    - No statistically significant effects on weight and length of F1 generation.
    - No statistically significant effects on sex ratio in the F1 generation.
    - Reproduction significantly reduced at 0.558 mg/L in both the F0 and F1 reproductive groups (NOEC = 0.188 mg/L).
    - Delayed maturation/time to first spawn in F1 generation at 0.558 mg/L.

  - Increased gonado-somatic-index (GSI) in F1 males at 0.558 mg/L.
  - Increased GSI in F1 females at 0.063, 0.188 and 0.558 mg/L.
  - Increased tubercle score in F1 males at 0.022, 0.063, 0.188 and 0.558 mg/L.
  - Statistically significant decrease in F1 female VTG plasma concentration starting from 0.188 mg/L.
  - No effects on F1 male VTG plasma concentration.
  - Gonadal histopathology results:
    - Decreased yolk formation, decreased post-ovulatory follicles, and decreased mean ovarian stage scores in the ovaries of females at 0.558 mg/L;
    - Increased interstitial cell hyperplasia (number)/hypertrophy (volume) at 0.063, 0.188 and 0.558 mg/L, and increased spermatozoa at 0.558 mg/L in male testis
  - Liver histopathology results:
    - Increased nuclear pleomorphism, multi-nucleation, cystic degeneration, necrosis, pigmented macrophages, aggregates, and anisocytosis in hepatocytes of males and females at 0.558 mg/L.
    - Instances of nuclear pleomorphism in males at 0.188 mg/L.
    - Decreased basophilia (vitellogenesis) in female hepatocytes at 0.558 mg/L.
    - No effects on basophilia in male livers.

- Fish short term reproduction assay with fathead minnow *Pimephales promelas* (FSTRA, OECD TG 229, reliability 1, 21-day exposure, test concentrations: 0, 0.01, 0.12 and 1.0 mg/L):
  - Decreased fecundity and fertilisation success at 1.0 mg/L (note increased fecundity observed at 0.12 mg/L but this was not deemed biologically significant).
  - Increased male and female GSI at 1.0 mg/L.
  - Decreased vitellogenin in females at 1.0 mg/L.

- Study with elements of OPPTS Guideline 890.1350 and OECD 229 with fathead minnow *Pimephales promelas* (21-day exposure, test concentrations: 0, 0.005, 0.05, 0.5 and 1 mg/L, reliability 1):
  - No effects on nuptial tubercles.
  - Increased male and female GSI at 0.5 and 1 mg/L.
  - Decrease in cumulative number of eggs per female at 0.5 and 1 mg/L (a decrease was also noted at 0.05 mg/L but without concentration-response).
  - Decreased 17β-estradiol in females at 0.5 and 1 mg/L.
  - Decreased vitellogenin in females at 0.05, 0.500 and 1 mg/L.
  - Gonad histopathological results:
    - increased prevalence of spermatozoa,
    - distended seminiferous tubules at 1 mg/L.
  - Some limited increase and decrease in ovarian expression of several genes related to steroidogenesis (increase in: fshr, star, cyp11a, cyp17, and cyp19a1a; decrease in: hmgr and cyp51). These were generally inconsistent and very small changes, in most instances ≤1 fold difference, and were considered not biologically significant. The up-regulation observed in genes coding for cyp19a1a was around 2-3 fold at 0.5 and 1 mg/L. This was statistically significant and could be considered biologically significant.
  - Some limited increases and decreases in hepatic expression of several genes coding for proteins related to metabolism (increases in: cyp3a; decreases in: hmgr, fasn, fdps and cyp51). These changes were generally small and inconsistent and the limit of quantification (LOQ) of the methodology could not be established. Statistically significant up-regulation in the gene coding for

cyp1a1 (xenobiotic metabolising enzyme) appeared dose responsive at all concentrations, and was up-regulated in the region of 4-fold in the highest concentration.

- Non-guideline study with newly fertilised fathead minnow *Pimephales promelas* embryos exposed to concentrations of 0.069, 0.12, 0.21, 0.43 and 0.97 mg/L for 4 days and after hatching were exposed for a further 31 days (study reliability 2):
  - No effects on hatching success.
  - Larval growth (length and weight) significantly reduced at concentrations of 0.97 mg/L.
  - Larval survival significantly reduced at concentrations of 0.97 mg/L.
  - Any growth/development effects only observed at concentrations equivalent to those at which effects on survival were observed.

- Rapid Androgen Disruption Activity Reporter assay with spg1-gfp transgenic medaka eleutheroembryos (RADAR assay, OECD TG 251, reliability 1, 3 days exposure, test concentrations: 0, 0.003, 0.009, 0.027, 0.081, 0.243 mg/L):
  - No mortality at any test concentration. No observation of malformations or behavioural effects.
  - Unspiked condition: no statistically significant change in fluorescence.
  - Spiked condition: statistically significant concentration-dependent decrease in fluorescence indicating inhibition of 17α-methyltestosterone-induced spiggin production in transgenic medaka eleutheroembryos.

In vitro information:

All assays reported below have a reliability 1-2 and no cytotoxicity was reported.

- Inhibition of CYP19 activity (IC50=6.5 µM) in human placental microsomes.
- Competitive inhibition of CYP19 activity in H295R cell line.
- Positive in recombinant human microsome aromatase activity inhibition assay
- Inconclusive results on aromatase activity inhibition in a JEG-3 cell line.
- Negative for agonism and positive for antagonism modulation of testosterone- in MCF-7 cell line proliferation assay.
- 185-fold selectivity for inhibition of yeast (*Candida albicans*) CYP51 compared to human CYP51 in yeast and human CYP51 expressed in bacteria.
- Binding to zebrafish CYP51 with a much lower affinity than yeast.
- Negative for both agonism and antagonism ER activation in human ERα or ERβ transfected into CHO cell line.
- Weak positive for agonism ER activation in yeast estrogen screen.
- Negative for agonism, positive for antagonism ER activation in MCF-7 cell line proliferation assay.
- Negative for binding in rat uterine ER.
- Weak positive for agonism, negative for antagonism ER activation in MVLN cell line.
- Positive for AR binding in immuno-immobilised human AR.
- Negative for agonism, positive for antagonism AR activation in human AR transfected into CHO, CHO-K1, and MDA-kb2 cell lines.
- Inhibition of estrone biosynthesis in human ovarian granulosa tumour cells.
- Decreased oestradiol and testosterone biosynthesis in H295R cell line.
- Decreased estrogen biosynthesis at ≥1000 µg/L (≥3 µM).
- No effect on testosterone biosynthesis in ovary explants from fathead minnow.
- Positive toxcast in NCGC_ERalpha_Antagonist and NVS_NR_hAR.

Assessment

Adverse effect(s):

A pattern of potentially endocrine-related adverse effects relevant at the population level was observed across studies and species: decrease in fecundity was observed, accompanied by changes in gonad histopathology in both males and females.

The endocrine-related adverse effects were observed in the absence of other toxic effects. Although some effects in liver were observed in one of the available studies, currently there is no proven correlation between hepatotoxicity and effects due to endocrine disruption.

Endocrine activity:

Several *in vitro* assays are available showing positive evidence for androgen antagonism and aromatase inhibition (inhibition of CYP19).

In addition, a FSTRA and a 21-d assay were available. In one of the 2 available FFLCTTs *in vivo* mechanistic parameters were also measured.

Estradiol and testosterone were only measured in the 21-d assay. Decrease in the level of estradiol was observed in a dose response manner (0.5 and 1 mg/L) both *ex vivo* and in plasma. A decrease in testosterone was only observed *ex vivo* at the highest tested concentration.

VTG was measured in 3 studies and a decrease was observed in females in all of them. The decrease observed is empirically supported by the dose response. Difference between studies can be explained by the study design and dose spacing.

The endocrine activity gives indication of activity through A and S modalities.

Biologically plausible link:

Considering the observed endocrine activity and adverse effect(s), two MoAs can be postulated: aromatase inhibition leading to reproductive failure and androgen antagonism leading to reproductive failure.

For the first MoA:

	Brief description of key event	**Supporting evidence**
MIE	Inhibition of aromatase	Several *in vitro* assays showing positive evidence
KE1	Decreased level of estradiol ex vivo in ovaries	Decrease observed in one 21-day assay with fish
KE2	Decreased level of estradiol in plasma	Decrease observed in one 21-day assay with fish
KE3	Decreased VTG level in plasma	Decrease observed in 2 level 3 studies and one FFLCTT

KE4	Change in female gonad histopathology	Change in gonad histopathology observed in 1 level 3 study and one FFLCTT
Adverse effect	Decrease in fecundity	Decrease observed in 2 FFLCTTs and 2 level 3 studies

An additional MoA for androgen antagonism was postulated. However, this is not completely supported by the available data. No decrease in testosterone was observed *in vivo*. No changes in male secondary sex characteristics were recorded or on fertility. Therefore, the substance is not likely to be acting as an androgen antagonist. The most plausible MoA is the aromatase inhibition leading to reproductive failure.

Conclusion:

Overall, in all the available studies and in two species, a decrease in fecundity was observed in a dose response manner. When assessed, this was accompanied by changes in female gonad histopathology.

Endocrine activity, *i.e.*, inhibition of aromatase, was also observed *in vitro* and *in vivo*.

Considering all the available information on *in vitro* and *in vivo* mechanistic parameters and EAS-mediated parameters it can be concluded that the substance meets the CLP ED criteria Category 1 for the EAS-modalities for the environment.

SCL calculation:

No observed effect concentration ($NOEC_{reproduction}$ = 0.05 mg/L), thus according to Table 4.2.2, section 4.2.2.6.1 of this guidance, substances with 0.001 mg/L<NOEC≤0.1 mg/L result in a medium potency group corresponding to a GCL (0.1%). Therefore, no SCL will be set.

4.2.5.1.3. Example 3 - ED ENV 1 (EAS modalities)

Available information:

The substance was concluded not to meet the CLP criteria as ED HH due to the absence of a pattern of '*EATS-mediated*' adversity.

In vivo information

- Fish sexual developmental test with fathead minnow *Pimephales promelas* (study similar to OECD 234, exposure over 128 days, test concentrations of 0, 9.6, 27, 83, 255 µg/L, reliability 1):
  - Secondary sexual characteristics (proportion of male fish with a pigmented spot on dorsal fin, with pigmentation on the nose/lip, with a fat pad present, fat pad score of male fish, proportion of male fish with one or more tubercles present) in male fish significantly decreased at 27, 83, 255 µg/L (NOEC = 9.6 µg/L).
  - No effect on sex ratio.
  - One fish with testis-ova observed at 255 µg/L. This fish also had feminized gonadal ducts.
  - Retained peritoneal attachments/gonadal duct feminization of the testis: at 255 µg/L almost all male fish (42 out of 45) exhibited feminization of gonadal ducts.

- Stage testis development affected with the highest proportion of fish in all treatments in entirely immature phase or even juvenile phase (54 to 69 %) compared to control fish with 33 %.
- Length and weight slightly reduced at 27 µg/L and higher concentrations in males and females; (NOEC=9.6 µg/L).
- Time to hatch significantly increased at 255 µg/L.
- Significant decrease (90%) in larvae/juvenile survival from post-hatch to thinning on day 33 at 255 µg/L.
- Statistically significant VTG induction observed only in females at 83, 255 µg/L.

- Modified juvenile growth test with pikeperch *Sander lucioperca* (fish were exposed from 60 dph to 88 dph and further reared without exposure until 144 dph, test concentrations 0, 10, 100, 200 µg/L, reliability 2 as well conducted study but no raw data available):
  - Statistically significant and concentration dependant sex ratio shift towards more females and less males at 10 µg/L and above (from 58% females at 10 µg/L to 98% females at 200 µg/L).
  - No males were observed at the highest test concentrations (100 and 200 µg/L).
  - Results at day 144 show that the effects on sex ratio persist even after exposure has ceased.
  - Statistically significant and concentration dependant VTG induction observed both in males and females in all treatments.

- Modified reproduction assay with Japanese medaka *Oryzias latipes* (14 days, tested concentrations 0, 151, 453, 1510 µg/L, reliability 3):
  - Significant decrease in number of hatchings and unfertilized eggs at the lowest concentration of 151 µg/L.
  - Reduced average number of hatchings at higher concentrations (453 and 1510 µg/L), but not significant due to high replicate variances.

In vitro information:

All assays reported below have a reliability 1-2 and no cytotoxicity was reported.

- All available competitive binding assays using fish receptors showed that the substance binds to the ER receptor. The relative binding affinity (RBA) was 1.4 – 7.7E-5.
- Binding to sex steroid binding proteins (in plasma of rainbow trout).
- Dose-dependent increase in vitellogenin expression in primary fish hepatocytes.
- Weak ER agonist in a reporter gene assay based on recombinant yeast cells.
- Induction of human breast cancer cell (MCF-7) proliferation in four studies and thus acts as ER agonist in these cells.
- No interference with growth or survival of the immature rat ovarian follicles (from 14-day-old rat) but decreased estradiol and testosterone secretion in a dose-dependent manner.

Assessment

Adverse effect(s):

A pattern of potentially endocrine-related adverse effects relevant at the population level was observed across studies and species: change in sex ratio and decreased secondary sex characteristics in males accompanied by changes in male gonad histopathology. Decreased fertility was observed in one study not considered reliable. The effects were observed below the concentration at which excessive toxicity was observed.

Endocrine activity:

In vitro data unambiguously show that the substance acts as a ligand of the estrogen receptor in fish and mammalian cells. Modulation of ER-mediated gene expression was observed on transcriptional, protein and cell physiological levels showing that the substance activates fish and mammal estrogen receptors. Moreover, based on the available mechanistic information (*e.g.* VTG) it can be concluded that the substance has the potential to exert estrogen-like effects and disrupt endocrine homeostasis.

Biologically plausible link:

Information on endocrine activity of the substance points to an estrogenic mechanism of action. Endpoints indicative for an estrogenic MoA were assessed in three fish species (*P. promelas*, *S. lucioperca* and *O. latipes*) and a pattern of adverse effects was observed. A change in the sex ratio towards females was observed in at least one species (*S. lucioperca).* This change is both indicative of an endocrine MoA and adverse. This substantiates that the substance alters the function of the endocrine system in fish via an estrogenic MoA. Such an effect was observed in at least one species (*S. lucioperca).*

Conclusion:

There is convincing evidence for endocrine-related adverse effects in different fish species such as reduction of secondary sexual characteristics in males, accompanied by changes in gonad histopathology in one species and sex ratio shift towards more females and less males in another species; there is convincing evidence indicating that the substance has estrogenic activity; there is a plausible link with both adverse effect(s) and endocrine activity observed in the same study.

Based on the above, the substance meets the CLP criteria for ED ENV 1.

SCL calculation:

The No observed effect concentration is $NOEC_{growth}$=9.6 µg/L = 0.0096mg/L,, thus according to Table 4.2.2, section 4.2.2.6.1 of this guidance, substances with 0.001<NOEC≤0.01 result in a medium potency group corresponding to a GCL. Therefore, no SCL will be set.

It should be noted that a LOEC in a juvenile growth test (10 µg/L) is similar to the NOEC from the fish sexual development study used for SCL calculation. Thus the actual NOEC value could be significantly lower.

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4.3. PERSISTENT, BIOACCUMULATIVE AND TOXIC OR VERY PERSISTENT, VERY BIOACCUMULATIVE (PBT/VPVB) AND PERSISTENT, MOBILE AND TOXIC OR VERY PERSISTENT, VERY MOBILE (PMT/VPVM) PROPERTIES

4.3.1. Definitions and general considerations for PBT/vPvB and PMT/vPvM substances

Commission Delegated Regulation (EU) 2023/707, Annex I*: 4.3.1. and 4.4.1.* *For the purposes of sections 4.3 and 4.4 the following definitions shall apply:*

"PBT" means a persistent, bioaccumulative and toxic substance or mixture that meets the classification criteria set out in section 4.3.2.1.

"vPvB" means a very persistent and very bioaccumulative substance or mixture that meets the classification criteria set out in section 4.3.2.2.

"PMT" means a persistent, mobile and toxic substance or mixture that meets the classification criteria set out in section 4.4.2.1.

"vPvM" means a very persistent and very mobile substance or mixture that meets the classification criteria set out in section 4.4.2.2.

"log *K*oc" means the common logarithm of the organic carbon-water partition coefficient (i.e. *K*oc).

Annex I*: 4.3.1.2.* The hazard class Persistent, Bioaccumulative and Toxic or Very Persistent, Very Bioaccumulative properties is differentiated into:

- PBT properties and,
- vPvB properties.

Annex I: 4.4.1.2. The hazard class Persistent, Mobile and Toxic or Very Persistent, Very Mobile properties is differentiated into:

- PMT properties and,
- vPvM properties.

Definitions

Persistence (P) can be described as the resistance of chemicals to transformation and degradation processes. In addition, Annex II of REACH on the requirements for the compilation of safety data sheets defines persistence "*as the lack of demonstration of degradation, as defined in Annex XIII, sections 1.1.1 and 1.2.1.*" Degradability is further defined in REACH Annex II, 12.2 as "*the potential for the substance or the appropriate substances in a mixture to degrade in the environment, either through biodegradation or other processes, such as oxidation or hydrolysis*". Degradation may be biotic or abiotic and may take place in both aerobic and anaerobic conditions.

Bioaccumulation (B) is the net result of uptake, transformation and elimination of a substance in an organism due to all routes of exposure (*i.e.* air, water, sediment/soil and food) (CLP Annex I, 4.1.1.1.(e)). Annex I specifies that 'bioconcentration' means the net result of uptake, transformation and elimination of a substance in an organism due to waterborne exposure (CLP Annex I, 4.1.1.1.(f)).

Mobility (M) refers to the potential of a substance once released to the environment to reach water bodies, including drinking water resources and groundwater. REACH Annex II, section 12.4 defines mobility in soil as "*the potential of the substance or the components of a mixture, if released to the environment, to move under natural forces to the groundwater or to a distance from the site of release*". Mobile substances possess moderate to (very) low adsorption potential, as indicated by the organic carbon-water partition coefficient (*i.e.* K_{OC}, see section 4.3.3.3.1).

Toxicity (T) refers to the intrinsic property of a substance to cause adverse effects to humans, wildlife, plants and/or other environmental organisms as a result of the exposure to the substance itself.

CLP refers explicitly to the combination of these properties that poses concern, for example the combination of not easy to break down in the environment and tendency to accumulate in living organisms (for PBTs/vPvBs) and high persistence and high mobility (for PMTs/vPvMs). More definitions of the relevant terminology are included in the respective sections of this Guidance.

Historical developments on PBT/vPvB and PMT/vPvM assessment

For more than 30 years, regulatory Authorities throughout the world have been assessing the hazards caused by substances that possess persistent, bioaccumulative and toxic (PBT) and very persistent, very bioaccumulative (vPvB) properties. These properties indicate that such substances remain in the environment, they tend to accumulate in living organisms and they could give rise to toxic effects. Additionally, exposure to the environment (including pristine/remote regions and humans) is difficult to reverse, as environmental concentrations do not readily decrease by lowering emissions (Commission Delegated Regulation (EU) 2023/707, recital (7)). Between 1994 and 2007, 141 risk assessments have been performed and concluded by different Member States[39] under Council Regulation (EEC) No 793/93, Existing Substances Regulation (ESR). Since the entry into force of Regulation (EC) No 1907/2006, the 'REACH' Regulation, the identification of substances with PBT and/or vPvB properties entailed the comparison with the criteria stipulated in Annex XIII of REACH, where all available information is assessed in a weight of evidence determination (WoE), in particular where the criteria set out in section 1 of Annex XIII cannot be applied directly to the available information. The same applies to the PBT/vPvB assessment under the Biocidal Products Regulation (BPR, Regulation (EU)

[39] https://echa.europa.eu/information-on-chemicals/information-from-existing-substances-regulation

528/2012)) and the Regulation (EC) No 1107/2009 (Plant Protection Products Regulation, 'PPP' or 'PPPR')[40].

The experience and accumulated scientific knowledge in PBT/vPvB assessment and the need of protection for the environment regarding Substances of Very High Concern (SVHCs) were the trigger for the European Commission to propose the introduction of a new hazard class (HC) in Regulation (EC) No 1272/2008 ("CLP" Regulation) regarding substances with PBT and/or vPvB properties. Due to the similarity of their properties, with the exception of toxicity, the Commission has proposed one single new hazard class, with differentiation, while establishing common rules for the scientific assessment of the intrinsic properties related to persistence and bioaccumulation. The overall aim of PBT/vPvB assessment undertaken either under the REACH Regulation or under CLP is to ensure a high level of protection for human health and the environment.

In recent years, substances that break down slowly in the environment and have a high environmental mobility, often reaching water resources, have received increased scientific and regulatory attention. The German Authorities (UBA) first proposed to name such substances in the regulatory context of REACH as PMT/vPvMs (Neumann *et al*., 2015, Neumann and Schliebner, 2019). These substances possess persistent, mobile and toxic (PMT) and/or very persistent and very mobile (vPvM) properties, often reaching (drinking) water resources, they are only partly removed by wastewater and drinking water treatment processes, they can spread over long distances and also cause environmental exposures that are difficult to reverse (Commission Delegated Regulation (EU) 2023/707, recital (8), Neumann and Schliebner, 2019). The European Commission proposed a new hazard class (with differentiation) to be introduced in CLP also regarding substances with PMT and/or vPvM properties, with the overall aim being to ensure a high level of protection for human health and the environment, focussing on waters, including drinking water.

The following sections of the present Guidance document will outline the respective CLP criteria, identify the different sources of relevant information, detail the different assessment elements to be taken into account by Authorities and data holders and provide guidance on how to compare the available information with the CLP criteria to come to a conclusion on whether classification in either of the related hazard classes may apply. The following apply to single substances (mono-constituent substances under REACH and CLP) and their relevant impurities, constituents and/or degradation products, with further considerations on mixtures described in section 4.3.6. As clearly indicated in CLP, the two new hazard classes (PBT/vPvB and PMT/vPvM) apply only to all organic substances, including organo-metals. This is because PBT/vPvB assessment under REACH was defined in Annex XIII as "generally applicable to any substance containing an organic moiety. Based on the common definition of an organic substance in chemistry, PBT and vPvB criteria are not applicable to inorganic substances" (ECHA Guidance on IR&CSA, Chapter R.11.2.1). Furthermore, inorganic substances are out of the scope of the PBT/vPvB and PMT/vPvM assessment under CLP[41].

4.3.2. CLP criteria for PBT/vPvB and PMT/vPvM substances

The following sections (green texts) merely reproduce CLP Annex I regarding the CLP criteria for the individual properties. Further elaboration on these can be found in subsequent sections of the Guidance (4.3.3).

[40] For the explicit Regulatory context of approval/renewal under Reg. 1107/2009, European Commission (2012) outlines the POP, PBT and vPvB assessment elements of new/existing active substances and the initial establishment of a list of Candidates for Substitution (CFS). These principles differ to the respective hazard assessment under CLP.

[41] It is noted that this does not automatically apply to related principles in other Regulations. For example, inorganic substances are subjected to PBT assessment according to Regulation (EC) No 1107/2009.

4.3.2.1. Persistence criteria

Annex I: 4.3.2.1.1. and 4.4.2.1.1. A substance shall be considered to fulfil the persistence criterion (P) where any of the following conditions is met:

(a) the degradation half-life in marine water is higher than 60 days;

(b) the degradation half-life in fresh or estuarine water is higher than 40 days;

(c) the degradation half-life in marine sediment is higher than 180 days;

(d) the degradation half-life in fresh or estuarine water sediment is higher than 120 days;

(e) the degradation half-life in soil is higher than 120 days.

Annex I: 4.3.2.2.1 and 4.4.2.2.1 A substance shall be considered to fulfil the 'very persistent' criterion (vP) where any of the following situations is met:

(a) the degradation half-life in marine, fresh or estuarine water is higher than 60 days;

(b) the degradation half-life in marine, fresh or estuarine water sediment is higher than 180 days;

(c) the degradation half-life in soil is higher than 180 days.

4.3.2.2. Bioaccumulation criteria

Annex I: 4.3.2.1.2. A substance shall be considered to fulfil the bioaccumulation criterion (B) where the bioconcentration factor in aquatic species is higher than 2000.

Annex I: 4.3.2.2.2. A substance shall be considered to fulfil the "very bioaccumulative" criterion (vB) where the bioconcentration factor in aquatic species is higher than 5 000.

4.3.2.3. Mobility criteria

Annex I: 4.4.2.1.2. A substance shall be considered to fulfil the mobility criterion (M) when the log K_{OC} is less than 3. For an ionisable substance, the mobility criterion shall be considered fulfilled when the lowest log K_{OC} value for pH between 4 and 9 is less than 3.

Annex I: 4.4.2.2.2. A substance shall be considered to fulfil the 'very mobile' criterion (vM) when the log K_{OC} is less than 2. For an ionisable substance, the mobility criterion shall be considered fulfilled when the lowest log K_{OC} value for pH between 4 and 9 is less than 2.

4.3.2.4. Toxicity criteria

Annex I: 4.3.2.1.3. and 4.4.2.1.3. A substance shall be considered to fulfil the toxicity criterion (T) in any of the following situations:

(a) the long-term no-observed effect concentration (NOEC) or ECx (e.g EC10) for marine or freshwater organisms is less than 0,01 mg/l;

(b) the substance meets the criteria for classification as carcinogenic (category 1A or 1B), germ cell mutagenic (category 1A or 1B), or toxic for reproduction (category 1A, 1B, or 2) according to sections 3.5, 3.6 or 3.7;

(c) there is other evidence of chronic toxicity, as identified by the substance meeting the criteria for classification as specific target organ toxicity after repeated exposure (STOT RE category 1 or 2) according to Section 3.9;

(d) the substance meets the criteria for classification as endocrine disruptor (category 1) for human health or the environment according to sections 3.11 or 4.2.

4.3.3. Identification and assessment of hazard information for PBT/vPvB and PMT/vPvM substances

The following sections will present in detail the information that can be used for classification and labelling purposes when assessing the PBT/vPvB and PMT/vPvM properties, as well as other related assessment elements (corresponding to the section named "Interpretation of data" in this Guidance, section 4.1). Before proceeding to the identification of the relevant information and its regulatory assessment, a number of general points have been assembled that are relevant for the consideration of all hazard properties discussed. These include:

(i) **data availability and quality**

CLP refers to the identification of all relevant available information for the purposes of determining whether the substance entails a physical, health or environmental hazard as set out in its Annex I. Available data should be based on methods referred to in Article 13(3) of the REACH Regulation[42], or sound scientific principles that are internationally recognised or methods validated according to international procedures (CLP Article 8). The CLP Article further expands on the scientific principles that should be followed by manufacturers, importers or downstream users during the performance of any new tests for the purpose of determining whether a substance or a mixture entails a human health or environmental hazard, provided that all other means of generating information have been exhausted. Furthermore, scientific information must be in accordance to standardised test methods, where available. In the presence of such information, results from reliable experimental studies conducted under Good Laboratory Practice (GLP), as well as data from comprehensively reported, peer-reviewed academic studies, generally receive higher weight over estimated/predicted values for the classification and labelling of the substance.

CLP Annex I, 4.1.1.2.2 and section 4.1.3.1.2 of this Guidance further expand on the use of other data than from standardised studies, stating that "*in practice data from other standardised test methods such as national methods shall also be used where they are considered as equivalent*". Data from non-standard studies and non-testing methods shall be considered in classification provided that they fulfil the requirements specified in section 1 of Annex XI to the REACH Regulation (1.1.2). Based on these legal provisions, RAC has previously formed opinions on the harmonised classification and labelling of substances referring to aquatic hazards using data from non-standard test methods. In all cases, the classification should be based on the best available data (CLP Annex I, 4.1.1.2.2; see also part 1 of Annex I to CLP).

Concerning active substances in accordance with the PPP Regulation, the Commission Regulation (EU) No 283/2013 of 1 March 2013 (European Commission, 2013) sets out the data requirements, while the related Commission Communication provides test methods and guidelines for the active substances in plant protection products. Concerning active substances in accordance with the BPR, ECHA (2022c) further details the information requirements and relevant test methods for biocides.

CLP does not introduce any direct responsibilities to generate new information, but in case of any new testing being carried out for the purposes of the CLP Regulation, Article 7 explicitly states that any testing on animals within the meaning of Directive 86/609/EEC shall only be undertaken where no other alternatives exist that would provide reliable, high quality data. In the absence of adequate experimental information, other information indicative of P, B, M or T properties such as qualitative or quantitative structure-activity relationships ((Q)SARs), suitable *in vitro* tests, information from the application of the category approach (grouping, read-across) and other

[42] Regulation (EC) No 440/2008 further lays down test methods pursuant to REACH Article 13(3)

types of available information (for example, monitoring data, if appropriate) may be used in a WoE determination (see point below, but also within the sections for the individual properties).

Furthermore, the European Court of Justice has confirmed that the application of the precautionary principle can be taken into consideration in the context of the classification of a substance under CLP, where the assessment of the risks of that substance to the environment and to human health gives rise to uncertainty[43]. In this context, when more than one reliable experimental study result (value) is available for the same property, in most cases the most conservative value is used in order to account for the uncertainties of the test method and differing experimental conditions. This is in line with both the long-established PBT/vPvB assessment approach used, for example, for the identification of Substances of Very High Concern (SVHC) under REACH Article 57 (d)/(e) and with the approach used for harmonised classification of substances under CLP. Section 4.3.4 further outlines some general considerations on the application of the WoE.

There may be exceptional situations where it is appropriate to combine several study results to generate a value for comparison with the CLP criteria. This is discussed in section 4.3.4, as well as under the respective sections 4.3.4, 4.3.4.1, 4.3.4.2, 4.3.4.3, 4.3.5.1, where the conditions that need to be met for combining results from reliable studies are detailed.

(ii) relevant conditions

Sections 4.3.2.3 and 4.4.2.3 of Annex I of CLP state that the information used for the purposes of assessment of the PBT/vPvB and PMT/vPvM properties shall be based on data obtained under relevant conditions that allow for an objective assessment of these substance properties instead of under particular environmental or 'realistic' conditions that may vary considerably across the European Union. In other words, as confirmed by both ECHA's Board of Appeal and the European Court of Justice, the purpose of the PBT/vPvB assessment is meant to clarify the intrinsic property of the substance irrespective of the local/specific environmental conditions and taking into account the physico-chemical properties of the substance[44]. Furthermore, a study is considered to be performed under relevant conditions if it is performed in accordance with the testing conditions provided for in the Test Methods Regulation ((EC) No 440/2008)[45], in line with Article 13(3) of the REACH Regulation and bullet point (i) above. These considerations also hold true for the PBT/vPvB and PMT/vPvM assessment under CLP.

Property specific considerations of relevant conditions are presented in this Guidance under each respective property, when relevant, and in ECHA Guidance on IR&CSA, Chapters R.11, R.7b and R.7c.

(iii) use of (Q)SARs and read-across approaches

(Q)SAR predictions can be used together with other information in the WoE determination. When using (Q)SARs to predict a substance property, an assessment of both the model and the prediction is needed. A (Q)SAR model must be scientifically valid (using OECD principles (OECD, 2004; OECD, 2007)) and adequate and reliable documentation must be provided. A valid (Q)SAR

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[43] See paragraphs 96 to 98 of the judgment of the Court of Justice in *SGL Carbon and others v.* Commission, joined Cases C-65/21 P and C-73/21 P to C-75/21 P, not yet published, EU:C:2022:470 accessible at the following link: https://curia.europa.eu/juris/document/document.jsf?text=%2522coal%2Btar%2522&docid=260991&pageIndex=0&doclang=EN&mode=lst&dir=&occ=first&part=1&cid=1798278#ctx1..

[44] See judgment of the General Court in 3V Sigma v. ECHA, Case T-176/19, not yet published, EU:T:2020:621 (https://curia.europa.eu/juris/liste.jsf?language=en&td=ALL&num=T-176/19) and the summaries of the relevant ECHA Board of Appeal decisions in section 11.4 of the Board of Appeal digest of decisions available at https://echa.europa.eu/documents/10162/2314761/digest_of_decisions_of_boa_en.pdf.

[45] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008R0440

model does not necessarily produce an acceptable prediction. For an acceptable (Q)SAR prediction, the input is correct, the substance falls within the applicability domain of the model, the prediction is reliable, and the outcome is fit for the regulatory purpose. The validity of models and predictions can be assessed by using the OECD (Q)SAR assessment framework (QAF) (OECD, 2023).

Transparent documentation of the validity of the models, as well as for reporting information relevant for judging the reliability of predictions for individual compounds or other comparable documentation must be provided. A (Q)SAR Model Reporting Format (QMRF) displays a description of the (Q)SAR model relative to the five OECD (Q)SAR validation principles in a systematic and summarised way (OECD 2004, 2007; minor update OECD 2023). The information about the (Q)SAR prediction is reported in the (Q)SAR Prediction Reporting Format (QPRF). An updated QPRF template was published in 2023 and it reflects the newly established OECD (Q)SAR Prediction Principles (OECD, 2023).

More information can be found in OECD (Q)SAR assessment framework documents (OECD, 2023), in the Guidance on (Q)SARs and grouping of chemicals, Chapter R.6[46] and in ECHA Practical Guide "How to use and report (Q)SARs"[47].

There are several recognised methodological challenges for P and B assessments. Despite a general preference on reliable experimental data over predicted data, (Q)SAR predictions may still be useful in the evaluation and interpretation of the laboratory studies.

Read-across is a technique for predicting endpoint information for one substance (target), by using data from the same endpoint from (an)other substance(s) (source). It needs to be clear how the read-across addresses the endpoint or property under consideration. The term "analogue approach" is used when the read-across approach is employed between a small number of structurally similar substances. As the number of substances is small, trends may not be apparent. As a result of structural similarity, a given (eco)toxicological/ environmental fate property of the source substance is used to predict the same property of the target substance. The "category approach" is used when read-across is employed between several substances that are grouped together based on defined structural similarity and allowable differences between the substances. Because of the structural similarity, the results will be either similar or follow a regular pattern. (Q)SAR predictions may furthermore be applied to support read-across, made both by the analogue and the category approach. They may provide information which can be used in the trend analyses as they often/typically extract trends over a larger span of chemicals, as well as in the establishment of the read-across hypothesis, and to analyse how the differences in source and target structures may change the property under analysis.

The basis for a prediction within the group for the target substance must be explicit (*e.g.* "worst case", or trend analysis). Use of the Read-Across Assessment Framework (RAAF, ECHA 2017a[48]) may help assess and, where necessary, improve the read-across. ECHA developed the RAAF based on the most frequently encountered types of read-across approaches in the different ECHA-managed regulatory processes.

The documents 'Practical Guide: How to use alternatives to animal testing' (ECHA 2016[49]) and 'ECHA Guidance on IR&CSA, Chapter R.6: QSARs and grouping of chemicals' (ECHA 2008), developed by ECHA, give further details on how to use and report read-across.

[46] https://echa.europa.eu/documents/10162/17224/information_requirements_r6_en.pdf

[47] https://echa.europa.eu/documents/10162/13655/pg_report_qsars_en.pdf

[48] https://echa.europa.eu/documents/10162/17221/raaf_en.pdf/614e5d61-891d-4154-8a47-87efebd1851a and https://echa.europa.eu/documents/10162/17228/raaf_uvcb_report_en.pdf/3f79684d-07a5-e439-16c3-d2c8da96a316

[49] https://echa.europa.eu/documents/10162/17250/pg_report_qsars_en.pdf/407dff11-aa4a-4eef-a1ce-9300f8460099

(iv) substances with more than one constituent, additives, impurities and UVCBs

CLP Annex I, 4.3.2.3 and 4.4.2.3 refer to the identification that "*shall also take account of the PBT/vPvB and PMT/vPvM properties of relevant constituents, additives or impurities of a substance …*". PBT/vPvB and PMT/vPvM assessment are exercises most easily performed on single substances with a well-defined identification. However, as discussed below, a substance may be composed by more than one single substance in a form of its constituents. The term UVCB is defined as substances of Unknown or Variable composition, Complex reaction products or Biological materials as further detailed in Chapter 4.3 of the Guidance for identification and naming of substances under REACH and CLP[50].

Constituents, impurities, and additives should normally be considered relevant for the PBT/vPvB and PMT/vPvM assessment when they are present in concentration of ≥ 0.1% (w/w). This limit of ≥ 0.1% (w/w) is set based on a well-established practice recognised in European Union legislation to use this limit as a generic limit[51]. Individual concentrations below 0.1% (w/w) normally do not need to be considered.

Importantly, a close structural similarity of individual constituents within a fraction of a UVCB substance, namely constituents with the same carbon number, chain lengths, degree and/or site of branching or stereoisomers, triggers the need to sum up the concentrations of these constituents and to compare the total concentration with the limit of ≥ 0.1% (w/w) in order to determine whether these constituents need to be covered in the PBT/vPvB assessment. This approach is also relevant for PMT/vPvM assessment. More detailed elaboration on the criteria for grouping or read across, is available in other sections of this and the REACH Guidance.

In order to comply with the CLP Annex I, 4.3.2.3 and 4.4.2.3 provisions on the PBT/vPvB and PMT/vPvM properties of the relevant constituents, characterisation and identification of UVCBs or fractions of impurities needs to take place, as comprehensively as possible. However, full characterisation may not always be possible or even necessary due to (i) the number of constituents/impurities being relatively large and/or (ii) the composition being, to a significant part, unknown, and/or (iii) the variability of composition being relatively large or poorly predictable. Regardless of whether full substance identification is possible or not for the whole composition, efforts should be made for carrying out a PBT/vPvB and PMT/vPvM assessment for all constituents, impurities and additives present in concentrations ≥ 0.1% (w/w). ECHA Guidance on IR&CSA, Chapter R.11: PBT/vPvB assessment includes further information on assessment of substance with complex composition.

The PBT/vPvB and PMT/vPvM assessment should be performed on each relevant constituent, impurity, and additives present in concentrations ≥0.1% (w/w). In order for the PBT/vPvB or PMT/vPvM criteria to be fulfilled, all respective criteria must be met for the same substance or at least one (but always the same one) individual constituent, impurity, additive or transformation/degradation product, if applicable. It cannot be concluded that a substance warrants a PBT/vPvB or PMT/vPvM classification when, for example, the assessment of persistence has been concluded for one constituent and the assessment of bioaccumulation, toxicity or mobility for another constituent.

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[50] https://echa.europa.eu/documents/10162/2324906/substance_id_en.pdf/ee696bad-49f6-4fec-b8b7-2c3706113c7d

[51] The limit of ≥ 0.1% (w/w) is indicated in the European Union legislation, where there is no specific reason (e.g. based on toxicity) to establish a concentration limit specific to the case. Examples of this generic concentration limit are, i.a., another category of substances of very high concern according to Article 57 of REACH, where the default concentration of Carcinogenic/Mutagenic (category 1A/1B) ingredients in a mixture requiring a Carcinogen/Mutagen (1A/1B) classification of the mixture under Regulation (EC) No 1272/2008 is ≥0.1% (w/w). Furthermore, Articles 14(2)(b), 31(3)(b) and 56(6)(a) of REACH apply a similar principle and the same concentration limit for PBT/vPvB substances in mixtures regarding some obligations under REACH. Additionally, the Judgments of the General Court (Seventh Chamber, extended composition) of 7 March 2013 in cases T-93/10, T-94/10, T-95/10 and T-96/10 (see in particular paragraphs 117 to 121) confirmed the validity of this approach for PBT/vPvB constituents of a substance.

As detailed in the ECHA Guidance on IR&CSA, Chapter R.11.4.2.2, there are three assessment approaches of substances containing multiple constituents, impurities and/or additives, namely the known constituent approach, the fraction profiling and the whole substance approach.

The **known constituent** approach can be applied when a substance is "*a priori*" known to contain specific constituents at relevant concentrations, these constituents are suspected based on available information to represent the worst case of these properties of all constituents of the substance, and these specific constituents can be isolated or separately manufactured. Depending on the quality and availability of information for all relevant constituents and properties, a conclusion as PBT/vPvB and/or PMT/vPvM for the whole substance may be drawn in case one or more constituent of the substance is proven to fulfil all the regulatory criteria. This approach has been applied in the SVHC identification of substances originating from coal tar distillation[52] (*e.g.* coal tar pitch, high temperature; anthracene oil) and also under Substance Evaluation. Advantages and disadvantages of this and the other two approaches are reported in ECHA Guidance on IR&CSA, R.11.4.2.2.2.

The **fraction profiling** approach is applied when, due to the complexity of the substance, it is not feasible to fully identify, assess or isolate single constituents but the substance can be divided into fractions/blocks. Within these blocks, the constituents must be structurally similar and their degradation, bioaccumulation and toxicity properties can be predicted to follow a regular predictable pattern.

The **whole substance** approach considers the substance to be one, assuming that all its constituents can be justified to be very similar and, therefore, can be expected to have reasonably similar PBT/vPvB and PMT/vPvM properties. Same principles in establishing similarity of constituents apply for mono-constituent, multi-constituent and UVCB substances. For such similarity criteria, refer to Chapter R.6 of the ECHA Guidance on IR&CSA, RAAF and advice on using read-across for UVCB substances.

In a regulatory context, information from the first two approaches is preferable to the last, as these provide more certain, transparent and detailed information. ECHA Guidance on IR&CSA, Chapter R.11 (R.11.4.2.2) further details certain circumstances that the whole substance approach can be used for certain endpoint-specific assessments. Regarding such substances containing more than one constituent where data on individual constituents are available, they should be evaluated and classified following the same classification rules as mixtures (section 4.3.6).

(v) relevant transformation/degradation products

CLP Annex I, 4.3.2.3 and 4.4.2.3 refer to the identification that "*shall also take account of the PBT/vPvB and PMT/vPvM properties of … relevant transformation or degradation products*". The PBT/vPvB and PMT/vPvM assessment should be performed on the substance and each of the relevant transformation/degradation product[53]. There is currently no set % w/w or molar threshold concentration for relevant transformation or degradation product in the CLP Regulation.

A transformation or degradation product can be considered relevant in the degradation tests for soil, water-sediment and water for example, when it is detected ≥10% of the applied concentration or radioactivity (dose) of the parent substance at any sampling time (principal transformation/degradation products) or when detected ≥ 5% in at least two sequential

[52] https://echa.europa.eu/registry-of-svhc-intentions

[53] Currently, Annex II of the EC No 1107/2009 refers only to the PBT/vPvB assessment of the active substance, safeners and synergists, while the transformation products and metabolites are not subject to a PBT/vPvB assessment. Under PPPR a metabolite is defined "relevant" if there is a reason to assume that it has intrinsic properties comparable to the parent substance in terms of its biological target activity, or that it poses a higher or comparable risk to organisms than the parent substance or that it has certain toxicological properties that are considered unacceptable.

measurements or the concentration is continuously increasing, or the concentration remains stable (once formed) in the test system indicating persistence (see also section 4.3.3.1.2.1, simulation tests in water, water-sediment and soil). In addition, lower percentages than these may be adopted in a case-by-case basis, with the assessment accounting for the overall hazardous profile of the substance and its relevant transformation/ degradation products, including the "*the rate of generation of the more hazardous degradation product (i.e., quantity produced and time frame) should be considered*" (section 4.1.3.3.1 of the current Guidance).

The PBT/vPvB and PMT/vPvM assessment should be carried out for each relevant transformation or degradation product. In all cases, any information that the substance may be mineralised quickly (not likely to form transformation/degradation products relevant for the assessment) or the opposite (based, for example, on results from hydrolysis studies or field data) must be carefully considered.

To provide some context of the set boundaries for the relevance of the transformation or degradation products, OECD test guideline (TG) requirements and data requirements in Regulation (EU) No. 283/2013 are shortly described below.

In simulation degradation tests, the concentration of the test substance and transformation products should be measured and reported at every sampling time. In general, transformation products detected at ≥ 10% of the applied concentration at any sampling time should be identified unless reasonably justified otherwise (OECD TGs 307, 308 and 309). OECD TGs 309 and 308 further specify that transformation products 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.

Regulation (EU) No. 283/2013, section 7 specifies that data on route of degradation in soil and aquatic systems shall be sufficient to identify:

- the individual components which in at least two sequential measurements, account for more than 5 % of the amount of active substance added;
- components present which at any time account for more than 10% of the amount of active substance added;
- and the individual components (> 5%) for which at the end of the study the maximum of formation is not yet reached.

For active substances in plant protection products, the Regulation (EU) No. 283/2013, section 7 further specifies that aerobic degradation (DegT50 and 90 values) from a minimum of three different soils shall be provided for metabolites, breakdown and reaction products which occur in soil if one of the following conditions is fulfilled:

- they account for more than 10% of the amount of active substance added at any time during the studies;
- they account for more than 5% of the amount of active substance added in at least two sequential measurements;
- the maximum of formation is not reached at the end of the study but accounts for at least 5% of the active substance at the final measurement;
- all metabolites found in lysimeter studies at annual average concentrations exceed 0.1 µg/L in the leachate.

(vi) Substances with nanoforms

Annex VI of REACH, on the basis of the Commission Recommendation of 18 October 2011, defines a nanoform as "*a form of a natural or manufactured substance containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in*

the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm, including also by derogation fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm".

When a form of a substance fulfils the criteria of the nanoform definition, specific considerations apply, with REACH Annex I currently noting that the PBT/vPvB assessment under REACH shall address also all relevant nanoforms.

More recently, Commission recommendation of 10 June 2022 on the definition of nanomaterial (2022/C 229/01) has updated the definition of nanomaterial as the one meaning "*a natural, incidental or manufactured material consisting of solid particles that are present, either on their own or as identifiable constituent particles in aggregates or agglomerates, and where 50 % or more of these particles in the number-based size distribution fulfil at least one of the following conditions:*

- *one or more external dimensions of the particle are in the size range 1 nm to 100 nm;*
- *the particle has an elongated shape, such as a rod, fibre or tube, where two external dimensions are smaller than 1 nm and the other dimension is larger than 100 nm;*
- *the particle has a plate-like shape, where one external dimension is smaller than 1 nm and the other dimensions are larger than 100 nm*".

ECHA Guidance on IR&CSA, Chapter R.11.4.2.1.4 reports on some key considerations regarding the PBT/vPvB assessment of substances with nanoforms. Appendices to ECHA Guidance on IR&CSA, Chapters R.7a, R.7b, and R.7c contain recommendations for assessment of nanomaterials in the context of the chemical safety assessment, under REACH. Future updates of the current CLP Guidance will include more information on the PBT/vPvB and PMT/vPvM assessment of nanoforms under CLP, once further experience on the regulatory handling of substances with nanoforms is gained.

(vii) assessment of "difficult" substances requiring special considerations

Some substance properties may lead to difficulties to both testing and the interpretation of study results. Thus, assessment of substances requiring special considerations refer to those that possess, for example, very high sorption potential, low solubility in octanol and/or water, high volatility, high instability in biotic or abiotic media, complex or multi-constituent substances including those in nanoforms, surface-active, ionisable and coloured substances. For some of these type of substances, standard test guidelines used to determine the different properties may not be directly applicable. Specific considerations for these substances are reported in ECHA Guidance on IR&CSA, Chapters R.11.4.2, but also in various sections in R.7b and R.7c, in section 4.1.3.2.2 of this Guidance, as well as in the "Guidance Document Aquatic Toxicity Testing of Difficult Substances and Mixtures" (no. 23) developed by OECD.

Several considerations relating to such substances will be incorporated in subsequent sections of this Guidance, for example, in 4.3.3.1-4.3.3.4, whilst specific considerations on ionisables are reported in detail in, among others, both the following bullet point and in section 4.3.3.3.6 of this Guidance.

(viii) specific considerations for ionisable substances

Ionisable substances are molecules able to dissociate, forming ionic compounds. In general, ionised organic substances do not readily diffuse across respiratory surfaces and other processes may play a role in uptake (*e.g.* complex permeation, carrier-mediated processes, ion channels, or ATPases). Dissociated and neutral chemical species can, therefore, have markedly different bioavailabilities. It is essential to know or estimate the dissociation constant pK_a to evaluate the

degree of ionisation in surface waters at environmentally relevant pH (pH 4-9, ECHA Guidance on IR&CSA, Chapter R.7a) and under physiological conditions (pH 3-9, ECHA Guidance on IR&CSA, Appendix R.7.10-3.

The balance between ionised and non-ionised forms of some substances varies with the pH of the solution in which a substance is dissolved. Since ionised and non-ionised forms can have different solubility in water, small changes in the pH can significantly alter the bioavailability of a substance in a toxicity test. When reviewing data from toxicity tests, the test design should consider the effects on dissociation equilibrium due to changes in the pH of test solution. Information on the toxicity of the two forms of a substance from preliminary tests can help in deciding the pH of the solution in the definitive test, that should be conducted in condition where the test organisms are exposed to the most toxic form, providing that this condition allows a healthy maintenance of the test organisms. Thereby, test solutions might have to be buffered in order for the test to be "*conducted at a pH consistent with the more toxic form of the substance, whilst remaining within the range required to maintain the health of the control organisms*" (EFSA, 2013). Specific indications on how to conduct toxicity tests with ionisable substances are reported in the OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (no.23).

Different sections of this Guidance, especially the one relevant to mobility (4.3.3.3.6), will elaborate further, more property-related considerations for ionisable substances.

4.3.3.1. Persistence assessment

Commission Delegated Regulation (EU) 2023/707, Annex I: 4.3.2.3.1. and 4.4.2.3.1. The following information shall be considered for the assessment of P or vP properties:

(a) results from simulation testing on degradation in surface water;
(b) results from simulation testing on degradation in soil;
(c) results from simulation testing on degradation in sediment;
(d) other information, such as information from field studies or monitoring studies, provided that its suitability and reliability can be reasonably demonstrated.

Annex I: 4.3.2.4.2. and 4.4.2.4.2. In applying the WoE determination, the following information, in addition to the information referred to in sections 4.3.2.3.1 and 4.4.2.3.1 shall be considered as part of the scientific assessment of the information relevant for the P, vP properties:

Indication of P or vP properties:

(i) Results from tests on ready biodegradation;
(ii) Results from other degradation screening tests (e.g. enhanced ready test, tests on inherent biodegradability);
(iii) Results obtained from well-developed and reliable biodegradation (Q)SAR models;
(iv) Other information, provided that its suitability and reliability can be reasonably demonstrated.

4.3.3.1.1. Persistence terminology

Abiotic degradation is transformation or degradation of a substance modified by non-biological mechanisms (*i.e.* physico-chemical processes) such as hydrolysis, oxidation and photolysis, etc.

Biodegradation is biologically mediated degradation of a substance, usually carried out by microorganisms. It can proceed in the presence of oxygen (aerobic biodegradation) or in the absence of oxygen (anaerobic biodegradation).

Degradation is a abiotic or biotic process by which a substance is transformed from one chemical species to another.

A **degradation half-life (DegT50[54])** is the time taken for 50% degradation of a test substance when the degradation can be described by (pseudo) first-order kinetics, *i.e.* where the degradation rate constant (k) is independent of concentration.

Degradation products are all substances resulting from biotic and abiotic transformation reactions of a substance.

Rate constant is a kinetic parameter describing an aspect of the rate (per time) at which a substance dissipates from the environment or an environmental compartment. Such parameter may be non-specific, simply describing net dissipation due to degradation and transfer processes,

[54] DegT50 abbreviation is used only for the purpose of guidance documents published by ECHA to describe the degradation half-life and may differ from abbreviations of half-life used in other guidance documents.

or it may be specific, describing dissipation due to degradation, formation, or transfer (FOCUS, 2014).

Degradation rate constant is typically a first-order or pseudo first-order kinetic rate constant, k (d^{-1}).

Dissipation is a result of one or more loss process leading to the disappearance of a substance from an environmental matrix, test system or one compartment of a test system by biotic and/or abiotic processes, such as degradation processes (microbial degradation, hydrolysis and/or photolysis) and transfer processes between different compartments (such as volatilisation, leaching, adsorption/desorption, and plant/organism uptake).

DT50 is a generic term to describe the time required for disappearance/dissipation of 50% of a substance.

First-order kinetics: is when the rate of a degradation follows a first-order equation, where the rate of degradation is proportional to the concentration of the substance which declines over time.

Pseudo-first order kinetics: behaves mathematically like a first-order reaction even though it may be mechanistically a higher order reaction. In chemical reaction kinetics, this applies when other reactants are present in large excess compared to the substance. The concentration of each reactant in large excess will not change appreciably during the course of the reaction and will, therefore, not limit the reaction rate. The reaction rate will then stay proportional to the concentration of the substance. In biodegradation reactions, the microorganisms or enzymes catalysing the biodegradation reaction can be considered as one "reactant" and, therefore, biodegradation reactions are mechanistically not first-order reactions as the rate is dependent on the amounts of more than one reactant. Pseudo first-order kinetics can apply to biodegradation when the amounts of the other reactants, including the relevant microorganisms/enzymes, do not change appreciably (FOCUS, 2014, Schmidt *et al.*, 1985 and Alexander, 1999).

Hydrolysis is decomposition or degradation of a substance by reaction with water.

Inherent biodegradation describes the potential for biodegradation under optimised aerobic conditions designed to promote biodegradation.

Mineralisation (ultimate degradation) is the complete degradation of an organic compound to CO_2, H_2O, other inorganic compounds and biomass under aerobic conditions, and to CH_4, CO_2, H_2O, other inorganic compounds and biomass under anaerobic conditions.

Photolysis is chemical decomposition or degradation induced by light or other radiant energy.

Primary degradation is the initial structural change (transformation) of a substance resulting in the loss of the original chemical identity, and formation of a transformation/degradation product.

Readily biodegradable substance is a substance that reaches the required pass level of 60% CO_2 evolution or O_2 demand, or 70 % dissolved organic carbon (DOC) removal in a 10-day window within 28 days in standard ready biodegradability tests. **The 10-day window** is the 10 days immediately following the attainment of 10% biodegradation (DOC removal, ThOD or $ThCO_2$) in ready biodegradability tests.

4.3.3.1.2. Data on persistence

Data on degradation of a substance may be available from standardised tests, or from other types of information, such as field and monitoring studies, screening studies or (Q)SAR models. The interpretation of such degradation data for classification purposes often requires detailed evaluation of the (test) data.

There are three types of tests that measure biological degradation that are the most relevant for the persistence assessment:

1. Tests on simulation degradation and transformation (OECD TG 309 surface water, OECD TG 308 sediment, and OECD TG 307 soil)
2. Tests on inherent biodegradation (OECD TG 302 series)
3. Tests on ready biodegradation (*e.g.* OECD TG 301 series, OECD TG 306, OECD TG 310 and enhanced ready test)

Simulation tests provide information on degradation kinetics, degradation half-lives, mineralisation, non-extractable residues (NERs) and transformation/degradation products. Simulation tests are the most relevant information for deriving a definitive DegT50 value, whilst tests on ready and inherent biodegradability contribute supporting information, at a screening level.

Abiotic degradation tests provide also relevant information to be included in the assessment. Tests, for example, for hydrolysis and photolysis are presented in more detail in section 4.3.3.1.2.5 of this Guidance.

The ECHA Guidance on IR&CSA, Chapters R.7b and R.11 further detail the availability, applicability, adequacy (reliability and relevance), reporting and scientific and regulatory considerations for the use of different test methods on degradation. Difficult to test substances may require additional measures in reporting and assessment of the data. For example, volatility of a substance potentially leading to dissipation of the substance plays an important role and may bring challenges in the persistence assessment. Therefore, in interpretation of the degradability test results it is crucial to differentiate between disappearance of the substance from the test system due to degradation and other dissipation processes. It is also important to acknowledge that not all tests are applicable to volatile substances and some modifications of the test system may be warranted. For example, OECD TG 301 describes six different methods to measure ready biodegradability but only three of the methods are applicable to volatile substances. Simulation biodegradation tests, such as OECD TGs 307, 308 and 309, have been developed for non-volatile or slightly volatile substances, but they may be adapted to volatile substances using precautions (see ECHA Guidance on IR&CSA, Chapter R.11.4.2.1.3 and ECHA (2022b) for further information).

The following sections will also briefly summarise the key studies and considerations on their conduct and regulatory use.

The scope of P/vP assessment covers all following environmental compartments:

- fresh, estuarine and marine water
- fresh, estuarine and marine sediment and
- soil

Once reliable and relevant information is available resulting in a degradation half-life value in any of these environmental compartments above the regulatory threshold(s) set for P and/or vP, the substance can be concluded as fulfilling the CLP criteria for P and/or vP, respectively. section 4.3.4 of this Guidance will present the assessment of the WoE determination to reach a conclusion if substance meets the CLP criteria for P/vP.

The following sections provide description of methods to derive degradation half-life and presents short overview of different type of studies most commonly used for determining the degradation potential of substances.

Degradation half-life (DegT50) derivation

Degradation half-life (DegT50) can be directly compared with the numerical P/vP criteria. DegT50 values are most commonly based on data derived from simulation biodegradation tests. It is important to note that a dissipation half-life (DT50) is referring to the overall process leading to the disappearance of the test substance from the test system (or one compartment of the

system). If transfer processes have occurred simultaneously with degradation, the derived DT50 value is not representative of the DegT50 value.

Lag phase of degradation could be occasionally observed in simulation studies. A lag phase describes the phase when microbes are 'adjusting' to the new substrate (food source) and/or new environment conditions and depends on the cell density in tests, the possible pre-adaptation of the inoculum and the total amount of specifically degrading bacteria (Ingerslev *et al.*, 2000). When a lag phase occurs in simulation tests, the estimated length of the lag phase should be reported, together with the explanation on how it is determined (*e.g.* based on detection limit of the method or another definition, or whether the value is derived from data analysis software).

OECD TG 309 includes a lag phase definition and specific advice on the lag phase length estimation. In addition, efforts should be made to distinguish whether the observed lag phase can be attributed to any experimental artefacts. Justification for the treatment of the lag phase length in the DegT50 derivation should be provided. When the lag phase is attributed to experimental artefacts, the validity of the study needs to be assessed carefully as this might indicate issues related to the test design and performance.

A kinetic model that best fits and/or most appropriately describes the experimental data should be used for estimating the degradation half-life[55,56]. A qualitative assessment should describe whether the degradation pattern observed from the experimental data is representative of the degradation of the substance under the test conditions and not the result of experimental artefacts. The selection of a degradation kinetic model should be based on the assessment of the metrics for determining the "goodness of fit" which include visual assessment of goodness of fit, $\chi 2$ error and t-test statistical metric. Detailed description for the criteria for the acceptability of the fit is included in Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration (FOCUS, 2014).

When the kinetic of decline is first-order and no lag phase occurs, the DegT50 predicted by SFO (Single First-Order Rate) kinetic model can be used for direct comparison with the P/vP criteria. When the kinetics of decline are bi-phasic and acceptable single-first order (SFO) fitting is not possible, the best-fit bi-phasic model (e.g. DFOP, HS)[57] should be selected and used for predicting a DegT50. When DFOP (Double First-Order) or the HS (Hockey-Stick) kinetic model (both models allow deriving slow phase DegT50) is selected as the best fitting model, the DegT50 predicted from the slow phase where it is assumed that degradation follows (pseudo) first-order kinetics should be preferred for comparison with the P/vP criteria. The First Order Multi-Compartment (FOMC) model, also mentioned in the FOCUS degradation kinetics guidance, is a bi-phasic mechanistic model based on the soil heterogeneous nature (FOCUS, 2014). Considering the uncertainties associated with DegT50 values derived using the FOMC model, this model is the less preferred one to be used for comparison to the P/vP criteria. The use of the FOMC-derived DegT50 can be considered in a WoE approach only if the other models do not fit the data adequately. Furthermore, a pseudo-DegT50[58] (DegT90/3.32) also derived from FOMC should not be used as it is considered highly uncertain (section R.11.4.1.1.3 in *Chapter R.11* of the Guidance on IR&CSA). In any case, a justification for the selection of the model should be provided with

[55] In the context of the Plant Protection Products Regulation (EC 1107/2009) and specifically within the FOCUS kinetic guidance (2014), a distinction is made between trigger and modelling endpoints. For the purpose of the P/vP assessment under CLP, this distinction does not apply and the kinetic model that most appropriately describes the observed data should be used.

[56] It is noted that use of bi-phasic kinetic models is recommended only in cases where an acceptable single-first order (SFO) fitting is not possible.

[57] The DFOP and HS biphasic kinetic models are based on first-order degradation kinetics. The DFOP model (Double-First-Order in Parallel model) consists of two SFO models in parallel (the sum of two first order equations), and the HS model (Hockey-Stick model) consists of two SFO models in series (two sequential first order curves).

[58] Pseudo-DegT50 should not be confused with the Pseudo first order kinetics and any DegT50 derived from such kinetic analysis.

adequate and reliable documentation such as the key parameters of the kinetic analysis and assessment of the goodness of fit.

The extrapolation of DegT50 beyond test duration is common (e.g. for slow phase of bi-phasic degradation) and often also necessary, considering the duration of the standard tests and the P/vP criteria. Extrapolation will increase the uncertainty of the derived DegT50 value that should be acknowledged in the interpretation of the data. In general, when a DegT50 obtained from a properly justified kinetic model fulfils the P or vP criterion, even if the DegT50 is extrapolated over study duration, the substance can be concluded P or vP. When there is no significant measurable degradation observed during the test and the kinetic model indicates that the relevant rate constant is not significantly different from zero, the calculated degradation half-lives should be carefully interpreted. In such cases, it is still possible to reach a conclusion on persistence (P or vP) as demonstrated in the cases of substances included in the REACH candidate list (e.g. Melamine, EC 203-615-4 and 1,4-dioxane EC 204-661-8).

Any deviations from the recommended mass balance/recovery, as they are described in the corresponding testing guidelines (OECD TG 309, OECD TG 308 and OECD TG 307), should be reported and justified. Further guidance on handling mass balance/recovery data is provided in ECHA Guidance on IR&CSA, Chapters R.11, section R.11.4.1.1.3 and Table R.11-6. Furthermore, when dissipation through volatilisation is observed, correction procedures can be applied and for this purpose, correction procedures are available for the parent substance for the SFO kinetics model which could be also applied in the case of HS and DFOP kinetic model (ECHA Guidance on IR&CSA, Chapters R.11, section R.11.4.2.1.3 and related Appendix R.11-7).

A good knowledge of the degradation pathway leading to the formation of transformation/degradation products is essential for deriving a reliable degradation half-life for the transformation/degradation product. When a study is performed on the parent substance and transformation/degradation products are formed, the pathway model approach as described in the FOCUS degradation kinetic Guidance (2014) should be used, as it accounts for both formation and removal (degradation) of transformation/degradation products. In the pathway approach, the parent and transformation/degradation data are assessed together. Evaluation of the transformation/degradation products data individually by using only the decline phase (Decline model) is another available option and it should be used only if the pathway model does not provide visual and statistically satisfactory representation of the data (FOCUS, 2014).

Further information on the degradation kinetic models, the data handling, the assessment of the goodness of fit and the general recommendations on the kinetic analysis can be found in ECHA Guidance on IR&CSA, Chapters R.11, section R.11.4.1.1.3. and the Generic Guidance Document for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration (FOCUS, 2014). Furthermore, EFSA Guidance provides further advice especially on derivation on DegT50 from field studies (EFSA, 2014)[59].

4.3.3.1.2.1. Simulation tests in water, water-sediment and soil

Simulation degradation tests attempt to assess degradation in a specific environment by use of indigenous biomass, media and relevant solids (e.g. soil and sediment) in relevant test conditions. As detailed in section 4.3.4 of this Guidance, degradation simulation studies performed in relevant environmental media specified in Annex I (4.3.2.1.1 and 4.4.2.1.1) of CLP and at relevant conditions are the tests considered as the ones with the highest regulatory relevance. These tests provide a definitive degradation half-life that can be compared to the numerical persistence

[59] To note that there are some deviations in how abbreviations are used for degradation half-live between this Guidance and EFSA Guidance (2014) and FOCUS (2014). It should also be noted that there are some differences in the approaches for deriving a degradation half-life between this Guidance and EFSA Guidance (2014) and FOCUS (2014), such as the consideration of non-extractable residues (see also section 4.3.3.1.2.1 of this Guidance).

criteria as defined in CLP. Such tests allow both biotic and abiotic degradation processes to operate.

The following tests can be used to simulate the biodegradation of organic substances under relevant conditions in soil, sediment or surface water: Aerobic and Anaerobic Transformation in Soil (OECD TG 307); Aerobic and Anaerobic Transformation in Aquatic Sediment Systems (OECD TG 308); and Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test (OECD TG 309).

The simulation degradation studies include two types of investigations: a) a degradation pathway study where degradation products (*i.e.* degradation transformation/degradation products) are identified and quantified, b) a kinetic study where the degradation rate constants (and degradation half-lives) of the parent substance and, if applicable, of the transformation/degradation products are experimentally determined. In the simulation study, the test concentration is low (close to the expected in the environment) to anticipate that the biodegradation kinetics (first order or pseudo first-order) obtained in the test reflect degradation rates expected in the environment. Higher concentrations of the test substance (e.g. above >100 µg/L) are relevant preferably to overcome potential analytical limitations when identifying and quantifying the transformation/degradation products. The endpoints that need to be addressed and reported are primary or ultimate degradation rate and degradation half-lives (DegT50) or disappearance/dissipation half-lives (DT50) for the compartments included in the test system, as well as the route of degradation, transformation/degradation products and non-extractable residues (as relevant). Determination of non-extractable residues is relevant in soil and water-sediment studies (OECD TG 307, OECD TG 308 and Kästner *et al.*, 2014). Determination of non-extractable residues is also recommended in surface water simulation degradation studies (OECD TG 309) especially when relevant for mass balance calculations and derivation of degradation half-life. In addition, a mass balance and quantity of possible losses from the test system during the test period need also to be reported. An incomplete mass balance will introduce severe uncertainty to the interpretation of data. This, in turn, can ultimately impede the substance assessment with sufficient certainty and give a lower weight to the test and its results in the P/vP assessment as part of a WoE approach.

The use of both radiolabelled and non-labelled test substances is acceptable. For assessing total mineralisation, a ^{14}C-labelled test substance is typically used and $^{14}CO_2$ evolution is measured. If a ^{14}C-labelled substance is used, the most relevant location of the label depends on the goal of the study, e.g. label in the most recalcitrant part of the molecule ensures the determination of the total mineralisation. This must be considered in the assessment. If the used analytical method is sensitive enough to detect low concentrations applied in simulation tests, such data can be used to report on the total residual concentration of the test substance. Disappearance of the parent substance however does not necessarily imply its degradation. Other dissipation processes, for example volatilisation or adsorption, may also cause disappearance of the parent substance and they should be taken into account when assessing results on the primary degradation rate. Data on chemical analyses can be used in parallel with radiolabelling techniques. Specific chemical analyses are also needed to identify and quantify transformation/degradation products.

When a substance is not fully degraded or mineralised, the persistence of relevant transformation/degradation products must be considered in the assessment. Identity, stability, behaviour, molar quantity relative to the parent substance of the transformation/degradation products are important parameters to be included in the assessment. There is no set regulatory % (w/w) threshold concentration for transformation/degradation products in persistence assessment under CLP. However, a transformation/degradation product can be considered relevant in the simulation degradation test for soil, water-sediment and surface water at least when detected at ≥10% of the applied concentration of the parent substance at any sampling time (principal transformation/degradation products) or when detected ≥ 5% in at least two sequential measurements or the concentration is continuously increasing, or it seems to be stable during a degradation study (see also section 4.3.3 (v) of this Guidance).

If the primary degradation or mineralisation half-life for the whole system is below the respective degradation half-life value of the P/vP criteria, the parent substance can be considered not persistent in the tested environmental compartment (surface water, sediment or soil). However, investigation of degradation pathways/transformation/degradation products would be needed since it cannot be excluded that a second transformation route forms a persistent transformation/degradation product in concentrations relevant for the P assessment.

ECHA Guidance on IR&CSA, Chapter R.7b, section R.7.9.4.1 "Data on degradation/biodegradation" provides Guidance on the key results to be reported on each of these tests. In biodegradation studies, information gained from sterile controls is also useful for the interpretation of the study results (see sections R.11.4.1.1.2 and R.7.9.4.1 in Chapters R.11 and R.7b of the ECHA Guidance on IR&CSA and ECHA (2022) for more details).

According to the OECD simulation degradation Test Guidelines (TG), the radiolabelled mass balance should target to 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. An incomplete mass balance will introduce severe uncertainty to the interpretation of data. The simulation test results should be considered as not valid or at least treated with caution if the mass balance is not fulling these criteria. ECHA Guidance on IR&CSA, Chapter R.11 (section 4.1.1.3 and Table R.11-6) describes DegT50 calculation methods for studies with incomplete mass balance.

Degradation half-lives (DegT50) obtained in the simulation tests conducted in the relevant conditions and in accordance with the respective TGs may be directly compared with the numerical P/vP criteria. When bi-phasic models are considered more appropriate to describe the degradation, the DegT50 from the slow phase should be preferred for comparison with the numerical P/vP criteria. In the context of simulation degradation tests, by "relevant conditions", relevant testing conditions are generally meant (see also section 4.3.3 (ii) of this Guidance). For the simulation test conditions, among others, the following factors should be considered: temperature, test concentration, test design, physico-chemical properties of the substance, etc. Any deviation from the relevant/standard test conditions should be taken into account in weighting of the relevance and reliability of the information as part of the WoE assessment.

The simulation test is considered relevant to derive degradation half-life when

- no pre-exposure (pre-adaptation) of the water, soil or sediment microorganism has taken place;
- low concentration (e.g. ≤ 100 µg/L) reflecting the one expected in the environment is used;
- the study is considered to be performed under relevant conditions;
- the study is performed in accordance with the testing conditions provided for in the test methods Regulation, in line with Article 13(3) of the REACH Regulation.

Non-extractable residues (NERs) may be formed during the degradation simulation tests. Total NERs are defined as the residues remaining in the matrix after defined exhaustive extractions. The Total NERs are considered as non-degraded parent substance in DegT50 derivation unless further characterisation of the Total NER is performed. Total NER consist of potentially remobilisable NER (Type 1: strongly sorbed and physically entrapped), irreversibly bound NER (Type 2: covalently bound) and NER incorporated into biomass (Type 3: biogenic) (Löffler *et al.*, 2022, ECHA, 2019, Kästner *et al.*, 2018). The potentially remobilisable fraction of the Total NER (NER Type 1) poses a potential risk for the environment. If the quantity of the remobilisable fraction (Type 1) is available, the total extractable fraction of the parent substance together with the Type 1 NER are considered for the DegT50 estimation. If such DegT50 is above the P/vP criterion, it can be further refined by taking into account only the quantity of the parent substance concentration in the Type 1 NER together with extractable fraction of the parent substance. Appendix R.11-4 "Approach on non-extractable residues (NER) quantification and characterisation

in persistence assessment" of ECHA Guidance on IR&CSA, Chapter R.11 provides a stepwise assessment approach on how to quantify and take the different types of NERs into account.

Temperature has an influence on the degradation rate. In Europe, due to wide range of environmental temperatures, it must be taken into account in the estimations of the degradation rate in the different environmental compartments. When biodegradation rates or half-lives have been determined in simulation tests, it should be considered to recalculate the degradation rates obtained to reflect an average EU outdoor temperature (European Commission, 2003). Standard environmental characteristics for Europe, including average environmental temperature of 12° (9°C for the marine environment), have been established, for example, in van de Meent (1993), Schoorl *et el.* (2014) and European Commission (2003). Unless other evidence is provided, degradation rates in a test conducted in the laboratory at 20-25°C are in general considered higher than those measured in the field in Europe or in a test conducted in the laboratory at the reference temperature.

Temperature correction to the average environmental temperature of 12°C in Europe (9°C for the marine environment) should be applied to the DegT50 obtained in a water, sediment or soil simulation test conducted at other temperatures. It is noted that the General Court of the European Court of Justice in its judgment in Case T-177/19[60] considered that there was no manifest error of assessment by ECHA in applying a temperature correction of 12°C for determining the degradation half-life of a specific substance. In another case (Case T-176/19), the Court accepted ECHA's explanation that a substance's degradation rate, must be obtained through such studies being conducted at an environmentally 'relevant' temperature and that this temperature is 12 °C by default[61]. According to the three OECD test guidelines (TGs 307, 308 and 309), the studies can be performed at a range of temperatures, typically between 10 and 25 °C. It is acknowledged that temperature correction may induce uncertainty on the derived degradation rate. The closer to the average European temperature the test is conducted, the less uncertainty due to the temperature correction can be expected.

In the absence of structural substance class-specific equations/models reflecting the temperature dependence of biodegradation, the Arrhenius equation (or a similar appropriate equation designed to normalise physico-chemical degradation rates) can be used for the normalisation. This is:

$\ln k = lnA - (E_a/RT)$

Where

k = rate constant (day^{-1})

A = factor equal to the rate coefficient at infinite temperature (day^{-1})

E_a = activation energy ($kJ\ mol^{-1}$)

R = gas constant ($8.314.10^{-3}\ kJ.K^{-1}.mol^{-1}$)

T = temperature (K)

For first-order kinetics, the equation can be reformulated to:

$$DegT50env = DegT50test.e^{\left(\frac{Ea}{R}\left[\frac{1}{Tenv}-\frac{1}{Ttest}\right]\right)}$$

[60] See judgment of 9 June 2021 Exxon Mobil v. ECHA, T-177/19, not yet published, EU:T:2021:336 Link to T-177/19.

[61] See judgment of 16 December 2020 3 V Sigma v. ECHA, T-176/19, not yet published, EU:T:2020:621, at paragraphs 76 and 77, Link to T-176/19

where

$DegT50_{env}$ = half-lives at environmental temperature *Tenv* (typically 285K = 12°C) and

$DegT50_{test}$ = half-lives at test temperature *Ttest* (typically 293K = 20°C).

There are potential uncertainties resulting from the use of the Arrhenius equation because:

1) It was designed for simple chemical reactions rather than biological processes;
2) The specific activation energy (E_a) for a substance or a chemical group is rarely known

A generic E_a of 65.4 kJ/mol has been derived by EFSA (2007) for soil degradation studies. It corresponds to the median value of available pesticide E_a data. If the Arrhenius equation is used for temperature correction, in the absence of valid substance specific E_a value, the generic E_a value should be used.

Other relevant test conditions depend on the type of study conducted. Test dependent considerations on the relevant test conditions are further described below.

Surface water simulation test (OECD TG 309)

The purpose of OECD TG 309 is to measure the time course of biodegradation of a test substance at low concentration in aerobic natural water and to quantify the observations in the form of kinetic rate expressions. This simulation test is a laboratory shake flask batch test to determine rates of aerobic biodegradation of organic substances in samples of natural surface water (fresh, brackish or marine). To ensure the presence of an active microbial population, a substance which is normally easily degraded under aerobic conditions (e.g. aniline or sodium benzoate) should be used as reference substance.

The test is performed in batch by incubating the test substance with either surface water only ("pelagic test") or surface water amended with suspended solids/sediment of 0.01 to 1 g/L dry weight ("suspended sediment test") to simulate a water body with suspended solids or re-suspended sediment. For the purpose of CLP, OECD TG 309 with water amended with sediment 'suspended sediment test' is generally not preferred over pelagic test conditions, as the subsequent addition of suspended matter may significantly enhance biodegradation of some substances (Ingerslev and Nyholm, 2000).

Test conditions of OECD TG 309 can be considered relevant for classification purposes, when the test

- is performed at concentrations e.g. ≤ 100 µg/L and preferably in the range of ≤1-10 µg/L (to ensure that biodegradation follows first-order kinetics);
- inoculum is collected from natural surface water preferably containing suspended particulate matter (SPM) between 10 and 20 mg_{dw}/L in freshwater and c.a. 5 mg_{dw} SPM/L in marine water;
- is conducted in relevant temperature in accordance with the test guideline (temperature correction applied in accordance with the text above);
- determines the degradation half-life in at least one surface water sample and at two different concentrations of the test substance.

If any other conditions are used, the relevance of the information must be justified as part of the WoE assessment.

For low solubility substances, even if their water solubility is within the range reported above, it is acknowledged that the feasibility of the test depends, *inter alia*, on the possibility to develop with reasonable efforts appropriate analytical methods with suitable sensitivity to detect relevant changes in concentration (including transformation/degradation products).

OECD TG 309 simulation test is applicable to non-volatile or slightly volatile organic substances tested at low concentrations. The relevance of the test conducted with volatile substances depends on the means taken to minimise volatilisation and the maintenance of the test substance in the water phase accessible for microorganisms to the extent that a reliable degradation half-life can be determined. The volatilised fraction should be adequately trapped and quantified in order to be able to interpret the results reliably. Further information on how to address volatilisation in simulation testing and data handling can be found in ECHA Guidance on IR&CSA, Chapters R.11, section R.11.4.2.1.3 and Appendix R.11-7,R.7, section R.7.9.4 and ECHA (2022b).

Aerobic and Anaerobic Transformation in Aquatic Sediment Systems (OECD TG 308)

OECD TG 308 describes a laboratory test method to assess aerobic and anaerobic transformation of organic chemicals in aquatic sediment systems. The surface layer of aquatic sediments can be either aerobic or anaerobic, whereas the deeper sediment is usually anaerobic. These conditions in sediment may be simulated by using aerobic or anaerobic tests described in OECD TG 308. The aerobic test simulates an aerobic water column over an aerobic sediment layer that is underlain with an anaerobic gradient. The anaerobic test simulates a completely anaerobic water-sediment system.

The sediment degradation test according to OECD TG 308 includes the determination of the degradation half-lives in two different types of water-sediment systems. OECD TG 308 allows;

i. the measurement of the transformation rate of the test substance (and relevant transformation products) in a water-sediment system;

ii. the measurement of the transformation rate of the test substance (and relevant transformation products) in the water and in sediment;

iii. the measurement of the mineralisation rate of the test substance and/or its transformation products (when a ^{14}C-labelled test substance is used);

iv. the identification and quantification of transformation products in water and sediment phases including mass balance; and

v. the measurement of the distribution of the test substance and its transformation products between the two phases during a period of incubation in the dark (to avoid, for example, algal blooms) at constant temperature.

The method is generally applicable to chemical substances (non-radiolabelled or radiolabelled) for which an analytical method with sufficient accuracy and sensitivity is available. It is 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, cannot be kept in water and/or sediment under the experimental conditions of this test. Further guidance on the assessment of volatile substances is provided in ECHA Guidance on IR&CSA, Chapters R.11, section R.11.4.2.1.3 and Appendix R.11-7, R.7, section R.7.9.4 and ECHA (2022b).

OECD TG 308 outcome can be affected both by test vessel and system geometry and the associated water-sediment interface size. Headspace volume and height of the water and sediment columns can influence the partitioning and consequently degradation of the test substance (Hennecke *et al*., 2014; Shrestha *et al.*, 2016), especially for volatile substances. The system geometry should be consistent with the range indicated in OECD TG 308 (*i.e.* water:sediment volume ratio between 3:1 and 4:1, height of 2.5 cm (±0.5) layer and minimum weight of 50g of the sediment). Sediment spiking instead of addition of the test substance via

water may, in some cases, be acceptable to ensure exposure of sediment in the test system. This may be the case, for example, for substances which would transfer significantly quicker to the atmospheric compartment via volatilisation compared to transfer to the sediment compartment.

According to OECD TG 308, the aerobic test simulates an aerobic water column over an aerobic sediment layer with an anaerobic gradient. Aeration of the test system is needed in order to maintain aerobic conditions in the water column and surface layer of the sediment throughout the study. OECD TG 308 recommends aeration by gentle bubbling or by passing air over and gently stirring the water surface in open test vessels (for non-volatile substances), and by gentle stirring of the water surface in biometer type systems (for slightly volatile substances). When results of a closed systems test with a volatile substance are interpreted, the assessment should consider if the oxygen was distributed from the headspace to the water layer to maintain aerobic test conditions. However, any aeration method should disturb as little as possible the sediment layer and its stratification. For example, visual resuspension/cloudiness of the overlying water is one indication of disturbed sediment. The aeration methods recommended in OECD TG 308 are acceptable. If any other methods are used, their influence in stratification should be taken into account. In OECD TG 308, shaking method is not appropriate as it may modify the stratification of the sediment, affecting the maintenance of the anaerobic layer and, therefore, may have an influence on the degradation process in the sediment simulation test.

The total water-sediment system DegT50 derived from an OECD TG 308 is a robust indicator for persistence in sediment compartment and should be used in the context of persistence assessment (Honti and Fenner, 2015). For adsorptive substances (e.g. log K_{OC}>4) that will partition primarily to the sediment phase, a degradation half-life in the sediment can reasonably be assumed to be equal to the degradation half-life for the total water-sediment system. This degradation half-life ($DegT50_{Total\ system}$) would compare to relevant marine, fresh or estuarine sediment criteria set in CLP Annex I. Because of the low volume and depth of water relative to the volume of sediment and the surface of the water-sediment interface used in OECD TG 308, even substances with moderate or low adsorption potential will tend to partition from the water phase to the sediment phase. Due to the complexity of the phase transfer processes in the water-sediment test system, the specific degradation half-lives calculated for the sediment phase ($DegT50_{sediment}$) and the water phase ($DegT50_{water}$) separately are highly uncertain (Honti and Fenner, 2015). Therefore, such results must be interpreted with caution considering the partitioning of the substance between water and sediment when selecting the most representative value for DegT50. OECD TG 308 test setup does not reflect well the surface water conditions. It states that it is not suitable to simulate conditions in flowing water (e.g. rivers). Also Honti and Fenner (2015) reflected that OECD TG 308 has limited relevance for the open sea conditions and for water bodies having high water:sediment ratios. Therefore, DegT50 values from OECD TG 308 is not recommended to be used for comparison with the Annex I CLP criteria for persistence in marine, fresh or estuarine waters. Nevertheless the relevance of its use to reflect the degradation potential in surface waters should be considered on a case-by-case basis under the WoE assessment.

The fact that the parent substance may degrade to more soluble and less adsorptive degradation products that can be released from the sediment to the water phase should also be taken into account in the assessment.

Generally, it would be expected that an anaerobic half-life would be greater than an aerobic half-life, where the main route of degradation is aerobic, *i.e.* if there is no oxygen, biodegradation will be hindered. Therefore, it is not recommended to use only strictly anaerobic sediment degradation simulation data to conclude whether a substance has a degradation half-life exceeding the P and/or vP thresholds. Nevertheless, if anaerobic water sediment data are available, they may be used as supporting information.

Aerobic and Anaerobic Transformation in Soil (OECD TG 307)

OECD TG 307 describes a method designed for evaluating aerobic and anaerobic transformation of chemicals in soil. The experiments are performed to determine (i) the rate of transformation of the test substance and (ii) the nature and rates of formation and decline of transformation/degradation products to which plants and soil organisms may be exposed.

The soil simulation degradation test according to OECD TG 307 includes the determination of the degradation half-lives in 4 different types of soils. Aerobic and anaerobic studies with one soil type are generally sufficient for the evaluation of transformation pathways. Aerated soils are aerobic, whereas water-saturated or water-logged soils are frequently dominated by anaerobic conditions. These conditions in soil may be simulated by using aerobic or anaerobic tests described in OECD TG 307. Under anaerobic incubation conditions, the test system must allow for measurements of oxygen concentration and redox potential.

As noted in OECD TG 307, aerobic conditions are dominant in surface soils and even in sub-surface soils. Anaerobic conditions may occur only occasionally during flooding of soils after heavy rainfalls or when paddy conditions are established in rice fields. Thus, it is not recommended to conclude on persistence using only anaerobic soil simulation data. Nevertheless, if anaerobic soil data is available, it may be used as part of the WoE approach.

Results of OECD TG 307 may be used for classification purposes, when conducted in relevant test conditions maintaining relevant temperature (temperature correction to be applied in accordance with the text above) and relevant moisture conditions in accordance with the TG. The method is applicable to all chemical substances (non-labelled or radiolabelled) for which an analytical method with sufficient accuracy and sensitivity is available. It is 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. Further information on how to address volatilisation in simulation testing can be found in ECHA Guidance on IR&CSA, Chapter R.11, section R.11.4.2.1.3 and R.7, section R.7.9.4).

Degradation rate of ionisable substances can depend on the soil pH and should, thus, be considered in the assessment regarding the relevance of test conditions. For example, for weakly acidic substances, a faster degradation has been observed at higher pH and a slower degradation at low pH.

Other simulation tests

Section 4.3.3.1.2.6 of this Guidance (Non-standard biodegradation studies) provides further information on how to use non-standard simulation tests conducted in water, sediment or soil for the P/vP assessment.

The relevance of the test conditions to simulate degradation potential in water (marine, fresh or estuarine), sediment (marine, fresh or estuarine) and soil should be considered when simulation test data derived from any other compartment e.g. wastewater or treated effluent is used in persistence assessment. However, such information should not be used on its own to demonstrate that the substance is or is not P/vP.

The studies described below provide important information on degradation during wastewater treatment process and mixing zone after the release of the effluent. Whilst these studies are more relevant for risk assessment than hazard identification, they can be considered as supporting information in the WoE approach.

Other simulation test standards include:

- OECD TG 303: Simulation Test - Aerobic Sewage Treatment,
    - A: Activated Sludge Unit
    - B: Biofilms

- OECD TG 314: Simulation Tests to Assess the Biodegradability of Chemicals Discharged in Wastewater
    - A: Biodegradation in a Sewer System Test
    - B: Biodegradation in Activated Sludge Test
    - C: Biodegradation in Anaerobic Digester Sludge Test
    - D: Biodegradation in Treated Effluent-Surface water Mixing Zone Test
    - E: Biodegradation in Untreated Wastewater-Surface water Mixing Zone Test

OECD TG 314 (A-E) allows checking of the fate of a substance on its way through the sewer system and sewage treatment plant to the mixing zone in surface water. Such studies are neither screening studies nor equivalent to a simulation study on degradation in the respective environmental compartments *i.e.* soil, water or sediment. They do not employ relevant environmental conditions for assessing the persistence of the substance in the compartments relevant for the PBT/vPvB or PMT/vPvM assessment, namely natural surface water, sediment or soil. Furthermore, they do not provide information on ready biodegradability or on degradation rates in the individual environmental compartments (*i.e.* natural surface water, sediment or soil). Therefore, as stated above, such information is considered as supporting information in the WoE approach.

4.3.3.1.2.2. Field and mesocosm studies

Field studies, mesocosm, or lysimeter experiments can provide relevant information for the persistence assessment. In contrast to laboratory studies, field studies allow degradation testing under more natural conditions and over long periods up to several years. In field studies the risk of decreasing microbiological activity is lower than in longer-lasting extended laboratory studies due to the differences in test conditions. With field studies, it is also possible to investigate the accumulation potential of substances over several years.

There are several Guidance documents available on how to perform and interpret terrestrial field dissipation studies. The NAFTA Guidance (Corbin *et al*., 2006) refers to the degradation behaviour of substances under realistic exposure conditions considering all possible dissipation and degradation pathways. EFSA Guidance Document (EFSA, 2014) is used for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil[62]. OECD Guidance document 232 (OECD, 2016) considers aspects from both the NAFTA and the EFSA Guidances. Guidance on how to derive DegT50 values from meso- or macrocosm studies is provided in Deneer *et al*. (2015).

Compared to laboratory studies, field studies are semi-controlled with a range of varying environmental factors and particularly dependent on local conditions including varying temperature and moisture conditions. Derivation of DegT50 from field dissipation studies is complicated and has uncertainties related to dissipation processes such as volatilisation, leaching, surface run-off or uptake into plants during the test (EFSA, 2014). These uncertainties can significantly influence the disappearance of the substance from the test matrix and should be

[62] To note that there are some deviations in how abbreviations are used for degradation half-live between this Guidance and EFSA Guidance (2014) and FOCUS (2014). It should also be noted that there are some differences in the approaches for deriving a degradation half-life between this Guidance and EFSA Guidance (2014) and FOCUS (2014), such as the consideration of non-extractable residues (see also section 4.3.3.1.2.1 of this Guidance).

taken into account in the assessment and in considerations of the reliability of the derived DegT50 when compared to the numerical P/vP criteria under CLP. Therefore, DegT50/DT50 values from field studies are (in many cases) not directly comparable with one another or laboratory tests. Information may, however, be used as part of WoE. In some cases, if dissipation e.g. due to volatilisation from soil, leaching, surface run-off or uptake into plants can be excluded, mesocosm or field studies may be used to derive reliable DegT50 (EFSA, 2014). For existing field studies (legacy studies), EFSA (2014) recommends conducting inverse modelling using time -step normalisation procedure.

In cases where field data clearly demonstrate that more than 50% of a compound remains in the environment for a period longer than the criteria for P/vP, even though a numerical degradation half-life is not possible to calculate, the substance could be concluded P/vP.

Consideration should be given especially to whether temperature and moisture correction should be applied by taking into account normalisation factors to relevant conditions. Moreover, it should be considered how the formation of NER could influence the DegT50 derivation.

Temperature correction to relevant temperature (12°C for water, soil and sediment and 9°C for marine environment) should be applied to the DegT50 obtained, with more information on how to perform temperature correction provided above in this Guidance. FOCUS Kinetics Guidance (FOCUS, 2014), Chapter 9 explains the normalisation of field dissipation half-lives to the reference moisture conditions. It explains that it is useful to normalise the data not only to a reference temperature, but also at moisture conditions (*i.e.*: 100% FC = pF2). Normalised input parameters will allow field dissipation data collected under specific environmental conditions to be used to simulate likely behaviour under different conditions if dissipation is mainly due to degradation. The normalisation can be conducted using measured or simulated values for soil moisture content (e.g. daily experimentally measured data or calculated from standard weather data using a pesticide leaching model). These simulation models are based on Walker (1974). In order to permit the broadest possible use of field dissipation data, suitable for calculation of DegT50, the assessment of the likely impact of loss processes (volatilisation, soil surface photolysis, leaching out of the sampled soil layers and possible uptake into plants) is also described in FOCUS (2014).

Lysimeter studies, which are often carried out with radiolabelled substances (OECD, 2002), can also provide useful information about the degradation behaviour of a substance to be used as supporting information. Guidance Document for the Performance of Out-door Monolith Lysimeter Studies (OECD No. 22) describes a method for obtaining information on the fate and behaviour of a chemical in an undisturbed soil under outdoor conditions. Lysimeter studies are dose-dependent, they cannot fully control the varying climatic conditions and they are not suitable to all soil types. The output of this method is a concentration, expressed as maximum of average, in µg/L. The derivation of DegT50 from lysimeter and mesocosm studies is challenging. Inverse modelling could allow estimating the DegT50 instead of disappearance DT50 from such studies (SANCO, 2014). The derived DegT50 can be related to reference conditions (e.g. reference temperature at 12°C and reference moisture at field capacity). However, inverse modelling can have the problem of non-uniqueness (namely the fact that the same model output can be obtained with different parameter combinations). This can make the estimation of only DegT50 uncertain (SANCO, 2014) and needs to be considered by an appropriate assessment of the data.

More information on lysimeter studies can be found under section 4.3.3.3.2 Other experimental information on deriving a K_{oc} value. In addition to the above, see also ECHA Guidance on IR&CSA, Chapter R.7b, section R.7.9.4.2 and Chapter R.11, section R.11.4.1.1.4.

4.3.3.1.2.3. Monitoring studies

There are many sources of monitoring data. Information may be found for example from national monitoring programmes of Member States (e.g. Swedish national monitoring data collection[63]), from European monitoring programmes (e.g. NORMAN Network[64]), Information Platform for Chemical Monitoring (IPCheM)[65] or internationally acknowledged organisations (such as OSPAR or the Danube Convention).

Findings of significant concentrations of a substance in remote and pristine environments such as the Arctic or Alpine lakes may be indicative of high persistence. Also, significant concentrations of a substance in higher levels of the food chain may indicate high persistence, besides the potential to bioaccumulate.

Trends of rising concentrations in environmental media or biota may be observed. Information on volumes, uses and releases along the same period, if available, compared with these trends can provide relevant information. Archived samples from environmental specimen banks, dated sediment cores and ice cores can be used to gain understanding on temporal changes. The reliability of data from archived samples should consider the compatibility of the methods of sample collection, processing and storage with the known properties of the substance of interest.

Monitoring data obtained in areas closer to the sources may also be useful for persistence assessment and can be used as one line of evidence for supporting the conclusions on this property. Use of monitoring data in persistence assessment encompasses several uncertainties. Conclusions on the basis of monitoring studies should be drawn only when there is sufficient understanding of the substance distribution and transport behaviour and under the condition that the uncertainties in the monitoring data are adequately addressed. Monitoring programmes may be designed to cover a large spatial area (high number of stations over a large territory), to achieve a high spatial resolution (high number of stations per area unit), or to assess temporal trends (high sampling frequency). Dependent on the design of the monitoring programme, information on detection (frequency) of a specific substance may be used as part of evidence for the persistence assessment. Several factors such as fate of the substance, potential sources and extent of emissions in relation to sampling site(s), sample size and sampling frequency should be considered when detection frequency is used in the persistence assessment. The lack of detection of a substance in monitoring studies should be considered carefully as it does not necessarily mean that a substance is not persistent. This is because shortcomings in analytical methods may affect monitoring of substances in the environment. Uneven distribution of the substance in the media, such as soil or sediment may also lead to lack of detection or variation in presence of the substance in the environmental samples. Among other factors, dissipation by transfer processes (e.g. volatilisation or plant/organisms uptake) influence the uneven distribution in the environmental samples.

Monitoring data from sewage treatment plants (e.g. percentage of removal during the residence time in the sewage treatment plant, determination of transformation/degradation products or adsorption to sludge) may provide useful information for persistence assessment. However, it cannot be considered relevant in estimating degradation rates in the environmentally relevant conditions.

EFSA (2023b) and Gimsing *et al.* (2019) provide guidance on how to evaluate groundwater monitoring studies for regulatory purposes in the context of Regulation (EC) 1107/2009. Use of

[63] http://dvsb.ivl.se/dvss/DataSelect.aspx

[64] http://www.norman-network.net/

[65] https://ipchem.jrc.ec.europa.eu/#discovery

groundwater monitoring data in the weight of evidence assessment for mobility is further elaborated in sections 4.3.3.3 and 4.3.5.1 of this Guidance.

An overall assessment of the reliability of any monitoring data set as well as its relevance to the assessment purpose should be conducted and the outcomes of this reliability and relevance assessment included in the weight of evidence reasoning. In addition to the above, see also ECHA Guidance on IR&CSA, Chapter R.11, section R.11.4.1.1.1 and R.11.4.1.1.6.

4.3.3.1.2.4. Screening studies

There are several standard degradation test methods that can be used in the WoE assessment in addition to the information referred to in CLP Annex I: 4.3.2.3.1. and 4.4.2.3.1.

Short description of the available screening methods is provided below. ECHA Guidance on IR&CSA, Chapters R.7b, Section R.7.9.4.1 and Chapter R.11, section R.11.4.1.1 provide more detailed guidance on the available screening tests and their use in persistence assessment. sections 4.1.3.2.3.2 and Annex II section II.2 of this Guidance describes the use of such information to assess rapid degradation as part of the aquatic hazard classification.

Ready biodegradability tests

The existing methods for testing ready biodegradability are OECD TG 301 A-F and OECD TG 310. These test guidelines are not equally applicable to all types of substances. Difficulties may especially occur during tests on substances which have low water solubility, high volatility or adsorbing properties. The applicability of the ready biodegradability tests for poorly water soluble, volatile and adsorbing substances has been summarised by the OECD (2006) and in respective TGs.

Additionally, the origin of the inocula should be examined in order to verify whether the inocula is adapted to the tested substances. Differences in the adaptation of the inocula may explain the differences in the results (OECD, 2006). Results from tests based on adapted inocula are generally regarded as inappropriate information to support non-persistence but depending on the reliability and relevance they may be used as a part of a weight-of-evidence in P assessment. For example, data derived with inocula from wastewater treatment plants (WWTPs) influenced by point sources must not be used as information supporting non-persistence (e.g. if effluents from an industrial site using the substance are connected to the municipal WWTP).

A test substance concentration lower than generally recommended by the test guideline/method should only be used for substances toxic to microorganisms and when it is still possible to reliably assess biodegradation through the measurement of carbon dioxide evolution, oxygen demand dissolved organic carbon removal. To improve sensitivity, degradation of the parent compound can also be followed, *e.g.* by using radiolabelled test material. Reliable assessment of ready biodegradability could be possible if ratio of test substance to biomass is kept to the standard of the respective test guideline. If the biomass-substance ratio deviates from the respective test guideline, results should be treated with caution. Such results are considered to be more relevant for concluding whether the substance is not readily biodegradable. The following pass levels of biodegradation, obtained within 28 days (fulfilling 10-day window[66]), may be regarded as evidence of ready biodegradability: 70% DOC removal (OECD TG 301 A and TG 301 E); 60% theoretical carbon dioxide ($ThCO_2$; TG 301 B); 60% theoretical oxygen demand (ThOD; TG 301 C, TG 301 D and TG 301 F). In OECD TG 310, the CO_2 evolution resulting from the ultimate aerobic biodegradation of the test substance is determined by measuring the inorganic carbon (IC) produced in sealed test bottles, and the pass level has been defined as 60% of theoretical

[66] The 10-day window begins when the degree of biodegradation has reached 10% DOC removal, ThOD or ThCO2 and must end before or at day 28 of the test. It does not apply to OECD TG 301 C.

maximum IC production (ThIC). In general, for a substance with more than one constituent, ready biodegradability of the constituents cannot be assessed measuring only DOC removal, ThOD, ThIC or $ThCO_2$ in a study conducted with the whole substance. If the test is conducted with substances with more than constituent and it is anticipated that a sequential biodegradation of the individual constituents is taking place, 10-day window concept does not apply.

If the substance is readily biodegradable, or if the criteria for ready biodegradability are fulfilled with the exception of the 10-day window, the substance may be considered as not P. However, in case of contradicting results within the WoE, screening information indicating not P and not vP may not always exclude the substance from being persistent or even very persistent. Furthermore, a negative result in a test for ready biodegradability does not necessarily mean that the substance will not be degraded under relevant conditions.

Ready biodegradation studies are conducted in stringent test conditions and are known to be highly variable in measuring ready biodegradability. When faced with conflicting results on ready biodegradability, differing results always have to be assessed considering the test conditions, substance properties and reliability of the data (see also section II.3.5 of this Guidance, ECHA Guidance on IR&CSA, Chapters R.7b and R.11). The overall results should be assessed in a WoE approach.

Other screening tests

Information on enhanced ready biodegradability tests is relevant when the substance is poorly soluble and/or adsorptive and enhancement is used to compensate for poor bioavailability. The enhancements can be an extended test duration or an increased test vessel size. The test should be performed with non pre-adapted/non pre-exposed inocula. The test duration should never be extended beyond 60 days, and the test criteria set for ready biodegradability tests should be applied, *i.e.* 60% or 70% degradation, depending on analyte (DOC, $ThCO_2$ or ThOD), without the 10-day window. Prolongation of the test duration up to 60 days is considered acceptable if some initial, slow but steady biodegradation is observed not reaching a plateau by the end of the ready biodegradability test, *i.e.* after 28 days. Comparable to ready biodegradability test, positive results from enhanced ready biodegradability tests may be used to conclude that the substance is not P/vP when supported by the other available information. If the results from an enhanced ready biodegradability test are negative, they are not on their own enough to demonstrate that the substance fulfils the P criteria. Consequently, result from enhanced ready biodegradability test should be assessed in a WoE approach.

OECD TG 306 "Biodegradability in Seawater" includes shake flask and closed bottle tests. If the result is positive (>70% DOC removal; >60% ThOD), it may be concluded that there is a potential for biodegradation in the marine environment. OECD TG 306 indicates that "*results of this test are not to be taken as indications of ready biodegradability, but are to be used specifically for obtaining information about the biodegradability of chemicals in marine environments*". These are not ready biodegradability tests since no inoculum is added in addition to the microorganisms already present in the seawater. Neither do the tests simulate the marine environment since nutrients are added and the concentration of test substance is very much higher than would be present in the sea. If the ratio of inoculum to substrate in the test system is enhanced by increasing the concentration of microorganisms this also increases the degradation potential. In this case the test system does not resemble a pelagic water body anymore and is, thus, less stringent. This has consequences for interpretation of the data with respect to conclusion on ready biodegradation behaviour.

Degradation of substances in seawater has generally been found to be slower than in freshwater inoculated with activated sludge or sewage effluent due to lower amount and diversity of microorganisms. Therefore >60% ThOD or >70% DOC removal obtained in OECD TG 306 (sea water without added inoculum) after 28 day (Closed Bottle Method) or 60 day (Shake Flask Method) is considered an indication of ultimate biodegradation in the marine environment. Results

can also be regarded as a piece of evidence that the substance is likely to fulfil the criteria for ready biodegradability even if, as described in the TG, they cannot be directly compared with criteria of ready biodegradability described above. A result of >20% ThOD or DOC removal in OECD TG 306 (seawater with no added inoculum) is indicative of a potential for primary biodegradation in the marine environment (ECHA Guidance on IR&CSA, Chapter R.7b, section 7.9.4.1).

Tests from OECD TG 302 series determine the inherent biodegradability of organic substances and include three methods: the Modified SCAS Test (OECD 302 A), the Zahn-Wellens/EMPA Test (OECD 302 B) and the Modified MITI Test (II) (OECD 302 C). Inherent tests are similar to ready biodegradability tests as they usually measure the same parameters and are conducted with a high test substance concentration and an even higher microbial concentration. In general, they use more favourable, if not optimal, conditions than ready biodegradability tests (e.g. with increased biomass to test substance ratio and allowing pre-adaptation of the microbial inoculum) and are hence designed to show whether a potential for degradation exists. Even if pre-adaptation is allowed in the TG, it is not allowed to be used in the assessment to conclude not P/vP.

Two of these methods, OECD TG 302 B and OECD TG 302 C, may be used to confirm that the tested substance does not fulfil the criteria for P provided that the following conditions are fulfilled. In OECD TG 302 B biodegradation above 70% of theoretical (measured as DOC removal or O_2 uptake) may be regarded as evidence of inherent, ultimate, biodegradability (non-persistence) provided that ≥70 % mineralisation (DOC removal) is reached within 7 d, lag phase is not longer than 3d, removal before degradation occurs is below 15% and inoculum is not pre-adapted. In OECD TG 302C, ≥70 % mineralisation (O_2 uptake) is reached within 14 d, lag phase is not longer than 3d, and inoculum is not pre-adapted. Careful interpretation of the data must be performed when considering the use of DOC removal as a degradation sum parameter to ensure that elimination did not occur due to adsorption or volatilisation (both of which are physical removal processes which should not be misinterpreted as transformation or biodegradation). If supported by other weight of evidence, lack or low mineralisation (<20% degradation) in an inherent biodegradability test (OECD TG 302 series) may provide sufficient experimental information to confirm that the P-criteria are fulfilled. Additionally, in specific cases it may be possible to conclude that the vP-criteria are fulfilled with this result if there is additional specific information supporting it (*e.g.* specific stability of the chemical bonds). Care should be taken to the interpretation of such tests, since, for example, a very low water solubility of a test substance may reduce the availability of the substance in the test medium. These issues are discussed in more detail in ECHA Guidance on IR&CSA, Chapter R.7b, sections R.7.9.4 and R.7.9.5.

ECHA Guidance on IR&CSA, Appendix R.7.9—1 in Chapter R.7b contains a list of the ISO and OPPTS tests that are equivalent to the OECD guidelines listed above. This Chapter also lists some of the important attributes of each described degradation test.

Results obtained from the ready biodegradability, enhanced ready biodegradability, and inherent biodegradability test can be mainly used as indication of persistence or evidence of non-persistence or as supporting information in the persistence assessment with other lines of evidence (note some exceptions based on inherent degradation tests above).

Interpretation of screening studies with substances containing multiple constituents, impurities and/or additives is challenging if the study is conducted with the whole substance. If the concentration of the constituents is analytically monitored during the study, it may be possible to assess the degradation potential of the relevant constituents separately. If only, for example, evolved CO_2 or consumed O_2 is measured, it is not possible to demonstrate which constituents of the substance have degraded and which not.

Differences in degradation potential of constituents, impurities and additives must also be assessed as part of the biodegradation screening test results. section 4.3.3 (iv) of this Guidance provides further insights into the assessment of substances with more than one constituent.

4.3.3.1.2.5. Abiotic degradation

Abiotic processes such as hydrolysis, oxidation and photolysis may transform substances in aquatic environments, soil and air. Abiotic transformation can be an important step in the pathway for degradation of substances in the environment (OECD, 2006b).

The following guideline exist to assess hydrolysis:

- OECD TG 111: Hydrolysis as a function of pH

In general, the hydrolysis reactions are relatively sensitive to temperature. According to OECD TG 111 tier 2, tests should be carried out with a minimum of three temperatures and preferably at least one temperature below the standard reporting temperature of 25°C. For the persistence assessment purposes, the hydrolysis rate at temperature of 12°C[67] is required. When hydrolysis kinetics has been assessed at different temperatures, the activation energy for the hydrolysis of the specific substance can be estimated using equation given in the Annex 2 of OECD TG 111. If a substance specific activation energy (E_a) is not available or cannot be derived, hydrolysis temperature correction may be done by using the Arrhenius equation (see section 4.3.3.1.2.1) by applying generic estimated E_a of 65.4 kJ/mol.

Rapid hydrolysis needs to be shown across all environmentally relevant pHs. Additional evidence is also needed to consider whether the fate properties (as adsorption) of the substance would cause attenuation of the hydrolysis rate in sediment or soil, or whether suspended solids would similarly affect the hydrolysis rate in aquatic media such as river or sea water.

The degradation half-lives obtained in a hydrolysis test (OECD TG 111) can be used as supporting information in the WoE assessment. Loss of parent substance by hydrolysis alone cannot remove the concern for P/vP. As abiotic degradation is primary degradation, careful consideration will need to be given to the potential formation of stable degradation products with PBT/vPvB or PMT/vPvM properties. Identified hydrolysis products should be reported in accordance with the recommendations contained in the test guidelines (e.g. OECD TG 111).

The following guidelines exist to assess phototransformation:

- OECD TG 316: Phototransformation of Chemicals in Water – Direct Photolysis;
- Draft OECD guidelines on Phototransformation of Chemicals in Water – Direct and Indirect Photolysis (draft August 2000) and on Phototransformation of Chemicals on Soil Surfaces (draft January 2002);
- US EPA 1998: Phototransformation of substances in water by indirect photolysis;
- EFSA Journal (2022): Scientific guidance on soil phototransformation products in groundwater–consideration, parameterisation and simulation in the exposure assessment of plant protection products

Data derived from abiotic studies cannot be used on their own within the persistence assessment, but may be used as part of a WoE approach. Due to the large variation in the light conditions between the different environmental compartments, the use of photolysis data is not generally recognised for the persistence assessment. This is discussed in more details in the ECHA Guidance on IR&CSA, Chapter R.7b. Nevertheless, the relevance of phototransformation products for the persistence assessment should be included in the assessment, if the phototransformation products are expected to be formed under relevant environmental conditions.

[67] Reference temperature for marine environment is 9°C.

4.3.3.1.2.6. Non-standard biodegradation studies

In addition to the standardised data described above, there is a vast amount of non-standardised biodegradation data that has been published in the scientific literature. Many of these studies share some common principles with the standard biodegradability tests, for example the fact that the test substance is usually provided to the microorganism or microbial community as the sole source of carbon for growth and energy. Non-standard data may be valuable, as part of a WoE assessment provided that they are relevant and reliable. Reporting and use of non-standard information is given in section 4.3.3 (i) of this Guidance.

4.3.3.1.2.7. Databases with available data

The ECHA REACH database includes public and disseminated information on ready biodegradability and simulation biodegradation studies, from the registration dossiers, submitted by companies to ECHA in the framework of the REACH Regulation. The data is available on ECHA's dissemination website[68] and OECD QSAR Toolbox[69]. Information on Biocidal active substances and Biocidal products is also available via the ECHA website[70]. The Japanese National Institute of Technology and Evaluation (NITE) database[71] collated experimental biodegradation, photooxidation and hydrolysis data. NITE biodegradation data is also available via the OECD QSAR Toolbox under 'Biodegradation NITE'.

The Global Portal to Information on Chemical Substances (eChemPortal)[72] provides free public access to information on properties of chemicals, and direct links to collections of information prepared for government chemical programmes at national, regional, and international levels. Access to information on existing chemicals, new industrial chemicals, pesticides and biocides is provided. eChemPortal also makes available national/regional classification results according to national/regional classification and labelling schemes or according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

The EU Pesticides database[73] includes information on active substances used in plant protection products, Maximum Residue Levels (MRLs) in food products, and emergency authorisations of plant protection products in Member States. The Pesticides Properties DataBase (PPDB)[74] is a relational database of pesticide chemical identity, physicochemical, human health and ecotoxicological data.

The information in these databases is not necessarily curated and when used in the assessments its quality and reliability must be carefully considered.

4.3.3.1.3. Non-testing data on degradation

Quantitative Structure Activity Relationships ((Q)SARs)

A variety of models have been developed to predict biodegradation and potential degradation products. (Q)SAR predictions can be used in the event that the applied model is scientifically valid, the input is correct, the substance is within the applicability domain of the model, the

[68] https://echa.europa.eu/

[69] https://www.qsartoolbox.org/home

[70] https://echa.europa.eu/information-on-chemicals

[71] http://www.nite.go.jp/en/chem/qsar/evaluation.html

[72] https://www.echemportal.org/echemportal/

[73] https://food.ec.europa.eu/plants/pesticides/eu-pesticides-database_en

[74] https://sitem.herts.ac.uk/aeru/iupac/index.htm

prediction is reliable, the outcome is fit for the regulatory purpose (see ECHA Guidance on IR&CSA, Chapter R.6, section R.6.1; OECD, 2023), and the results are adequately reported.

Models for biodegradation estimation include:

- The EPI (Estimation Programs Interface) Suite™ is a Windows®-based suite of physical/chemical property and environmental fate estimation programs developed by US EPA's and Syracuse Research Corp. (SRC) (https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface). EPI Suite™ is a screening-level tool. It includes two individual models for biodegradation estimation
    - BIOWIN™: Estimates aerobic and anaerobic biodegradability of organic chemicals using 7 different models. Two of these are the original Biodegradation Probability Program (BPP™). The seventh model estimates anaerobic biodegradation potential. The MITI models BIOWIN5 and BIOWIN6 models were updated in June 2017 using a much larger dataset of experimental data. The updated model is contained in the EPI Suite update file[75].
    - BioHCwin: Estimates biodegradation half-life for compounds containing only carbon and hydrogen (*i.e.* hydrocarbons).
    - HYDROWIN™: Estimate aqueous hydrolysis rate constant and half-life.
    - AOPWIN™: Estimates the atmospheric half-lives.
- The CATALOGIC software suite (commercial, requires licence) is a platform for models and databases related to the environmental fate of substances such as abiotic and biotic degradation, bioaccumulation and acute aquatic toxicity.
- The EAWAG Pathway Prediction System (PPS)[76] predicts degradation pathways using biotransformation rules established from the reactions compiled in the EAWAG-BBD database.
- VEGA HUB[77] is a platform offering a collection of QSAR models for (eco)toxicological and environmental fate endpoints, and an independent tool helping the user in the evaluation of the result, through the Applicability Domain Index. The QSAR prediction models derive from CAESAR, T.E.S.T., SARpy, EPISuite, Toxtree, and other tools.

The OECD QSAR Toolbox is a freely available software tool to perform transparent and reproducible hazard assessment. It includes publicly available databases for many chemical properties. Databases in the Toolbox containing experimental data related to persistence are ECHA REACH, Biodegradation NITE, and Biodegradation in Soil Oasis. Furthermore, the QSAR Toolbox can be used to predict properties using (Q)SAR models which have been made available via the QSAR Toolbox, or by building regression based (Q)SAR models based on experimental information available in the QSAR Toolbox. QSAR Toolbox ECHA P screening (BETA) profiler[78] identifies substances with the potential for P/vP properties using experimental data and QSAR models available within the QSAR Toolbox. The results of this screening are based on single threshold values for P/vP according to Annex XIII to REACH Regulation (EC) No 1907/2006 and ECHA Guidance on IR&CSA, Chapter R.11: PBT/vPvB assessment (June 2017).

The above list of models is not exhaustive, and other valid models may also be used. With more experimental data becoming available, and a better understanding of the relationship between

[75] https://www.epa.gov/tsca-screening-tools/download-epi-suitetm-estimation-program-interface-v411

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

[77] https://www.vegahub.eu/

[78] ECHA P screening (BETA) - Toolbox Repository (qsartoolbox.org)

structure and endpoint, (Q)SAR models are being updated or new models developed. In every case, it needs to be verified that both, the (Q)SAR model and the prediction are acceptable.

In line with CLP Annex I: 4.3.2.4.2. and 4.4.2.4.2., results obtained from well-developed and reliable biodegradation (Q)SAR models can be used when applying the WoE determination as part of the scientific assessment of the information relevant for the P, vP properties. When EPI Suite™ is used for this purpose, it is recommended to use combined results from three estimation models in the EPI Suite™ (US EPA, 2012; R.11).

The results of the following three freely available estimation models BIOWIN 2, 6 and 3 in the EPI suite™ may be used in WoE to indicate persistence as follows:

- Non-linear model prediction (BIOWIN 2): does not biodegrade fast (probability < 0.5)[79] and ultimate biodegradation timeframe prediction (BIOWIN 3): ≥ months (value < 2.25 (to 2.75)[80]), **or**
- MITI non-linear model prediction (BIOWIN 6): does not biodegrade fast (probability < 0.5) and ultimate biodegradation timeframe prediction (BIOWIN 3): ≥ months (value < 2.25 (to 2.75))

Borderline cases should be carefully examined, e.g. when the estimate of the ultimate degradation time predicted by BIOWIN 3 gives a result in the range of 2.25 to 2.75 (see sections R.7.9.4 and R.7.9.5 in Chapter R.7b of the ECHA Guidance on IR&CSA). In every case, the prediction needs to be verified that both, the QSAR model is valid and the prediction is acceptable. The use of (Q)SAR model predictions is of particular relevance and interest when test data are lacking and when assessing multi-constituent substances for which it may often be difficult to find or even to generate test data on relevant individual constituents (including impurities) due to analytical, technical, practical and cost implications.

Further information can be found in ECHA Guidance on IR&CSA, Chapters R.6 (QSARs and grouping of chemicals), R.7b sections R.7.9.3.1 and R.7.9.4.1, R.11 sections R.11.4.1.1.4, and OECD (2023).

[79] The probability is low that the substance biodegrades fast.

[80] For substances fulfilling this but BIOWIN 3 indicates a value between 2.25 and 2.75 more degradation relevant information is generally warranted.

4.3.3.2. Bioaccumulation assessment

Commission Delegated Regulation (EU) 2023/707, Annex I: 4.3.2.3.2. The following information shall be considered for the assessment of B or vB properties:

(a) results from a bioconcentration or bioaccumulation study in aquatic species;

(b) other information on the bioaccumulation potential, provided that its suitability and reliability can be reasonably demonstrated, such as:

(i) results from a bioaccumulation study in terrestrial species;
(ii) data from scientific analysis of human body fluids or tissues, such as blood, milk or fat;
(iii) detection of elevated levels in biota, in particular in endangered species or in vulnerable populations or subpopulations, compared to levels in their surrounding environment;
(iv) results from a chronic toxicity study on animals;
(v) assessment of the toxicokinetic behaviour of the substance.

(c) information on the ability of the substance to biomagnify in the food chain, where possible expressed by biomagnification factors or trophic magnification factors.

Annex I: 4.3.2.4.2. In applying the WoE determination, the following information, in addition to the information referred to in section 4.3.2.3.2 shall be considered as part of the scientific assessment of the information relevant for the B, vB properties:

Indication of B or vB properties:

(i) Octanol-water partitioning coefficient experimentally determined or estimated by well-developed and reliable (Q)SAR models;
(ii) Other information provided that its suitability and reliability can be reasonably demonstrated.

4.3.3.2.1. Bioaccumulation introduction

According to CLP, Annex I, section 4.1.1.1, 'bioaccumulation' means the net result of uptake, transformation and elimination of a substance in an organism due to all routes of exposure (*i.e.* air, water, sediment/soil and food). Annex I specifies that 'bioconcentration' means the net result of uptake, transformation and elimination of a substance in an organism due to waterborne exposure.

Bioaccumulation can lead to internal concentrations of a substance in an organism that cause toxic effects over long-term exposures even when external concentrations are very low. Highly bioaccumulative substances may also transfer through the food web, which in some cases may lead to biomagnification (ECHA Guidance on IR&CSA, Chapter R.11). Biomagnification refers to accumulation of a substance via the food chain, from prey to predator. It may be defined as an

increase in the internal concentration of a substance in organisms at succeeding trophic levels in a food chain.

A range of terms are used to describe accumulation of substances in biota, as described below.

4.3.3.2.2. Bioaccumulation terminology

Annex 1 of OECD TG 305 contains the following definitions for Fish BCF (OECD, 2012):

The **fish steady-state bioconcentration factor (Fish BCF_{SS})** is the ratio of the concentration of a substance in an organism to the concentration in water once a steady state has been achieved:

Fish BCF_{SS} = Co/Cw

where BCF is the bioconcentration factor (L/kg)

Co is the substance concentration in the whole organism (mg/kg, wet weight)

Cw is the substance concentration in water (mg/L)

Note that corrections for growth and/or a standard lipid content are not accounted for in this definition of the BCF.

Fish BCF_{SS} does not change significantly over a prolonged period of time, the concentration of the test substance in the surrounding medium being constant during this period. Kinetic and steady-state BCFs should also be reported relative to a default fish lipid content of 5% (w/w), unless it can be argued that the test substance does not primarily accumulate in lipid. Fish concentration data, or the BCF, are normalised according to the ratio between 5% and the actual (individual) mean lipid content (in % wet weight). The figure of 5% lipid content has been widely used as this represents the average lipid content of fish commonly used in OECD TG 305 (OECD, 2012).

The **5% lipid normalised steady-state fish bioconcentration factor (Fish BCF_{SSL})** is normalised to a fish with 5% lipid content.

The **fish kinetic bioconcentration factor (Fish BCF_K)** is the ratio of the uptake rate constant (k_1) to the depuration rate constant (k_2) and can be determined under non-steady state conditions. In principle, the value should be comparable to the Fish BCF_{SS} but deviations may occur if steady state was uncertain or if corrections for growth have been applied to the kinetic BCF.

$$Fish\ BCFK = \frac{k1}{k2}$$

The **uptake rate constant (k_1)** is the numerical value defining the rate of increase in the concentration of test substance in/on test fish (or specified tissues thereof) when the fish are exposed to that chemical (k_1 is expressed in L kg^{-1} day^{-1}).

The **depuration (loss) rate constant (k_2)** is the numerical value defining the rate of reduction in the concentration of the test substance in the test fish (or specified tissues thereof) following the transfer of the test fish from a medium containing the test substance to a medium free of that substance (k_2 is expressed in day^{-1}).

The **5% lipid normalised kinetic fish bioconcentration factor (BCF_{KL})** is normalised to a fish with a 5% lipid content.

The **5% lipid normalised, growth corrected fish kinetic bioconcentration factor (Fish BCF_{KgL})** is the kinetic BCF which is corrected for fish growth observed during the study period

and is subsequently normalised to a fish with a 5% lipid content. Growth correction during the study period is described in Annex 5 of OECD TG 305.

Although there has been some discussion on the growth correction recently (Gobas and Lee, 2019), the approach described in Annex 5 of OECD TG 305 is considered valid. Therefore, growth correction should be applied. Explanations on correction for growth dilution are given in ECHA Guidance on IR&CSA, Chapter R.11, Appendix R.11-6.

Annexes 1 and 7 of OECD TG 305 contain the following definitions for results from a fish dietary test (OECD, 2012):

The **fish dietary biomagnification factor (dietary Fish BMF)** describes the result of dietary exposure test, in which exposure via the aqueous phase is carefully avoided and thus the dietary BMF from this test method cannot directly be compared to a BMF value from a field study (in which both water and dietary exposure may be combined).

$$dietary\ Fish\ BMFK = \frac{I \times \alpha}{k2}$$

$$dietary\ Fish\ BMFKg = \frac{I \times \alpha}{k2g}$$

where: α = assimilation efficiency[81] (absorption of test substance across the gut);

k2 = overall (not growth-corrected) depuration rate constant (day^{-1}), calculated according to Annex 5 of OECD TG 305

k2g = growth-corrected depuration rate constant (day^{-1});

I = food ingestion rate constant (g food g^{-1} fish day^{-1});

Dietary Fish BMF_K is the kinetic dietary BMF without growth correction

Dietary Fish BMF_{Kg} is the kinetic, growth corrected dietary BMF.

The **assimilation efficiency (α)** is a measure of the relative amount of substance absorbed from the gut into the organism (α is unitless, but it is often expressed as a percentage rather than a fraction). Annex 7 of OECD TG 305 explains how to calculate it from the test results.

The **food ingestion rate (I)** is the average amount of food eaten by each fish each day, relative to the estimated average fish whole body weight (expressed in terms of g food/g fish/day).

The **lipid- and growth-corrected fish kinetic dietary biomagnification factor (Fish dietary BMF_{KgL})** is the dietary BMF which has been growth corrected and corrected for lipid content of the fish and its food. For any use of the BMFkgL, it is important that the dietary lipid content and the feeding rate are reported alongside the value.

Annex I of OECD TG 315 includes the following definitions for bioaccumulation in sediment-dwelling organisms (OECD, 2008a):

OECD TG 315 indicates that the main endpoint of this test is the **sediment bioaccumulation factor (sediment BAF).**

The **steady-state sediment bioaccumulation factor (sediment BAFss, [$kg_{sediment} \cdot kg^{-1}_{worm}$])** is the BAF at steady state and does not change significantly over a prolonged period of time, the concentration of the test substance in the surrounding medium (Cs as g kg^{-1} of wet or dry weight of sediment) being constant during this period of time.

[81] In OECD TG 305 the term "assimilation efficiency" is used. However, as pointed out in OECD, 2017 assimilation is not the correct term, since it refers to uptake and subsequent incorporation into tissue, *i.e.* it refers to uptake and transformation.

$$sediment\ BAFss = \frac{Ca\ at\ steady\ state\ or\ at\ day\ 28\ (mean)}{Cs\ at\ steady\ state\ or\ at\ day\ 28\ (mean)}$$

Where

Ca = concentration in worms in g kg^{-1} wet or dry weight

Cs = concentration in sediment as g kg^{-1} of wet or dry weight of sediment

The **kinetic sediment BAF (sediment BAF_K)** is defined as:

$$sediment\ BAFK = \frac{k1}{k2}$$

where

k_1 = sediment uptake rate constant defining the rate of increase in the concentration of the test substance in/on the test organism resulting from uptake from the sediment phase [g sediment kg^{-1} of worm d^{-1}]

k_2 = elimination rate constant defining the rate of reduction in the concentration of the test substance in/on the test organism, following the transfer of the test organisms from a medium containing the test substance to a substance-free medium [d^{-1}]

The **biota-sediment accumulation factor (BSAF, [kg sediment OC kg^{-1} worm lipid content])** is the lipid-normalised steady-state concentration of test substance in/on the test organism divided by the organic carbon-normalised concentration of the substance in the sediment at steady state.

$$BSAF = BAFk \times \frac{foc}{flip}$$

where

f_{oc} = the fraction of sediment organic carbon based on either on dry weight or wet weight

f_{lip} = the fraction of worm lipid, both based either on dry weight or wet weight.

It should be noted that the term **biota-sediment accumulation factor (BSAF)** has been used in the literature to refer to bioaccumulation factors in sediment which have not been normalised to organism lipid and sediment total organic carbon content. Care should be taken to ensure it is clear what the reported value refers to.

Annex 1 of OECD TG 317 contains the following definitions for bioaccumulation in soil-dwelling organisms (OECD TG 317, 2010):

OECD TG 317 indicates that the main endpoint of this test is the **soil bioaccumulation factor (soil BAF)**.

The **steady-state soil bioaccumulation factor (soil BAFss, [$kg_{soil} \cdot kg^{-1}_{worm}$])** is the BAF at steady state and does not change significantly over a prolonged period of time, the concentration of the test substance in the surrounding medium (Cs as g kg^{-1} of wet or dry weight of soil) being constant during this period of time.

$$soil\ BAFss = \frac{Ca\ at\ steady\ state\ or\ at\ day\ 21\ (mean)}{Cs\ at\ steady\ state\ or\ at\ day\ 21\ (mean)}$$

where

Ca = concentration in worms in g kg^{-1} wet or dry weight

Cs = concentration in soil as g kg^{-1} of wet or dry weight of soil

The **kinetic soil BAF (soil BAF_K)** is defined as:

$$soil\ BAFK = \frac{k1}{k2}$$

where

k_1 = soil uptake rate constant defining the rate of increase in the concentration of the test item in/on the test organism resulting from uptake from the soil phase [g soil kg^{-1} of worm d^{-1}]

k_2 = elimination rate constant defining the rate of reduction in the concentration of the test item in/on the test organism, following the transfer of the test organisms from a medium containing the test item to a substance-free medium [d^{-1}]

The **biota-soil accumulation factor ($BSAF_{soil}$, [kg soil OC kg^{-1} worm lipid content])** is the lipid-normalised concentration of test substance in/on the test organism divided by the organic carbon-normalised concentration of the substance in the soil at steady state.

$$BSAFsoil = BAFk \times \frac{foc}{flip}$$

where

f_{oc} = the fraction of soil organic carbon based either on dry weight or wet weight

f_{lip} = the fraction of worm lipid, both based either on dry weight or wet weight.

It should be noted that the term **biota-soil accumulation factor ($BSAF_{soil}$)** has been used in the literature to refer to bioaccumulation factors in soil which have not been normalised to organism lipid and soil total organic carbon content. Care should be taken to ensure it is clear what the reported value refers to.

Field bioaccumulation metrics

The **field bioaccumulation factor (field BAF)** represents environmental exposure in the field to an aquatic organism from all routes and is referenced to the substance concentration in water (Arnot and Gobas, 2004; Burkhard *et al*., 2012b). The basis for the field BAF value is the ratio of the concentration in wet weight (ww) of the organism divided by the water concentration. The unit of the field BAF is L·kg ww^{-1}. It is recommended that the field BAF is reported in terms of wet weight as well as dry weight and is also normalised to lipid weight, with an explanation of how the normalisation was performed (European Commission, 2018).

Field measured biota-sediment accumulation factors (field BSAF) are derived by the concentration of a substance in biota divided by the concentration in the sediment (Burkhard *et al*., 2010).

The **field biomagnification factor (field BMF)** is the concentration of a substance in a predator relative to the concentration in the predator's prey (or food) originating from the same ecosystem at steady state and in which both, water and dietary exposure may be combined. (ECHA Guidance on IR&CSA, Chapters R.11, R.7c):

Field BMF = Co/Cd

where field BMF is the field biomagnification factor (dimensionless)

Co is the steady-state substance concentration in the organism (mg/kg)

Cd is the steady-state substance concentration in the diet (mg/kg).

Field BMFs for substances that partition into lipids should, as far as possible, be lipid normalised to account for differences in lipid content between prey and predator. It allows for a comparison of field BMF values in a direct and objective manner.

The **trophic magnification factor (TMF)** describes the average increase in biota concentration per trophic level (ECHA Guidance on IR&CSA, Chapter R.7c). The TMF for a food web is calculated as the exponent of the slope of the natural logarithm transformed concentrations for organisms in the food chain as a function of the trophic level of these organisms. The TMF represents the average biomagnification per trophic level within that food web. For substances that partition into lipids the TMF should be derived from lipid-normalised biota concentrations versus trophic level.

4.3.3.2.3. Data on Bioaccumulation

4.3.3.2.3.1. Fish bioaccumulation tests - aqueous exposure

The most commonly used test guideline for fish bioaccumulation is OECD TG 305 (OECD, 2012). Detailed guidance on interpretation of OECD TG 305 fish bioaccumulation test data is provided in the related OECD Guidance document (OECD, 2017), ECHA Guidance on IR&CSA, Chapters R.11 and R.7c and this Guidance, Annex III.2. The aqueous exposure test measures fish BCF. Reliable fish BCFs have been extensively used in a regulatory context to conclude that a substance meets the criteria for B or vB.

Principle of the test

To measure bioconcentration of a substance in fish, a sufficient number of fish are exposed to one or two sub-lethal concentrations of the test substance dissolved in water. Fish and water are sampled at regular time-intervals and the concentration of test substance is measured. Tests are generally conducted using a flow-through system. After reaching a steady-state tissue concentration (usually after 28 days, see paragraphs 17-18 of OECD, 2012), the remaining fish are transferred to clean water and the depuration is followed. A control group of fish is held under identical conditions except for the absence of the test substance, to relate possible adverse effects observed in the bioconcentration test to a matching control group and to obtain background concentrations of the test substance.

Where possible the bioconcentration factor is calculated both as the ratio of concentration in the fish (C_f) and in the water (C_w) at steady state (Fish BCF_{SS}) and as a kinetic bioconcentration factor (Fish BCF_K), which is estimated as the ratio of the rate constants of uptake (k_1) and depuration (k_2) assuming first order kinetics. The uptake rate constant, the depuration (loss) rate constant, the bioconcentration factor (steady-state and/or kinetic), and where possible, the confidence limits of each of these parameters are calculated from the model that best describes the measured concentrations of test substance in fish and water. The Fish BCF_{SS} is doubtful if the Fish BCF_K is significantly larger than the BCF_{SS}, as this can be an indication that steady-state has not been reached or growth dilution and loss processes have not been taken into account (OECD, 2012). Depending on the fish species and life stage, significant mass growth can occur throughout the testing period. The increase in fish mass during the test will result in a decrease of test substance concentration in growing fish (so-called growth dilution), and thus the kinetic BCF will be underestimated if not corrected for growth (see also ECHA Guidance on IR&CSA, Chapter R.11, Appendix R.11-6). OECD TG 305 explains how to correct the Fish BCF_K for growth dilution. If no information on growth is available, case by case assessment is needed, e.g. depending on the fish species and lifestage. There is currently no method to correct BCF_{SS} for growth dilution.

Fish BCF_{KgL} is the 5% lipid-normalised, growth-corrected, kinetic bioconcentration factor and is normally the preferred result for comparison with the numerical CLP B/vB criteria for substances accumulating mainly in lipids, since the kinetic BCF can be derived even if no steady state is reached and it can be corrected for fish growth. However, there may be cases where the Fish BCF_{SSL} is more appropriate. This must be assessed on a case by case basis.

OECD TG 305 specifies the applicability of the test and the conditions which must be met for a study to be valid.

Considerations when reviewing fish BCF tests (see also this Guidance, Annex III)

Exposure concentrations should not exceed the aqueous solubility of the test substance. In cases where test exposures significantly exceed aqueous solubility (e.g. due to the use of dispersants), and the analytical method does not distinguish between dissolved and non-dissolved substance, the study data should generally be considered unreliable. The total organic carbon and dissolved oxygen concentrations in the dilution water should be reported.

The concentration(s) of the test substance should be below its chronic effect level or 1% of its acute asymptotic LC_{50}, within an environmentally relevant range and at least an order of magnitude above its limit of quantification in water by the analytical method used. The highest permissible test concentration can also be determined by dividing the acute 96h LC_{50} by an appropriate acute:chronic ratio (e.g. appropriate ratios for some chemicals are about three, but a few are above 100) (Paragraph 51, OECD, 2012) . This is to avoid any toxic effect of the test substance during the test. The average growth in both test and control groups can be compared to check for toxic effects. Any decreased growth in the test groups would suggest toxic effects occurred. If no mortality information is provided for a study, one option is to designate the study as 'reliable with restrictions' if the exposure concentration used is at least a factor of 10 below the known or predicted fish LC_{50}.

If a radiolabelled test substance is used, total radioactivity measurements alone may overestimate the concentration of parent substance due to small amounts of radiolabelled impurities that may be present in the test substance, and/or formation of metabolites. Thus, a Fish BCF based on total radioactivity can be considered as a conservative value for the parent substance. To avoid overestimation of the BCF, it is preferable to have a substance-specific chemical analytical technique or selective clean-up procedure at the end of the exposure period and to report the Fish BCF based on parent and not on total radioactive residues. Further guidance is available in paragraphs 6 and 65 of OECD TG 305 (OECD, 2012). If the fish are not fed, high concentrations of (usually more polar) metabolites may build up in the gall bladder, which may lead to an overestimate of whole body levels (OECD, 2001).

The analytical method used for the quantification of the substance should be described. The recovery efficiency, precision, limits of quantification and detection and working range should be reported with an explanation of how they were determined.

The whole body lipid content should ideally be reported since many organic substances partition to lipid. Where reported, the BCF should be normalised to 5% lipid to allow comparison between studies, unless it is known that the substance does not primarily partition to lipids. BCF results should specify the units and tissue type (e.g. whole body, muscle, fillet, liver, fat). Whole body wet weight measurements are preferred for comparison with CLP B/vB criteria.

The kinetic Fish BCF (BCF_K) is normally preferred for regulatory purposes since for bioaccumulative substances a real steady state is often not attained during the uptake phase. The Fish BCF_K should be corrected for growth dilution (since some growth is expected, as the fish are fed to keep them healthy and maintain body weight). Where information on growth is not available, the likely significance of growth on the results should be assessed. The uncertainty in a BCF value derived from a fast-growing fish will be greater than that for a slow growing fish. For relevance and scientific justification of correction for growth dilution when deriving BCF see Appendix R.11-6 in ECHA Guidance on IR&CSA, Chapter R.11.

In conclusion, reliable and relevant fish BCFs can be compared directly with the numerical CLP B/vB criteria of BCF >2000 and BCF >5000, respectively.

4.3.3.2.3.2. Fish bioaccumulation tests - dietary exposure

Although they are less commonly conducted than aqueous exposure tests, dietary exposure tests may be available. The test is recommended for substances where the aqueous exposure methodology is not practicable. The only test guideline available currently is OECD TG 305-III: Dietary Exposure Bioaccumulation Fish Test. Such tests expose the fish via food only, avoiding aqueous exposure.

The primary endpoint measured in a fish dietary study is a dietary biomagnification factor (dietary BMF), which is the concentration of a substance in fish relative to the concentration in the food at steady state. The dietary fish BMF differs from the field BMF, one reason for this could be that exposure is through a combination of water and food in the field situation, while in the dietary exposure study the exposure through the water phase is excluded under controlled conditions. Since a field BMF covers exposure from several routes (including food and water) and a dietary BMF covers exposure only via food, dietary BMFs are generally lower than field BMFs.

In a study by Inoue *et al*. (2012) with carp, only two of the five substances that had a BCF value higher than 5000 L/kg, had a dietary BMF value in excess of 1. In a study by Martin *et al.* (2003 a,b) with perfluorinated compounds, one of the three substances with a BCF > 2000 had a dietary BMF of 1.0, while the two others had substantially lower BMF values. Therefore, a dietary fish BMF below 1 cannot be used to conclude that a substance is not bioaccumulative and it should be first assessed if the bioaccumulation potential can be concluded based on the estimated BCF, which can be directly compared to the CLP criteria. A dietary BMF <1 therefore does not mean that a substance is not bioaccumulative (ECHA Guidance on IR&CSA, Chapter R.11, section R.11.4.1.2.3).

The dietary BMF cannot be directly compared with the CLP criteria which are based on BCF values, but a BCF_K can be estimated from fish dietary studies using the Dietary Exposure Test Spreadsheet of OECD 305 TG[82]. Detailed guidance on estimation of the fish BCF from the dietary study is given below under "*Considerations when reviewing fish dietary exposure bioaccumulation tests*". Reliable and relevant fish dietary studies have been used in a regulatory context to conclude if a substance fulfils the CLP criteria for B or vB in a WoE approach, using the estimated BCF from the measured depuration rate constant/half-life (see Example B).

Principle of the test

In fish dietary exposure tests, a sufficient number of fish are exposed usually to one sub-lethal concentration of the test substance spiked on fish food. Both fish and experimental diet are sampled at regular time intervals and the concentration of test substance measured. An uptake phase of 7-14 days is recommended but it can be extended, if necessary. As fish may not reach steady state during the uptake phase, the data treatment and results are usually based on a kinetic analysis of tissue residues. The depuration phase begins when the dietary-exposed fish are fed for the first time with unspiked food and usually lasts for up to 28 days or until the test substance can no longer be quantified in whole fish, whichever is sooner. It is important to remove any uneaten food and faeces shortly after feeding to avoid the test substance partitioning to the water leading to exposure via the water.

A control group of fish is held under identical conditions and fed identically except that the commercial fish food diet is not spiked with test substance. This control group allows background

[82] https://www.oecd.org/en/topics/sub-issues/testing-of-chemicals/software-test-guidelines.html

(Last accessed: August 2024)

levels of test substance to be quantified in unexposed fish and serves as a comparison for any treatment-related adverse effects noted in the test group (OECD, 2012).

This method allows the determination of the substance-specific half-life ($t_{1/2}$, from the depuration rate constant, k_2), the assimilation efficiency (absorption across the gut; α), the kinetic fish dietary biomagnification factor (Fish BMF_K), the growth-corrected kinetic fish dietary biomagnification factor (Fish BMF_{Kg}), and the lipid-corrected kinetic fish dietary biomagnification factor (Fish BMF_{KL}) (and/or the growth- and lipid-corrected kinetic fish dietary biomagnification factor, Fish BMF_{KgL}) for the test substance in fish.

There has been recent discussion about the dietary BMF and the appropriateness of correcting for the lipid content of fish and their food according to the method in OECD TG 305 (Hashizume *et al*. (2018), Gobas *et al*. (2021), Environment Agency (2023)). As a result of these discussions, in line with ECHA Guidance on IR&CSA, Chapter R.11, the preferred endpoint from OECD TG 305 dietary exposure test is the BCF value estimated from experimentally derived depuration rate constant, which can be directly compared to the numerical CLP criteria, unless it can be demonstrated that the uptake rate constant k_1 cannot be reliably estimated with the available methods. For very hydrophobic substances, k_1 estimates may become increasingly uncertain. In that case other methods (direct application of k_2, or using a correlation of dietary BMF and BCF results to interpolate other dietary BMF results) as described in OECD (2017), Chapter 4.6.3 should be used and the results assessed in a WoE approach. The estimated BCF can be directly compared to the numerical CLP criteria.

In case the derivation of a BCF is not possible, the Fish BMF5%, which is the Fish BMF_{Kg} normalised to a fish with a 5% lipid content as recommended by Hashizume *et al*. (2018), may be useful to compare results from different studies (Environment Agency, 2023). For any use of the Fish BMF_{KgL}, it is important that the dietary lipid content and the feeding rate are reported alongside the value. Fish BMF5% and Fish BMF_{KgL} could be used in a benchmarking exercise.

Like in the aqueous exposure method, increases in fish mass during the dietary exposure test will result in dilution of the test substance in growing fish and thus the fish (kinetic) BMF will be underestimated if not corrected for growth (OECD, 2012). Annex 5 of OECD TG 305 (OECD, 2012) explains how to perform the growth correction. OECD TG 305 specifies the applicability of the test and the conditions which must be met for a study to be valid.

Considerations when reviewing fish dietary exposure bioaccumulation tests

For poorly soluble substances, if high concentration is spiked to the feed, e.g. in the upper limit indicated in OECD TG 305, there could be issues with the bioavailability of the substance due to potential crystallisation of the test substance. This could lead to underestimation of uptake and BMF. It is important that the spiked food is palatable to the fish. This can be checked by examining the growth of fish during the course of the study. There should be similar growth in the control and in the test groups of fish. The body burden of the test substance in the test fish should not reach a level which is sufficient to cause toxic effects.

Like in the aqueous fish bioaccumulation test, if radiolabelled test substance is used for the dietary exposure test, total radioactivity measurements alone may overestimate the concentration of parent substance due to small amounts of radiolabelled impurities that may be present in the test substance, and/or formation of metabolites.

The lipid content measured at least at the start and end of the uptake phase and at the end of the depuration phase should be reported, as well as the method used for its determination. The results should be expressed based on whole body, wet weight concentrations.

The fish dietary bioaccumulation test provides a BMF rather than a BCF, which is required for comparison with the numerical CLP criteria. Whenever possible, the kinetic BCF should be estimated based on the results of fish dietary test to compare with the CLP criteria. The BCF value can be estimated from a predicted uptake rate constant and the experimentally determined

depuration rate using the Dietary Exposure Test Spreadsheet of OECD TG 305[83], unless it can be demonstrated that the uptake rate constant (k_1) cannot be reliably estimated with the available methods.

A detailed description of the methods to estimate a BCF from a dietary study can be found in Annex 8 of OECD TG 305 (OECD, 2012) and the Guidance Document on Aspects of OECD TG 305 (OECD, 2017) in Chapter 4.6.3. The methods are 1) Uptake rate constant estimation method, 2) Relating depuration rate constant directly to BCF and 3) Correlating dietary BMF with BCF. OECD (2017) provides further information on the applicability domain of the three estimation methods.

Besides the calculation of a BCF from the depuration phase, the dietary BMF derived from OECD TG 305-III test can be compared with laboratory BMF values for substances with known bioaccumulation potential in a benchmarking exercise (see Correlating dietary BMF with BCF (Method 3) in OECD, 2017). For example, such an approach has been described for dietary bioaccumulation studies with carp (Inoue *et al.*, 2012). Based on a regression between BCF_L and BMF_{KgL} for nine compounds tested in this set-up, it was shown that a BCF_L value of 5000 L/kg, normalised to a lipid content of 5%, corresponds to a lipid corrected BMF_{KgL} from the dietary test of 0.31 kg food lipids/kg fish lipids, and a BCF_L of 2000 L/kg corresponds to a BMF_{KgL} of 0.10 kg food lipids/kg fish lipids. See OECD (2017), paragraph 288 for pros and cons of the method 3.

A different benchmarking could be obtained from aqueous and dietary bioaccumulation studies for perfluorinated compounds with rainbow trout (Martin *et al.*, 2003a, b). These studies emphasise the fact that even if a BMF from an OECD TG 305 dietary bioaccumulation study is found to be <1, it cannot be considered as a good discriminator for concluding substances not to be (very) bioaccumulative according to the BCF criteria. If benchmarking is used for comparing dietary BMF values with BMF values for substances with a known bioaccumulation potential, it must be ensured that these BMF values were obtained under similar conditions (*i.e.* fish species, fish weight/size, diet lipid content, feeding rate, fish lipid content and temperature).

Another endpoint from the dietary OECD 305 test is the depuration rate constant. The depuration rate constant has been proposed as an endpoint for the bioaccumulation assessment (e.g. Brooke and Crookes, 2012, Goss *et al*. 2013, Goss *et al*. 2018). For example, Brooke and Crookes (2012) presented lipid normalised depuration rate constants of 0.181 and 0.085 d^{-1} as critical values for lipid normalised BCF values of 2000 and 5000. Relating depuration rate constant directly to BCF is described as Method 2 in Guidance document on aspects of OECD TG 305 (OECD, 2017). The depuration rate constant is a useful metric for assessing bioaccumulation. However, it should be noted that the kinetics of uptake and depuration are still dependent on other factors, for example the size of the fish (e.g. Barber 2008, Brooke and Crookes, 2012). Indeed, from the analysis from Brookes and Crookes (2012) there is considerable scatter around the regression line between log BCF_L and log k_2 (lipid normalised), which may be caused by the variability in fish weight used in the underlying studies, at least partly. This implies that it is not possible to set one value for the depuration rate constant for different organisms. If aqueous bioconcentration is considered, an uptake rate constant of 520 L/kg/d could be estimated for fish with a weight of 1 g (Sijm *et al.*, 1995). The depuration rate constants that lead to bioconcentration factors of 2000 and 5000 could thus be estimated to be 0.26 d^{-1} and 0.10 d^{-1}. For fish weighing ten grams these values would be approximately half of these values (0.12 d^{-1} and 0.05 d^{-1}).

Detailed guidance on interpretation of OECD TG 305 fish bioaccumulation test data is provided in the test guideline and in the related OECD Guidance document (OECD, 2017). More information on the fish dietary bioaccumulation test and the use of the results can be found in the ECHA Guidance on IR&CSA, Chapter R.11, section R.11.4.1.2.3.

[83] accessible at https://www.oecd.org/en/topics/sub-issues/testing-of-chemicals/software-test-guidelines.html

(last accessed: August 2024)

In conclusion, reliable and relevant fish dietary tests provide useful information on bioaccumulation but the results cannot be directly compared with the numerical CLP B/vB criteria. The estimated BCF needs to be derived to allow a comparison with the criteria. If it is not possible to estimate the BCF, other toxicokinetic information from the study can be used in a weight-of-evidence approach to conclude on B or vB.

4.3.3.2.3.3. Invertebrate (*Hyalella azteca*) bioconcentration tests

Hyalella azteca is an epibenthic amphipod which is widespread in North and Central America and commonly used for ecotoxicity studies (Environment Canada 2013; US EPA 2000; ASTM International 2020). OECD TG 321 (OECD, 2024), *i.e. Hyalella azteca* bioconcentration test (HYBIT) was adopted in June 2024. This TG provides a non-vertebrate test to estimate the bioconcentration potential of substances. Since they are an aquatic species, reliable *Hyalella azteca* BCFs can be compared with the numerical CLP criteria for B/vB (CLP Annex I, section 4.3.2.1.2. and 4.3.2.2.2.).

Comparison between the metabolic rate of *H. azteca* with fish *in vitro* has shown that fish tend to have higher metabolic activity (Kosfeld *et al*., 2020). Since metabolism rates influence the BCF, this may explain why *H. azteca* tends to have higher BCFs than fish when normalised to a default 5% lipid content (Schlechtriem *et al*. 2019). However, OECD TG 321 recommends that *Hyalella azteca* BCF should be normalised to the species specific lipid content of 3% (based on whole body wet weight), unless there is evidence that the test chemical does not primarily accumulate in lipid. The BCF (normalised to 3% lipid where applicable) can be used for direct comparison with the CLP criteria. The test is discussed further in Chapter R.11.4.1.2.2 of ECHA Guidance on IR&CSA.

Principle of the test

The test follows a method similar to OECD TG 305 fish bioaccumulation test (aqueous exposure). Groups of adult male *H. azteca* are usually exposed for 3-14 days to one or more sub-lethal concentrations of the test substance dissolved in water. Only sexually mature males (> 8 weeks but < 6 months old) are used to avoid reproduction during the test and due to their more uniform size and lipid content compared to female *H. azteca*. Replicates of *H. azteca* and water are sampled at regular time-intervals and the concentration of test substance measured. Tests may be conducted using a flow-through or semi-static system. After reaching a steady-state tissue concentration (or after 14 days of exposure), the remaining *H. azteca* are transferred to clean water and the depuration phase is followed. The steady-state BCF_{SS} and kinetic BCF_K can be derived. If a steady state is not achieved, only the kinetic BCF_K is derived.

A correction of the kinetic BCF for growth dilution is not necessary because adult organisms are tested and their growth will be negligible. The lipid content of the tested *Hyalella azteca* should be determined. The BCF is based on the total concentration in *H. azteca* (*i.e.* per total wet weight of the sampled *H. azteca*). For many organic chemicals, there is a relationship between lipophilicity and the potential for bioconcentration. Correspondingly, there is a relationship between the lipid content of the test organism and the observed bioconcentration of such chemicals. The BCF should be normalised to 3% lipid to allow comparison between studies, unless it is known that the substance does not primarily partition to lipids. This is necessary to provide a basis from which results for different chemicals and studies can be compared against one another. OECD TG 321 specifies the applicability of the test and the conditions which must be met for a study to be valid.

Considerations when assessing H. azteca bioconcentration tests

Experience with use of this test is still limited and therefore, results should be assessed carefully.

If readily biodegradable solvents are used, they can cause problems with biofilm formation, leading to dietary uptake of the test substance which alters the uptake kinetics.

If radiolabelled test substances are used and only total radioactive residues have been measured, the BCF is based on the total of the parent substance, any retained metabolites and also assimilated carbon. Separation procedures, such as thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC) may have been used before radio-detection in order to determine a BCF based on the parent substance. When available, the BCF for the parent test substance should normally be used for assessment.

The tested concentration should be below the solubility limit of the test chemical in the test media. The exposure concentration should also be well below the toxicity limits, as toxic effects are not desired and should be avoided in bioconcentration studies.

In conclusion, reliable and relevant *H. azteca* bioconcentration tests provide a BCF which, normalised to its typical lipid content of 3%, can be directly compared with the numerical CLP B/vB criteria.

4.3.3.2.3.4. Bioconcentration tests in other aquatic invertebrates and species

Aquatic invertebrates

Other standard bioconcentration tests with aquatic invertebrates are available, for example ASTM E1022-22 Standard Guide for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Mollusks (ASTM International, 2022, previously ASTM E1022-94) and OCSPP 850.1710: Oyster Bioconcentration Factor (*Crassostrea virginica*) (US EPA, 2016)). These studies provide BCFs which can be compared with the numerical CLP B/vB criteria, if they are reliable. Invertebrate species may have a lower metabolic capacity than fish species, for example as is the case for polycyclic aromatic hydrocarbons (Bleeker and Verbruggen, 2009). Bioaccumulation in invertebrates may therefore be higher than in fish under the same exposure conditions.

Principles

BCF tests with aquatic invertebrates are similar to the fish and *H. azteca* bioconcentration tests where a number of organisms are exposed to sub-lethal concentrations of the test substance dissolved in water. The organisms and water are sampled at regular time-intervals and the concentration of test substance measured. After reaching a steady-state tissue concentration, the organisms are transferred to clean water and the depuration is followed.

Considerations when assessing BCF tests in aquatic invertebrates

The considerations described above relating to fish and *H. azteca* tests also apply to other standard BCF tests with aquatic invertebrates, namely the test concentration should not cause significant effects, steady state conditions should be used, the aqueous concentration in the exposure vessels should be maintained and should be below the water solubility of the substance. If radioanalysis was used, it should be checked whether parent compound analysis is also available to assess the contribution of metabolites.

Results should be reported on a whole body wet weight basis. For comparison with other BCF studies in the same species, lipid normalisation of the BCF to a representative lipid content for the tested invertebrate species should be considered, unless it is known that the substance does not primarily partition to lipids. Since bivalves such as oyster and mussel can shut and stop feeding in the presence of toxins, the study description should indicate the acute toxicity of the substance and whether closure has occurred. For test species which feed on particulates (including

microorganisms), the assessment of exposure concentrations may need careful consideration if the test system is not in equilibrium, especially for hydrophobic substances.

High-quality data on the BCF value for invertebrate species such as mussel, oyster or scallop can be used.

Further information on the evaluation of aquatic invertebrate studies is available in ECHA Guidance on IR&CSA, section, R.7.10.4.1. In addition to data from standard toxicity tests, data from reliable non-standard tests and non-testing methods indicative of B or vB properties may also be used if available.

In conclusion, reliable and relevant aquatic invertebrate bioconcentration tests can provide a BCF which can be directly compared with the numerical CLP B/vB criteria. For comparison with other BCF studies in the same species, lipid normalisation of the BCF to a representative lipid content for the tested invertebrate species should be considered, unless it is known that the substance does not primarily partition to lipids.

Other aquatic species

BCF for algae (single-celled) should not be used. Data on apparent accumulation in small organisms, such as unicellular algae and microorganisms, can be confounded by adsorption to cell or body surfaces leading to overestimates of bioconcentration (e.g. cationic substances may adsorb to negatively charged algal cells). Adsorption may also result in apparent deviation from first order kinetics and may be significant for small organisms because of their considerably larger surface/volume ratio compared with that for larger organisms.

Bioaccumulation data from aquatic plants should not normally be used, because it is currently not clear how observed accumulation in aquatic plants contributes to bioaccumulation for classification and labelling purposes. If such data exist, they could be included in the weight-of-evidence approach on a case-by-case basis.

4.3.3.2.3.5. *In vitro* fish toxicokinetic tests

In vitro methods such as fish liver S9 and primary hepatocyte assays provide information on biotransformation in the organism. Because biotransformation is considered to be a potentially important mechanism of elimination of hydrophobic substances, such *in vitro* clearance assays have the potential to support the assessment of bioaccumulation in a WoE approach assuming that the substance reaches the liver (ECHA Guidance on IR&CSA, Chapter R.11.4.1.2.4). To make use of *in vitro* fish toxicokinetic data for bioaccumulation assessment, the intrinsic clearance data from OECD TG 319A and B may be used as input to mechanistic (IVIVE) and/or relevant QSAR models to predict BCF, e.g. Laue *et al*. (2023) (see also section 4.3.3.2.6.3). A range of *in vitro* fish toxicokinetic tests are available in the scientific literature. Preference is given to results obtained from standard tests OECD TG 319 A/B (OECD 2018b; OECD 2018c).

Principle of the test

OECD TGs 319 A/B (OECD 2018b; OECD 2018c) describe the use of either cryopreserved rainbow trout hepatocytes or of liver S9 subcellular fractions for determining *in vitro* biotransformation kinetics in a detailed manner. In brief, the test chemical is incubated together with either hepatocytes or S9 fraction and substrate depletion is monitored over the duration of the experiment (maximum 4 hours). From the measured substrate depletion curve, the *in vitro* biotransformation kinetics can be determined. Detailed guidance on the performance of the tests is available in the test guidelines and related OECD Guidance document (OECD 2018a).

OECD TG 319 A/B specifies the applicability of the test and the conditions which must be met for a study to be valid.

Considerations when assessing in vitro fish toxicokinetic tests

The following information should be documented and provided in an IVIVE-based bioaccumulation assessment:

- *in vitro* test conditions (measured test chemical concentration, number of time points, species from which *in vitro* material originated, S9/hepatocyte concentration, total assay volume, open or closed system, assay duration, characterisation of *in vitro* material (Ethoxyresorufin-O-deethylase (EROD), glutathione transferase (GST) activities, etc.), incubation temperature);
- evidence that the depletion follows first-order kinetics or that the chemical starting concentration is below the Michaelis-Menten constant; and documentation of the behaviour of the negative control (if the negative control shows significant losses, the test should not be used);
- determined *in vitro* biotransformation kinetics (rate constants or clearances with units);
- estimated *in vivo* biotransformation kinetics (with units) and used extrapolation formalism (with reference);
- used IVIVE-bioaccumulation model (with reference) or used regression model (with reference, e.g. Laue *et al*., 2023).

Currently, *in vitro* tests cannot directly substitute *in vivo* data in terms of one for one replacement, for classification purposes. However, *in vitro* data can already play a role as supporting evidence in a WoE approach and there are ongoing efforts to develop and validate further *in vitro* methods which may add to our understanding of bioaccumulation (Laue *et al*., 2020). Although the standard guideline *in vivo* methods remain the most informative for classification and labelling purposes, all available and relevant information on bioaccumulation, including *in vitro* biotransformation data and non-guideline methods, should be assessed on their own merits and carefully balanced in the overall WoE.

4.3.3.2.3.6. Bioaccumulation tests in sediment-dwelling species

Bioaccumulation studies on sediment dwelling organisms measure the accumulation in sediment organisms via several uptake routes including direct contact, ingestion of contaminated sediment particles, porewater and overlying water (OECD, 2008a). The result is a bioaccumulation factor BAF which can be normalised to lipid content of organisms and organic carbon content of sediment to derive the BSAF, biota-sediment accumulation factor. These results cannot be directly compared with the numerical CLP B/vB criteria although the BSAF in combination with K_{OW}/ K_{OC} can provide evidence of bioaccumulation potentially indicating B or vB (ECHA Guidance on IR&CSA, Chapter R.11, Appendix R.11-3). BCF values can be calculated based on measured or estimated pore water concentrations according to ECHA Guidance on IR&CSA, Chapter R.11, Appendix R.11-3. A case-by-case assessment based on expert judgement of the reliability and relevance of the available information is required in order to be able to give BSAF values an appropriate weight in the WoE assessment.

Other indications of a high bioaccumulation potential, such as a bioaccumulation process not reaching the steady state at the end of the exposure period of an OECD TG 315 test or a low depuration rate, both representing slow kinetics, are relevant parts of a WoE approach when considering whether the B or vB criteria are fulfilled.

It should be noted that the term biota-sediment accumulation factor (BSAF) has been used in the literature to refer to bioaccumulation factors in sediment which have not been normalised to organism lipid and sediment organic carbon content. Care should be taken to ensure it is clear what the reported value refers to.

Principle of the test

A range of sediment bioaccumulation tests may be available in the published literature. OECD TG 315 Bioaccumulation in Sediment-dwelling Benthic Oligochaetes is the preferred test method.

For the uptake phase, worms are exposed to sediment spiked with the test substance which is covered with water and equilibrated. Using spiked sediment simulates a contaminated environment. Groups of control worms are held under identical conditions without the test substance. Worms and sediment are sampled at regular time intervals, and the concentration of test substance is measured. After reaching an apparent steady-state tissue concentration (or after 28 days, whichever is sooner), the remaining worms are transferred to clean sediment and the depuration is followed.

Considerations when assessing bioaccumulation tests in sediment-dwelling species

It is important that the test organisms burrow into the sediment and do not avoid the sediment since burrowing behaviour can influence the level of exposure (OECD, 2008a).

OECD TG 315 recommends the use of artificial sediment. If natural sediments are used, the sediment characteristics should be specifically reported as described in the test guideline. Substances with background sediment concentrations and potentially adaptable uptake mechanisms need careful consideration because sediment-dwelling organisms may have adapted to such substances, potentially affecting the bioaccumulation process.

For lipophilic substances, BAFs often vary with the organic carbon (OC) content of the sediment. Typically a substance will have greater availability to the organism when the sediment OC is low, compared to a higher OC. It should be considered to test at least two natural sediments with different organic matter content, and the characteristics of the organic matter, in particular the content of black carbon, should be reported. To ensure comparability of results between different sediments, the normalised BSAF normalised to total organic carbon content should be derived (See above, Bioaccumulation Terminology section 4.3.3.2.2; OECD, 2008a).

Many studies have shown that black carbon can substantially affect the strength of particle sorption and hence the bioavailability of a substance (Cornelissen *et al*., 2005). Observed black carbon partition coefficients exceed organic carbon partition coefficients by up to two orders of magnitude. When interpreting data where the exposure system includes natural sediments it is therefore important to account for the possible influence of black carbon partitioning to avoid underestimation of the substance's bioaccumulation potential from the freely dissolved phase (ECHA Guidance on IR&CSA, Chapter R.7.10.3.1).

If a radiolabelled test substance is used, total radioactivity measurements alone may overestimate the concentration of parent substance due to small amounts of radiolabelled impurities that may be present in the test substance, and/or formation of metabolites. To avoid overestimation of the BAF, it is recommended that BAF calculations be based on the concentration of the parent compound in the organisms and not only on total radioactive residues.

It is important to consider the implications of the worm gut contents when interpreting the study results (Mount *et al*, 1999; OECD, 2008a).

In conclusion, bioaccumulation tests in sediment-dwelling organisms provide a BAF or BSAF which cannot be compared directly with the numerical CLP B/vB criteria. However, BCF values can be

estimated from the BSAF based on measured pore water concentrations or estimated pore water concentrations.

4.3.3.2.3.7. Bioaccumulation tests in terrestrial (soil-dwelling) species

Bioaccumulation studies on soil-dwelling organisms such as OECD TG 317 measure the accumulation in soil organisms exposed through three phases: soil pore water, soil air and ingestion of soil. The resulting bioaccumulation factor BAF can be normalised to lipid content of worms and organic carbon content of soil to derive the $BSAF_{soil}$, biota-soil accumulation factor (OECD TG 317; OECD, 2010) which allows comparability between results from different bioaccumulation tests. These results cannot be directly compared with the numerical CLP B/vB criteria. Soil-dwelling species are different in their physiology than fish and may have a lower metabolic capacity than fish species.

The soil $BSAF_{soil}$ in combination with K_{OW}/ K_{OC} can provide evidence of bioaccumulation potentially indicating B or vB. BCF values can be calculated based on measured or estimated pore water concentrations as specified in ECHA Guidance on IR&CSA, Chapter R.11,_Appendix R.11-3. Considerations for benthic invertebrates are also applicable to terrestrial invertebrates. A case-by-case assessment based on expert judgement of the reliability and relevance of the available information is required in order to be able to give soil $BSAF_{soil}$ values an appropriate weight in the B and vB assessment.

Bioaccumulation data from **terrestrial plants** should not normally be used, because it is currently not clear how observed accumulation in terrestrial plants contributes to bioaccumulation in terrestrial food webs for classification and labelling purposes. If such data exist, they could be included in the weight-of-evidence approach on a case-by-case basis.

4.3.3.2.3.8. Field data - biomagnification in the food chain

Field bioaccumulation factors (Field BAF calculated from monitoring data, field measurements or measurements in mesocosms) or specific accumulation in food chains/webs expressed as biomagnification factors (BMFs) or trophic magnification factors (TMFs) can provide supplementary information indicating that the substance does or does not have bioaccumulation potential. Reliable and relevant field data should be given a high weight in a WoE approach where B /vB is indicated.

If field data indicate that a substance is effectively transferred in the food chain, this is a strong indication that it is taken up from food in an efficient way and that the substance is not easily eliminated (e.g. excreted or metabolized) by the organism (this principle is also used in the fish feeding test for bioaccumulation). This will lead to biomagnification from prey to predator (trophic magnification). A reliable field BMF or TMF value higher than 1 can be considered as an indication of very high bioaccumulation (ECHA Guidance on IR&CSA, Chapter R.11.4.1.2.6). For aquatic organisms, this value indicates an enhanced accumulation due to additional uptake of a substance from food along with direct accumulation from water. However, as dietary and trophic biomagnification represent different processes than bioconcentration in aquatic organisms, field BMF and/or TMF values <1 cannot be directly used to disregard a valid assessment based on reliable BCF data fulfilling the numerical CLP B/vB criteria, but in this kind of case all available data need to be considered together in a WoE approach. This is discussed further below under "Biomagnification (field BMF)".

Field bioaccumulation metrics are the field bioaccumulation factor (field BAF), field measured biota-sediment accumulation factor (field BSAF), field biomagnification factor (field BMF), trophic magnification factor (TMF). They are explained in section 4.3.3.2.2 of this Guidance.

Field BAF or field BMF of a substance may be greater than what is estimated based on BCF and BMF from laboratory experiments. This is because laboratory tests aim to expose fish either only via water or only via food, while under field conditions organisms are exposed to substances via all exposure routes depending on where they live (terrestrial or aquatic) and which taxa they belong to (air-breathers or water-breathers like fish).

Furthermore, apex (top) predators reflect biomagnification over the whole food chain while laboratory tests usually include only one trophic level in the biomagnification process from diet to test organism. This will ultimately lead to higher bioaccumulation in wild organisms feeding at higher trophic levels compared to the laboratory experiments for substances that are not rapidly metabolized and eliminated. The duration of exposure is expected to be substantially longer in wild animals as compared to the laboratory tests, which can play a substantial role in long-lived species such as many apex predators that accumulate hydrophobic substances over a lifetime. Bioaccumulation measurements of very hydrophobic, persistent substances that have not approached steady state in a field study, are considered to be underestimations (Burkhard *et al*., 2012a). Despite this, wildlife monitoring data (especially for endangered species) can give valuable indication of an increased bioaccumulation potential particularly for difficult to test chemicals. See also section 4.3.3.2.3.9.

Kelly *et al*. (2007) explained that apart from low rate of respiratory elimination to air, higher biomagnification of certain organic substances in air-breathing organisms is due to the greater ability to absorb and digest their diet, which is related to differences in digestive tract physiology and body temperature. In this context, field data on bioaccumulation and magnification in air-breathing biota again can provide valuable information for identifying substances that accumulate in wildlife and in human food webs (Czub and McLachlan, 2004).

Field bioaccumulation factors (BAFs/BSAFs)

For comparison of a fish field BAF with the CLP criteria, BAF values should be expressed on wet weight basis for whole body with a lipid content of 5%. If field BAF values (based on reliable information) are above 2000 or 5000, it might be sufficient to conclude that the substance fulfils the B or vB criteria as part of the Weight-of-Evidence approach.

Biomagnification (field BMF)

BMFs describe the increase in concentrations from prey to predator. For field data, BMF values are related to BAF values as both prey and predator are from the same environment (BMF prey-predator = BAFpredator/BAFprey). Food chain transfer and secondary poisoning are basic concerns in relation to PBT and vPvB substances, and therefore an indication of a biomagnification potential (BMF and/or TMF > 1) can on its own be considered as sufficient evidence to conclude that a substance fulfils the CLP B or vB criteria (ECHA Guidance on IR&CSA, Chapter R.11.4.1.2.6). On the other hand, absence of such a biomagnification potential cannot be used to conclude that these criteria are not fulfilled. This is because a field BMF only represents the degree of biomagnification in the specific predator/prey relationship for which it was measured. Biomagnification will vary between predator/prey relationships, so a low field BMF in one food chain does not mean that it will be low in other predator/prey relationship. Evidence of high biomagnification in one predator/prey relationship is an indication that biomagnification may also occur in other (unmeasured) predator/prey relationships.

Substances that partition into lipids should, as far as possible, be lipid normalised to account for differences in lipid content between prey and predator. It allows for a comparison of field BMF values in a direct and objective manner. It should however be noted that non-lipophilic substances such as PFAS may bioaccumulate by other mechanisms than partitioning/binding to lipids. In such a case, another reference parameter than lipid content may be considered for normalisation, e.g. dry weight or protein content. Normalisation of measured data with respect to lipid and dry weight content is described in Guidance Document No. 32 on Biota Monitoring prepared under the Water Framework Directive 2000/60/EC (European Commission, 2014).

Trophic magnification factor (TMF)

TMF can be used to understand the biomagnification potential of a substance as it represents the average increase or decrease of concentration levels in a food web per trophic level (TL): a TMF > 1 indicates that the substance biomagnifies in the food web (*i.e.* concentration increases with each trophic level) and thus can on its own be considered as sufficient evidence to conclude that a substance fulfils the CLP B/vB criteria; a TMF < 1 indicates that the substance undergoes trophic dilution (Weisbrod *et al*., 2009).

Currently, there is no standard procedure for measuring TMFs. Hence, the study design and sampling may vary considerably between different studies. The validity of the TMF is strongly dependent on the spatial and temporal scales over which the samples were retrieved. TMF can show variability related to ecosystem characteristics, organism biology and ecology, study design and the statistical methods used for TMF calculation (Kidd *et al.* 2019). More reliable TMFs may be derived from data for non-migratory species originating from a confined area and sampled in the same period, or from food chains for which low variability in time and space can be assumed (e.g. for vast remote areas).

Guidance on the assessment of TMF studies is given in ECHA Guidance on IR&CSA, Chapter R.11.4.1.2.7 and also Burkhard *et al*., 2013 and Borgå *et al*., 2012.

4.3.3.2.3.9. Detection of substances in wildlife and humans

Monitoring data for humans and biota are available in the open literature and some data can be accessed via the platform IPCHeM[84], the NORMAN network[85] or HBM4EU[86]. It is recommended to perform a literature search and to check these databases to check for available monitoring data on a substance. Guidance documents for assessing the quality of biomonitoring data, including interpretation of wildlife biomonitoring, have been elaborated by the EU project LIFE APEX (Badry *et al*., 2022a; Badry *et al*., 2022b ; Treu *et al*., 2022a) and Guidance Document No. 32 on Biota Monitoring prepared under the Water Framework Directive 2000/60/EC (European Commission, 2014). Further guidance on the use of field data for PBT/vPvB assessment is available in ECHA Guidance on IR&CSA, Chapters R.11.4.1.2.6 and R.11.4.1.2.7. It is important to consider the reliability and relevance of the data, in addition to the quality, for the WoE assessment.

The detection of substances in wild biota (concentration or occurrence data), in particular in apex species (top predators), provides a clear indication that it has been taken up by that organism. Care should be taken if gut content and adsorption to skin contribute significantly to the measured concentration (e.g. for smaller wildlife species). These data could be used within a WoE approach to assess bioaccumulation of a substance case by case (depending on the statistical power, quality and standardisation of the study). However, a detection of a substance as such does not necessarily mean that significant bioconcentration or bioaccumulation has occurred since an exposure level from the surrounding media and/or diet would be needed for such an assessment. Thus, concentrations measured in prey species or water in the surrounding media can be helpful to identify cases where bioaccumulation occurred in wild organisms. Furthermore, data from different time points as well as regions can give indications on temporal and spatial trends.

In cases where no data is available on sources and contemporary exposure levels, a high frequency of appearance of a substance in several biota species across different compartments could indicate bioaccumulation potential. In such cases, other available evidence of the substance's bioaccumulation potential should be thoroughly examined before reaching a conclusion.

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[84] https://ipchem.jrc.ec.europa.eu/

[85] https://www.norman-network.com/apex/

[86] https://www.hbm4eu.eu/

Detection of elevated levels of a substance in biota compared to levels in their surrounding environment indicates an increased concern for bioaccumulation. Reliable monitoring data can be used as line of evidence in the WoE assessment that the substance fulfils the CLP B/vB criteria.

Concentrations in biota increasing with age due to exposure and accumulation over lifetime, particularly in long-lived apex species (top predators), indicate an increased concern for bioaccumulation.

Finally, it is important that the quality of monitoring data (detection or quantification of a substance in biota) needs to be assessed and interpreted correctly. Guidance on the use of field data for bioaccumulation assessment is given in ECHA Guidance on IR&CSA, Chapter R.11.4.1.2.7.

Human data

Information coming from scientific analysis of human body fluids or tissues, such as blood, milk, or fat can be used for bioaccumulation assessment in a WoE approach.

Concentrations of substances in blood, serum and urine of humans (workers or the general population) can be used to determine their elimination half-lives. section 4.3.3.2.3.11 explains that if the whole-body terminal elimination half-lives are above 20 days in humans, it is an indication that the substance has B properties for consideration in a WoE assessment.

For workers exposed to a chemical in their workplace, a significant positive correlation between the number of years in the profession and the chemical concentration in blood could be used as supporting evidence of bioaccumulation in a WoE assessment. Measured exposure concentrations would support such an assessment.

4.3.3.2.3.10. Chronic toxicity tests on animals

Existing data from chronic toxicity studies with mammals (e.g. repeated dose toxicity studies, prenatal developmental toxicity studies, one/two-generation reproduction toxicity studies, extended one-generation study and carcinogenicity studies) and birds can provide information on bioaccumulation potential. The complete absence of any effects in the long-term is an indication that the substance is either non-toxic and/or that it is not taken up to a significant extent (EFSA, 2023, section 6.5.1). Although this is only indirect information on the uptake of a substance, it may be used together with other indicators, e.g. referring to non-testing information, to conclude in a WoE approach that a substance is likely to be not bioaccumulative (ECHA Guidance on IR&CSA, Chapter R.11.4.1.2.9).

Toxicokinetic studies in mammals can also provide useful information for assessing the bioaccumulation properties, as discussed in section 4.3.3.2.3.11 below.

4.3.3.2.3.11. Bioaccumulation in air-breathing organisms including humans - toxicokinetics studies

Although for many substances the assessment of bioaccumulation in aquatic species is sufficient, some substances like endosulfan, beta-hexachlorocyclohexane, many perfluorinated alkyl substances or highly lipophilic substances may accumulate more than expected in air-breathing organisms and are not recognised as highly bioaccumulative if only aquatic data are used in the assessment (Kelly and Gobas, 2001, Kelly and Gobas, 2003, Czub and McLachlan, 2004). One reason may be the ability of gill-breathing organisms to eliminate substances into the water that cannot be eliminated by air-breathing organisms by respiration as they are not volatile. For mammals and birds, bioaccumulation essentially occurs through the dietary route, associated with elimination via urination and the gastrointestinal tract, metabolism, exhalation and growth (dilution) (Kelly and Gobas, 2003, Kelly *et al.*, 2007). In this context, air-breathing organisms also include marine mammals and humans. The main concern of bioaccumulation is that

concentrations in an organism reach levels that lead to adverse effects, especially in apex predators at the top of the food chain.

Relevant assessment endpoints are the biomagnification factor (BMF), the whole-body total (or terminal) elimination rate and the biotransformation rate. Assessment of the whole-body total (or terminal) elimination rate or corresponding elimination half-life can be assessed using biomonitoring studies in humans or toxicokinetic studies with rat (e.g. OECD TG 417).

The discussion paper "Bioaccumulation assessment of air-breathing mammals" available at the ECHA website (ECHA Working group on Toxicokinetics, 2022[87]) gives details on the scientific background. For assessment of bioaccumulation in terrestrial mammals and other air-breathing organisms, see also R.11.4.1.2.8 "Bioaccumulation in air-breathing organisms and approaches" of the Chapter R.11 of the ECHA Guidance on IR&CSA.

Relevant information on toxicokinetics

OECD TG 417 'Toxicokinetics' (2010) focuses on the investigation of the biological fate of a chemical including the formation of metabolites (Phase I and II metabolites).

This complex study is commonly performed with a ^{14}C radiolabelled test substance. Three different study designs are possible (Hofer, 2021): Single (high and low) dose with a duration of normally 7 days; repeated (low) dose studies commonly performed for at least 14 day, and preconditioning repeated dose studies (14 days unlabelled test substance plus one day ^{14}C radiolabelled test substance, 14+1 day study (OECD TG 417 §57).

OECD TG 417 offers quite some flexibility in study design to accommodate for different regulatory needs, but it does not include guidance on how to assess accumulation. Several factors will influence the clearance rate (or the corresponding elimination half-life), thus it is not a fixed value but relates to the test conditions, rat strain, animal age (lipid content), etc.

In repeated daily administration studies, clearance rates are preferably measured after steady state conditions have been reached, when the administration is stopped. The time to establish a steady state will differ depending on substance and dose. Repeated (compared to single) dosing should better ascertain a high radiolabelled substance load into peripheral organ/tissue compartments and establishment of steady state. This is because some large and/or deep organs or tissues may have slow influx rates due to little blood perfusion, unfavourable partitioning, little active or passive transport through the cell membrane or else. So-called preconditioning studies (repeated dosing with unlabelled substance followed by a single radiolabelled dose the last day (TG 417 §57) to investigate enzyme induction/inhibition, appear not appropriate for bioaccumulation assessment since the last administered radiolabelled dose (measured) will not be present at steady-state conditions, and be small in comparison to repeated administration using a radiolabelled substance (Hofer *et al*., 2021).

Considerations when assessing toxicokinetic studies

The terminal half-life is the time required for the concentration to fall by 50% during the terminal phase studied. A field BMF of 1 can be translated into a whole-body, terminal elimination half-life of about 4 days in rat, and/or about 50 days in humans (ECHA Working group on Toxicokinetics, 2022). If the terminal elimination half-lives are assessed to be longer than these, then this is an indication that the substance has vB properties. Tissue, organ, or body fluid specific elimination half-lives may be shorter than the total (or terminal) elimination half-life and therefore should be compared to above values with care. Declining concentrations in organs/tissues is often more relevant than in blood plasma/serum, which often underrepresents elimination half-lives in organs/tissues. Elimination in blood is relevant for substances with a high blood distribution such as PFAS (Hofer *et al*., 2021).

[87] https://echa.europa.eu/documents/10162/17228/bioaccumulation_assessment_of_air_breathing_mammals_en.pdf/

If whole-body terminal elimination half-lives are between 2.5 and 4 days in rat, and/or 20 and 50 days in human, the assessment of the B property should be accompanied by a T assessment (PBT concern). The 2.5 days value was derived using equations as explained in ECHA Working group on Toxicokinetics (2022), with conservative assumptions as input (dietary uptake efficiency of 1, feeding rate of approximately 0.065 kg/kg/d, 0.05 kg lipid/kg rat, and 0.2 kg lipid/kg diet). The 20 days for human reflects the same ratio as for BCF 5000/2000.[87]

In conclusion, if a whole-body, terminal elimination half-life is longer than 4 days in rat, and/or 50 days in humans, then this is an indication that the substance has vB properties. There may be exceptional cases where the derived elimination half-life threshold values in rats or humans cannot be used as an indicator of vB, for example where there is very low dietary absorption efficiency. Such cases require an individual assessment to determine whether the substance is vB or not.

If whole-body terminal elimination half-lives are between 2.5 and 4 days in rat, and/or 20 and 50 days in human, it is an indication that the substance has B properties for consideration in a WoE assessment.

The use of toxicokinetic data in B-assessment is under scientific development and the recommendations above are based on current knowledge and experience. It is advised to follow-up recent and future developments in the field, e.g. via the ECHA website.

4.3.3.2.4. Considerations for ionisable substances, surfactants, substances not partitioning to lipids

Ionisable substances

Dissociated and neutral chemical species can have markedly different bioavailability. It is therefore essential to know or estimate the pK_a to evaluate the degree of ionisation in surface waters at environmentally relevant pH (pH 4-9) and under physiological conditions (pH 3-9). When assessing an aqueous BCF test performed on an ionisable organic substance, close attention should be paid to the pH at which the study was performed and therefore which chemical species the test was performed on. BCF tests most relevant to the aquatic environment will have been performed at environmentally relevant pH (pH 4-9) compatible with the test species, at which the highest fraction of non-ionised form was present. Further information is provided in ECHA Guidance on IR&CSA, Chapters R.7.10-3, R.7c and in OECD GD 23, as well as Armitage *et al.* (2017).

Surface active substances (surfactants)

A substance is *surface active* when it is enriched at the interface of a solution with adjacent phases (e.g. air) and when it lowers the surface tension of the medium/phase in which it is dissolved. In general, surfactants consist of an apolar and a polar moiety, which are commonly referred to as the hydrophobic tail and the hydrophilic headgroup, respectively. According to the charge of the headgroup, surfactants can be categorised as anionic, cationic, non-ionic or amphoteric (Tolls and Sijm, 2000).

It is well established that BCFs for neutral organic chemicals are positively correlated with the K_{OW}. However, K_{OW} is not a reliable parameter for predicting the BCFs of surfactants. Due to their amphiphilic properties, surfactants form aggregates in solution and have a tendency to accumulate at the interface of hydrophobic and hydrophilic phases. Surfactants can also emulsify the n-octanol/water system, making the measurement of log K_{OW} technically extremely challenging (Hodges *et al.*, 2019).

Log K_{OW} determination is further complicated by the fact that surfactants may form micelles in water (*i.e.* not dissolving exclusively as single molecules), so their 'solubility' cannot be properly defined and is hard to measure. The maximum monomolecular solubility is defined as the critical

micelle concentration (CMC), with formation of micelles occurring above this concentration. Although CMC is a commonly used surrogate for water solubility, CMC is not an appropriate solubility threshold, as micelles themselves are water-soluble (Hodges *et al.*, 2019). This can cause data interpretation problems for fish BCF tests, since the actual dissolved concentration of surfactant that the fish were exposed to may be uncertain.

Measured membrane lipid-water partitioning/distribution ratios, K_{MLW}/D_{MLW} (or K_{mw}), could be suitable to predict the bioaccumulation potential of surfactants (Droge, *et al*., 2021). Further information is provided in Appendix R.7.10 3 of ECHA Guidance on IR&CSA, Chapter R.7c.

Organic substances that do not partition to lipid

Bioconcentration is generally considered as a partitioning process between water and lipid, and other distribution compartments in the organism can usually be neglected (the water fraction may play a role for water-soluble substances, de Wolf *et al.*, 1994). However, proteins have been postulated as a third distribution compartment contributing to bioconcentration (SCHER, 2005), and may be important for certain types of substances (e.g. perfluorosulphonates, organometallic compounds, for instance methyl mercury, methyl arsenic, etc. or glutathione-compounds). Evidence for such a role may be available from mammalian toxicokinetics studies.

Protein binding in biological systems performs a number of functions (e.g. receptor binding, binding to modulate the catalytic activity of enzymes, binding to carrier-proteins to make transport possible, binding to obtain/sustain high local concentrations above water solubility, such as oxygen binding to haemoglobin, etc.). In some circumstances, binding may lead to much higher local concentrations of the ligand than in the surrounding environment.

Nevertheless, the picture may be more complicated because the process is not necessarily driven purely by partitioning (binding sites may become saturated and binding could be either reversible or irreversible). Indeed, it has been postulated that measured BCFs may be concentration dependent due to protein binding. In other words, bioconcentration is limited by the number of protein binding sites rather than by lipid solubility and partitioning.

In the absence of such studies, toxicokinetic information (e.g. human, rat) can be useful for comparing half-lives of substances that may accumulate via proteins with those for other substances that are known to be bioaccumulative.

4.3.3.2.5. Databases with available bioaccumulation data

The ECHA REACH database includes public and disseminated information on bioaccumulation studies, from the registration dossiers, submitted by companies to ECHA in the framework of the REACH Regulation. Data is available on ECHA's dissemination website and the OECD QSAR Toolbox.

The Global Portal to Information on Chemical Substances (eChemPortal) provides free public access to information on properties of chemicals, and direct links to collections of information prepared for government chemical programmes at national, regional, and international levels. Access to information on existing chemicals, new industrial chemicals, pesticides and biocides is provided. eChemPortal also makes available national/regional classification results according to national / regional classification and labelling schemes or according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

The Japanese National Institute of Technology and Evaluation (NITE) database collates experimental bioaccumulation data. NITE bioaccumulation data are also available via the OECD QSAR Toolbox as 'Bioconcentration and log *K*ow NITE' database.

Experimental BCF data in REACH dossiers are available in the OECD QSAR Toolbox in a normalised format as 'REACH Bioaccumulation database (normalised)'. This database is based on data up to the year 2017.

Further bioaccumulation databases available via the OECD QSAR Toolbox:

'Bioaccumulation Canada' is an empirical database of BCF values for non-mammalian aquatic organisms (algae, invertebrates and fish) for assessing the bioaccumulation potential of organic chemicals included in the Canadian Domestic Substance List (DSL). It has been implemented in the QSAR Toolbox in 2008.

'Bioaccumulation fish CEFIC LRI' contains experimental data for fish BCF values, which has been implemented in the QSAR Toolbox in 2008[88].

A further source of data is ECOTOX Knowledgebase[89]. ECOTOX is a comprehensive Knowledgebase providing single chemical environmental toxicity data on aquatic and terrestrial species, also including data on bioaccumulation.

The following scientific publications contain fish bioaccumulation databases including review of data:

- Jon A Arnot and Cristina L Quinn (2015) Development and Evaluation of a Database of Dietary Bioaccumulation Test Data for Organic Chemicals in Fish. *Environmental Science & Technology* **2015** *49* (8), 4783-4796. DOI: 10.1021/es506251q

- Jon A Arnot and Frank APC Gobas (2006) A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environ Reviews. 257-297.

4.3.3.2.6. Indicators of B or vB properties

4.3.3.2.6.1. Octanol-water partitioning coefficient *K*ow

In general, the potential of an organic substance to bioaccumulate is primarily related to the lipophilicity of the substance. A surrogate measure of lipophilicity is the n-octanol/water partition coefficient (K_{OW}) which, for lipophilic non-ionised and non-surface active organic substances, undergoing minimal metabolism or biotransformation within the organism, is correlated with the bioconcentration factor. Therefore, K_{OW} is often used for estimating the bioconcentration of non-ionised organic substances, based on the empirical relationship between log BCF and log K_{OW} (see section 4.1.3.2.3.3 of this Guidance).

For neutral organic substances, bioaccumulation is most often driven by partitioning to storage lipid. In these cases, the log K_{OW} can inform about the potential for bioaccumulation, and can be used together with other evidence in a WoE approach. A log $K_{OW} \geq 4.5$ indicates the potential for a BCF ≥ 2000 in aquatic organisms, while a log K_{OW} greater than 2 together with a log K_{OA} greater than 5 indicates the potential for B/vB for air-breathing organisms. If the log K_{OW} is less than 2, the substance can normally be regarded as not fulfilling the CLP B/vB criteria. If the substance has a log K_{OW} between 2 and 4.5, but log K_{OA} is below 5, then it can be expected that the substance is neither hydrophobic enough to reach a BCF of 2000 in aquatic species, nor that it is bioaccumulating in air-breathing mammals, because it can be eliminated rapidly enough by exhalation (Saunders and Wania, 2023; see also section 4.3.3.2.6.2 on octanol-air partitioning). Guidance on log K_{OW} is given in ECHA Guidance on IR&CSA, Chapter R.11.4.1.2.10 and Appendix R.11-5.

[88] see also https://cefic-lri.org/toolbox/bcf-database/ (last accessed: November 2023)

[89] available under https://cfpub.epa.gov/ecotox/ (last accessed: November 2023)

For organic substances, experimentally derived high-quality K_{OW} values are preferred over other determinations of K_{OW}. If multiple log K_{OW} data are available for the same substance, the reasons for any differences should be assessed before their use in a WoE approach. Generally, the most conservative valid value should take precedence.

For substances with very low solubility, specific methods exist to derive a K_{OW}, e.g. OECD TG 123 slow stirring method. However, this method is not always applicable due to experimental constraints caused e.g. by the low solubility and the available analytical methods.

The log K_{OW} generated by the HPLC-method according to OECD TG 117 (OECD, 2022) is an estimation method that is equivalent to theoretical models using descriptive information (like chemical structure, *i.e.* (Q)SARs) to estimate the log K_{OW}. These two methods are very close to each other in predictivity. For sufficiently soluble non-polar substances, HPLC results are generally within 1 log unit, with the applicability domain in the range of log K_{OW} 0-6. For the extremes (log K_{OW} <0 or >6) it is concluded that the molecular fragmental constants method (QSAR) is more trustworthy. The formation of intramolecular hydrogen bonds may impact the log K_{OW} by several orders of magnitude. Since EPI Suite does not consider the potential formation of intramolecular hydrogen bonds, the estimates for such substances are less reliable (see e.g. Wang *et* al., 2011, Buser *et al.,* 2013).

Examples of freely available (Q)SAR software programs that include models for the prediction of log K_{OW} are EPISuite[90], OECD QSAR Toolbox and VEGA. The data originating from calculations with the commercial quantum-chemical software COSMOconf and COSMOtherm have been shown to be more accurate than the data from many other estimation programs. Glüge and Scheringer (2023) have published COSMOtherm values for ca 4400 substances.

For some groups of substances, such as organometals, ionisable substances and surface active substances, log K_{OW} is not a valid descriptor for assessing the bioaccumulation potential (Armitage *et al*., 2017, Hodges *et al.*, 2019). Information on bioaccumulation of such substances should therefore take account of other descriptors or mechanisms than hydrophobicity. Guidance on consideration for bioaccumulation assessment of ionisable and surface active substances is given in Appendix R.7.10 3 of ECHA Guidance on IR&CSA, Chapter R.7c.

Furthermore, specific binding to proteins instead of lipids might result in an erroneously low BCF value, if this value is estimated from log K_{OW}. Per- and polyfluoroalkyl substances (PFASs) are examples of such partitioning behaviour, of which perfluorooctane sulphonic acid (PFOS) is a well-known example (e.g. Kelly *et al*., 2009). This also shows the importance to not limit the B identification to certain experimental values, but to acknowledge also any other evidence from field and monitoring studies. Guidance on consideration for bioaccumulation assessment of organic substances that do not partition to lipid is given in Appendix R.7.10 3 of ECHA Guidance on IR&CSA, Chapter R.7c.

4.3.3.2.6.2. Octanol-air partitioning coefficient K_{OA}

An indication of substances that might bioaccumulate or biomagnify in air-breathing organisms, is a combination of the octanol-water partition coefficient K_{OW} and octanol-air partition coefficient K_{OA} (Gobas *et al*., 2003). An efficiently absorbed, non-biotransformed neutral organic substance with a log $K_{OA} \geq 5$ in combination with a log $K_{OW} \geq 2$ has the potential to biomagnify in vertebrates of the terrestrial food chains and air-breathing marine wildlife as well as in humans, while the substances with log $K_{OW} < 2$ have a reduced gastrointestinal uptake or are efficiently excreted in urine, and therefore do not biomagnify even though their K_{OA} is high (Armitage and Gobas, 2007, Kelly *et al*., 2007, Gobas *et al*., 2009, McLachlan *et al*., 2011, Goss *et al.*, 2013). The numerical cut-off aligned to the screening criteria for prioritising bioaccumulating substances to air-breathing organisms are still subject to scientific review. Recently, Saunders and Wania (2023)

[90] https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface

evaluated thresholds for air-breathing animals across various species and predicted that animals with lower rates of respiration (e.g. manatees, sloths, and several temperate reptiles) and those ingesting high-lipid diets (e.g. polar bears and carnivorous birds) were able to biomagnify persistent chemicals with log K_{OA} < 5.

Baskaran *et al*. (2021a,b) have compiled all K_{OA} values reported in the published literature. Their dataset includes more than 2500 experimentally derived values and more than 10 000 estimated values for K_{OA}, in total covering over 1500 distinct molecules. A range of techniques can be used to predict K_{OA} of organic substances and are described in ECHA Guidance on IR&CSA, Chapter R.11.4.1.2.8. K_{OA} can furthermore be calculated reliably using LFERs (Baskaran *et al.*, 2021b) and OPERA[91] (Mansouri *et al.*, 2018). Another method is based on K_{OW} and Henry's Law Constant (H) (Meylan and Howard, 2005). In case H is also unavailable, H can be estimated based on water solubility (WS), vapour pressure (VP), and molecular weight (MW) (see equation R.16-4 of ECHA, 2016b). Sander (2015) published a compilation of 17350 Henry's law constants for 4632 organic and inorganic species in water, collected from 689 references, with further information made available online.

4.3.3.2.6.3. (Q)SAR models to predict BCF

BCF-(Q)SARs and other computer models may be used to address aquatic bioconcentration, provided that the model is appropriate for the chemical class. However, assessment of B or vB properties according to CLP Annex I (4.3.2.3.2.) clearly prefers experimental BCF data where available, and QSAR BCF data can only be considered as part of a broader WoE approach.

As for other endpoints derived using (Q)SARs, careful attention should be paid to the validity of the models and the acceptability of the predictions, which can be assessed against the established principles for the assessment of QSAR predictions and results presented in the OECD (Q)SAR assessment framework documents (OECD, 2023). Further information can be found in the Guidance on QSARs and grouping of chemicals, Chapter R.6[92] and in ECHA Practical Guide "How to use and report (Q)SARs"[93].

(Q)SAR BCFs derived using experimental input data (e.g. log K_{OW} and intrinsic clearance data from OECD TG 319A and B) (e.g. Laue *et al.* (2023)) should generally be given greater weight than those where the log K_{OW} and other source data is calculated. Examples of freely available QSAR software programs that include models for the prediction of log K_{OW} and BCF are EPISuite, OECD QSAR Toolbox and VEGA. OASIS Catalogic is also a valuable model for BCF prediction. The Bioaccumulation Assessment Tool, BAT[94] has built-in models including input of *in vitro* biotransformation rate to predict BCFs.

A reliable BCF prediction should not be used alone to decide whether a substance fulfils the CLP B/vB criteria but can be considered in the WoE assessment.

4.3.3.2.6.4. Biomimetic extraction procedures

Biomimetic extraction procedures with semi-permeable membrane devices (SPMD) and solid phase micro extraction (SPME) are used to mimic the way organisms extract substances from water. They extract only the freely dissolved (*i.e.* bioavailable) fraction of substances from water samples, in proportion to their partitioning coefficient, which is mainly related to the hydrophobicity of the substance and molecular size. In this way they simulate the potential for

[91] https://github.com/NIEHS/OPERA

[92] https://echa.europa.eu/documents/10162/17224/information_requirements_r6_en.pdf/

[93] https://echa.europa.eu/documents/10162/13655/pg_report_qsars_en.pdf/

[94] https://arnotresearch.com/bat-reg/ (last accessed: November 2023)

aquatic organisms to bioconcentrate organic substances by passive diffusion into storage lipids and cell membranes. These types of methods are at the moment only well described for hydrophobic substances. For more detailed information, see section R.7.10.3.1 in ECHA Guidance on IR&CSA.

4.3.3.2.6.5. Molecular size and octanol solubility

If average molecular size, log K_{OW}, and octanol solubility are above or below certain values (as described below), they may indicate a limited potential to be taken up by biota.

1. an average maximum diameter (Dmax aver) of greater than 1.7 nm
2. octanol-water partition coefficient as log10 (log K_{OW}) > 10 (calculated value, preferably by several estimation programs, for substances for which log K_{OW} can be calculated and the model is reliable)
3. a measured octanol solubility (mg/L) < 0.002 mmol/L × MW (g/mol) (without observed toxicity or other indicators of bioaccumulation)

Indicator 1. recommended here as non-testing information influences uptake and distribution of substances. The log K_{OW} (2.) is a general indicator for uptake, distribution and excretion whereas the octanol solubility (3.) reflects the potential for mass storage, which might further prevent uptake in significant amounts in the organism.

It is very important to note that the calculated log K_{OW} values above 10 are used simply to indicate a degree of hydrophobicity that is extreme. Such values should not be used in a quantitative manner.

These parameters should only be used as part of a WoE approach. In order to conclude on limited uptake, these parameters must be accompanied by experimental information from long-term exposure studies (e.g. birds, mammals, fish) confirming the low uptake of the substance to conclude that a substance is not bioaccumulative.

These types of information should be examined in a WoE approach together with the non-testing information on the substance to conclude whether the CLP B/vB criteria are fulfilled.

4.3.3.3. Mobility assessment

Commission Delegated Regulation (EU) 2023/707, Annex I: 4.4.2.3.2. The following information shall be considered for the assessment of M or vM properties:

(a) results from adsorption/desorption testing;

(b) other information, such as information from leaching, modelling or monitoring studies, provided that its suitability and reliability can be reasonably demonstrated.

Annex I: 4.4.2.4.2. In applying the WoE determination, the following information, in addition to the information referred to in section 4.4.2.3.2 shall be considered as part of the scientific assessment of the information relevant for the M, vM properties:

Information relevant for the M or vM properties:

(i) Organic carbon to water partition coefficient (K_{OC}) estimated by well-developed and reliable (Q)SAR models;

(ii) Other information, provided that its suitability and reliability can be reasonably demonstrated.

CLP defines the concern posed by PMT substances as a result of the combination of their persistence, mobility, and toxicity, and the concern posed by vPvM substances as a result of both their high persistence and high mobility in the environment. Due to the combination of these intrinsic properties, persistent and mobile substances may find their way into water bodies, and ultimately into drinking water, as wastewater treatment processes and drinking water purification processes may only partially remove them. CLP relates the criteria for M/vM to the log K_{OC} that reflects the potential of a substance to be adsorbed on the organic fraction of environmental matrices such as soil, sludge, sediment particles, and dissolved/particulate organic matter[95]. Therefore, it is inversely related to a substance's potential of entering water bodies (CLP Annex I, ECETOC, 2021, McCall *et al.*, 1981). Within the WoE assessment (see section 4.3.5.1), when reliable and relevant information is available resulting in a log *K*oc below the regulatory threshold(s) set for M and/or vM, the substance can be concluded as fulfilling the CLP criterion for M and/or vM, respectively.

Sorption describes the retention of a chemical species by a solid environmental compartment (Landrot and Sparks, 2023). Adsorption refers to the adhesion and binding capacity of a substance to a surface, absorption refers to the capacity of an amorphous phase to accommodate a substance within its bulk phase, while desorption refers to the release of a substance from a surface. Adsorption is the dominant process for shorter time scales (for example, 24 hours as in OECD TG 106) and usually occurs faster than absorption. However, whether adsorption or absorption is the dominant and rate limiting process will depend on the physico-chemical properties of sorbent and sorbate. For the sake of simplicity, adsorption will be considered to refer to both adsorption and absorption. The potential for adsorption/desorption of a chemical is an

[95] Organic matter is the set of organic compounds present in soil/sludge in various stages of decomposition. Organic carbon refers only to the measurable carbon component of the soil/sludge organic matter.

important environmental fate parameter and an indicator of partitioning of the substance in the various environmental compartments. The following sections will only further elaborate on adsorption and the corresponding distribution coefficient and not to desorption. In general, the capacity of organic substances to adsorb to solid organic matrices can be characterised by the organic carbon-water partition coefficient (K_{OC}, cm^3/g). For ionisable substances, other matrices (for example, clay particles) may also play a role on the adsorption of a substance (4.3.3.3.6). The K_{OC} value of a substance is known to be inversely related to the mobility in the environment (CLP Annex I, ECETOC, 2021, McCall *et al.*, 1981), it is related to the potential for sub-surface transport (e.g. in river bank filtration) and for entering ground and surface water bodies.

Different experimental (adsorption testing) and non-experimental methods are currently available for obtaining the K_{OC} value of a substance. These methods include soil leaching studies, lysimeter studies, modelling/ computational approaches, as well as analysis of monitoring data. It must be noted that modelling/computational approaches (for example, for estimating the exposure of groundwater or surface water) include use, emission, and exposure elements. In these methods, K_{OC} often constitutes an important input parameter for simulation models. Therefore, the results from these methods are not suitable on their own for the identification and assessment of M or vM properties.

The following sections specify the type of information that can be considered for the assessment of M/vM properties (sections 4.4.2.3.2 and 4.4.2.4.2 of the CLP Annex I legal text). Section 4.3.5 of this Guidance describes the WoE approach for concluding on these properties. Considerations regarding ionisable substances are presented in section 4.3.3.3.6 of this Guidance. Some of the methods in this section include both experimental and estimation elements to a derive a K_{OC}.

4.3.3.3.1. Experimental data on adsorption deriving a K_{OC} value

A description and interpretation of the relevant experimental studies to be used for classification purposes is provided below.

OECD TG 106 (Adsorption - Desorption Using a Batch Equilibrium Method)

OECD TG 106 is designed to estimate the adsorption/desorption behaviour of a substance on soils with different properties. It is used to obtain sorption kinetics and isotherms for different soil types that are used to determine equilibrium adsorption coefficients on the selected soils as a function of different soil characteristics, such as organic carbon content, pH, clay content, and soil texture.

OECD TG 106 does not differentiate between physical and chemical adsorption and absorption. Specific attention should be paid to poorly water soluble (water solubility below 0.1 mg/L), highly charged, and volatile substances (see OECD TG 106 for more details). For such substances, variations to OECD TG 106 may be needed, such as the use of passive samplers for poorly water soluble substances or devices to sample the headspace for highly volatile substances. For such cases, three phase partitioning, namely between water-soil-passive sampler, or water-soil-air, would need to be accounted for to derive Koc values.

Soil selection and characterisation are important steps in the adsorption testing. Specific guidance on soil selection is provided in OECD TG 106. As specified therein, the selected soils cover soil types from temperate geographical zones, but inclusion of soils from other geographical zones is also possible. For non-ionisable substances, selected soils should include soil organic carbon content ranging from low[96] to high organic carbon (*e.g.* >10%). The selected soils should be characterised in terms of organic carbon content, clay content, soil texture, and pH, as these

[96] OECD TG 106 notes that soils with less than 0.3% organic carbon may disturb the correlation between organic content and adsorption and recommends the use of soils with a minimum organic carbon content of 0.3%.

parameters are considered to be largely responsible for the adsorptive capacity of non-ionisable organic substances. The methods used to obtain these parameters should be provided. For ionisable substances that are present in their ionised form under environmental relevant pH (4-9), further information on the cation-exchange capacity (CEC) of the soil and the clay content and mineralogy should be provided. The specific considerations regarding the assessment of the ionisable substances are presented in the next section of this Guidance (4.3.3.3.6).

EFSA has published the outcome of a pesticide peer review meeting on issues to be considered by evaluators during the assessment of OECD TG 106 soil batch adsorption studies[97]. The document constitutes a checklist that was developed in order to ensure consistency and increase the quality of regulatory assessments, but also streamline guidelines for conducting the study and clarify some concepts when applying OECD TG 106. Note that this document and, in particular, the tool developed in relation to it, focusses on Tier 3 results of OECD TG 106.

As described in OECD TG 106, the test comprises of three testing tiers:

Tier 1 of the test method includes a preliminary study to determine (a) the soil/solution ratio, (b) the equilibration time for adsorption and the amount of test substance adsorbed at equilibrium and (c) the adsorption of the test substance on the test vessels' surfaces and the test substance stability.

Tier 2 investigates the adsorption kinetics at one concentration of the test substance. The aqueous concentration of the stock solution should preferably be three orders of magnitude higher than the detection limit of the analytical method used and should also be below the water solubility of the test substance (OECD TG 106). The selection of the aqueous concentrations (C_{Water}) for deriving K_d should be carefully considered and justified in accordance with OECD TG 106 specifications. The test is performed in five different soil types and the respective distribution/partition coefficients K_d and K_{OC} are calculated. K_d is the linear adsorption coefficient which describes the distribution of a substance between a solid and aqueous matrix after equilibration. After equilibrium is reached in Tier 2 testing, the distribution coefficient (K_d) is determined as the ratio of the concentration in the soil (C_{Soil}) to that in water (C_{Water}) at adsorption equilibrium.

$$Kd = \frac{C_{Soil}}{C_{Water}} \ (cm^3\ g^{-1})$$

C_{Soil}: concentration of the substance adsorbed on the soil at adsorption equilibrium (µg/g dry weight);

C_{Water}: concentration of the substance in the aqueous phase at adsorption equilibrium (µg/cm^3).

For non-ionisable substances, it is assumed that sorption is driven mainly by the soil organic carbon. Therefore, K_d values for soils with different organic carbon content will vary. In order to derive 'comparative' values across different soil types with varying organic carbon content, K_d can further be normalised to the fraction of organic carbon in the soil samples, by use of the following equation:

$K_{OC} = Kd \times \frac{100}{f_{OC}}\ (cm^3\ g^{-1})$, where f_{oc} is the soil organic carbon content (%)

The common logarithm ($\log_{10}$) of the K_{OC} value derived from K_d is then compared with the CLP mobility criteria.

Tier 3 investigates the adsorption isotherms and the desorption kinetics/desorption isotherms of a substance. The adsorption isotherms describe the relationship of the amount of the substance

[97] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2017.EN-1326

adsorbed on the soil and the concentration of substance in the solution when equilibrium has been reached at constant temperature. Tier 3 investigation is performed with the five different soil types used in Tier 2 investigation. The Freundlich adsorption isotherm equation is an empirical model that describes the adsorption isotherm of a substance as:

$$C_{Soil} = K_F \cdot C_{Water}^{\frac{1}{n}}$$

K_F is the Freundlich adsorption coefficient indicating the adsorption capacity of the sorbate onto the sorbent and n is the affinity-capacity coefficient. The K_F units are $cm^3\ g^{-1}$ only if $1/n = 1$; in all other cases, the slope $1/n$ is introduced in the dimension of K_F ($\mu g^{1-1/n}\ (cm^3)^{1/n}\ g^{-1}$). The Freundlich adsorption coefficient (K_F) derived from the sorption isotherms is equal to the distribution coefficient K_d only when the Freundlich exponent $1/n$ is equal to 1, in which case the sorption is assumed to be linear.

n is an exponent reflecting deviation from linearity of the relationship indicating the adsorption intensity (Pignatello, 2023). The value of $1/n$ is typically below 1 (typically ranges between 0.7-1.0) and may vary depending on the range of concentrations over which it is measured (Pignatello, 2023). If $1/n < 1$, sorption is less favourable at higher solute concentrations resulting in a concave downward isotherm shape. This is indicative of saturation of the adsorption sites available to the chemical resulting in relatively less adsorption. The case of $1/n > 1$ is generally rare and only occurs when previously sorbed molecules lead to a modification of the sorbent which favours further sorption. This only starts after a certain loading of the sorbent and occurs rarely under environmental conditions. If $1/n$ equals unity, sorption is mainly governed by absorption into the organic matrix and therefore does not decline with increasing solute concentration. This is mainly the case for partitioning of non-ionic, hydrophobic substances into an amorphous organic matrix (Schwarzenbach *et al.*, 2002).

In the same manner as for K_d, the K_F can be normalised to the organic carbon content of the soil (K_{FOC}). As the K_d and K_F are not equal coefficients, the calculated K_{FOC} is not equivalent to K_{OC} (Tier 2) and cannot be compared with the CLP mobility criteria.

However, the K_F can be used for deriving a specific K_d (K_d*) for a defined aqueous concentration (C_{Water}) using the following equation (Chen *et al.*, 1999, Schwarzenbach *et al*., 2002 and Mejia-Avendaño *et al*., 2020):

$$K_d^* = \frac{K_F \times C_{Water}^{\frac{1}{n}}}{C_{Water}} = K_F \times C_{Water}^{\frac{1}{n}-1}$$

An organic carbon normalised K_{OC}* can then be derived from the K_d* as described previously. The selection of the aqueous concentrations (C_{Water}) for deriving K_d* needs to be carefully considered and justified on a case by case basis. In any case, the selected concentrations should fall within the concentrations range used for deriving the respective K_F and 1/n (Tier 3) (Chen *et al*., 1999). Selection of aqueous concentrations outside the range tested in Tier 3 might lead to uncertain K_d* estimates and, therefore, they are not recommended. Both the K_{OC} (Tier 2) and K_{OC}* (Tier 3) can be used for comparing with the CLP mobility criteria.

<u>Studies on activated sewage sludge</u>

Recital (15) of the Commission Delegated Regulation (EU) 2023/707 states that the organic carbon-water partition coefficient (K_{OC}) reflects the ability of a substance to be adsorbed on the organic fraction of solid environmental compartments such as soil, sludge and sediment. Regarding activated sludge, studies such as OPPTS 835.1110 (Activated Sludge Sorption

Isotherm)[98] and the ISO 18749 standard (Water quality — Adsorption of substances on activated sludge - Batch test using specific analytical method)[99] may be compared to the CLP criteria within the WoE determination to provide useful information on the sorption of substances in sludge. Sorption is a key process in wastewater treatment plants and is most relevant for those substances that have been released to or have ended up in wastewater treatment plants.

Due to the differences in the composition and polarity between the organic matter in the sludge and soil and sediments, caution should be applied when interpreting results from such studies (Kile *et. al*., 1995). Therefore quantitative and qualitative information on the composition, the organic matter and the organic carbon content of the activated sludge should be reported. Generally sludge is characterised by high organic carbon content and, therefore, high K_d values are expected. The normalisation of the K_d to the organic carbon content (K_{oc}) allows for the comparison with the CLP criteria, taking into account the considerations related to the composition of the organic matter in sludge.

The Activated Sludge Sorption Isotherm method (OPPTS 835.1110), describes a procedure for the determination of the sorption potential of activated sludge solids by calculation of a sorption isotherm for different sorbent concentrations. The method derives a Freundlich sorption coefficient after the equilibrium between aqueous phase and sludge solids has been reached that must then be converted into a K_{OC} value. According to the ECHA Guidance on IR&CSA, Chapter R.7.1.15.3, caution should be exercised when interpreting any such results, as the method does not differentiate between adsorption and other elimination methods (such as complex formation, flocculation, precipitation, sedimentation, or biodegradation). The method does not describe the origin of the activated sludge, although differences in the composition of the activated sewage sludge from treatment plants receiving industrial or predominantly domestic sewage is expected. For this reason, and for the purpose of the mobility assessment under the CLP regulation, sludge originating from treatment plant receiving predominantly domestic sewage is recommended (von Sperling, M., 2007 and Ranade, V. and Bhandari, 2014).

The Water quality — Adsorption of substances on activated sludge - Batch test using specific analytical method (ISO 18749), derives a distribution coefficient K_d (L/kg) between the aqueous phase and sludge that can then be normalised to the organic carbon content to generate a K_{OC} value. The method is suitable for substances that are water soluble (or allow for stable suspensions/dispersions/emulsions), are not significantly removed by abiotic processes (e.g. stripping/foaming), do not de-flocculate activated sludge, are not readily biodegradable, and have a sufficiently sensitive analytical method. The test has been used as a screening method to determine the degree of adsorption of substances on activated sludge or primary sludge in wastewater treatment plants. As described in the method, sludge originating from treatment plant receiving predominantly domestic sewage should be used.

OECD TG 121 (Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC))

OECD TG 121 is an alternative approach that can derive K_{OC} values from indirect experimental measurements. OECD TG 121 is most applicable for substances that are neutral between pH 4-9, namely that are non-ionisable, or have the same ionic charge within this pH range. The method derives partition coefficients from the retention times measured on a specific HPLC column. The time it takes for the target substance to travel through the HPLC column (retention time) is determined by its partitioning between the stationary phase of the column (cyanopropyl stationary phase) and the mobile phase (liquid, e.g. water and methanol). The retention time is then compared to that of reference substances with known experimentally derived K_{OC} values and

[98] https://permanent.fdlp.gov/lps59946/835-1110.pdf

[99] https://www.iso.org/obp/ui/#iso:std:iso:18749:ed-1:v1:en

a K_{OC} value for the target substance is derived. The accuracy of the method is affected by the presence of reference substances used for calibration that are structurally similar to the test substance in order to address the same mechanisms of adsorption. If data on relevant reference substances are not available, relevant alternative calibration substances can be selected and their selection should be justified.

This method is designed for soils and sewage sludge, it can determine log K_{OC} values between 1.5 and 5 and may also be used for volatile, poorly water soluble and substances/mixtures with a high affinity to the surface of incubation systems (OECD TG 121 and ECHA Guidance on IR&CSA, Chapter R.7.1.15.3). As this is an estimation method with a limited set of reference substances, the uncertainty of such estimations should be assessed and considered when assessing the data. (see also European Commission (2002) SCP/KOC/002 Opinion)[100]. Furthermore, the method may not be applicable to strong acids and bases, surface-active substances, chemicals that react either with the mobile or the stationary phase, and those that interact in a specific way with inorganic components (for example, formation of cluster complexes with clay minerals).

OECD TG 312 (Soil leaching columns)

OECD TG 312 is based on soil column chromatography in disturbed soil and it describes a method to determine the potential for soil leaching of both test substance and its transformation products. K_{OC} values may also be obtained by use of different estimation techniques. For example, it can be estimated by using average leaching distance or established correlations between relative mobility factors (RMF) and K_{OC} values for reference substances[101] or based on the conversion-dispersion equation (*e.g.* as applied in Vereecken *et al.*, 2011). The choice of estimation technique should be justified. As mentioned above for OECD TG 121, the accuracy of the method is also affected by the choice of reference substances.

The test substance is introduced into soil columns of different soil properties and the leachate is collected after application of artificial rain. At the end of the leaching process, the soil is removed from the soil column for further analysis. The leaching of the substance can be evaluated in comparison with a reference substance on a relative scale using relative RMFs. The test is not applicable to volatile substances that might be lost under the experimental conditions of this test.

As with OECD TG 106, selection of soils with varying pH, OC, soil texture, etc. must be tested in order to evaluate the soil leaching. OECD TG 312 usually derives an amount of the test substance (measured as a percentage of the amount initially applied) and its transformation products as a percentage of soil depths. In other words, these types of experiments are used to determine the penetration depth, defined usually as the soil depth where half of the applied substance mass can be found. Additionally, the mobility classes as defined in Annex 3 of OECD TG 312 derived by the RMF are not directly comparable to the M/vM criteria under CLP and, thus, cannot be used as such. However, estimated *K*oc data based on the RMFs can be used within the WoE. Furthermore, the soil column leaching studies might underestimate adsorption due to difficulties in the exact determination of the relative rates of movement of the substance, the handling/packing of the soil and the probable non-equilibrium state of the test system.

In different regulatory regimes, such studies have been used to decide whether further field testing needs to be carried out but not to predict soil leaching behaviour under field conditions. For example, under the PPPR results from soil leaching column studies have been used in risk assessments, in a WoE approach for additional investigations of the pesticidal mobility within the overall risk assessment (Sanco, 2014). This is usually done in combination with a scenario modelling that also accounts for the use patterns and refers mainly to pesticidal-active substances

[100] https://food.ec.europa.eu/system/files/2020-12/sci-com_scp_out128_ppp_en.pdf

[101] https://www.oecd-ilibrary.org/docserver/9789264070561-en.pdf?expires=1691490605&id=id&accname=guest&checksum=F04D799468933A0FFB44B22ABD4AB1BC

with a low adsorption potential (namely K_{OC} below 25) and when no reliable K_{OC} can be obtained by OECD TG 106 (EC, 2002).

Soil thin and thick layer chromatography (TLC)

Soil thin and thick layer chromatography (TLC) studies have also been conducted to observe and measure the soil leaching of labelled pesticides through different soil types (Sánchez-Camazano *et al*., 1996, Kumar *et al*. 2013). In these studies, chromatographic techniques are used to separate the substances/compounds/constituents in the mixture and simulate the pesticide movement by the determination of a retardation/mobility factor (R_F). This factor is the ratio between the elution distance of the substance and the elution distance of the developing solvent (Mensink *et al*., 2008). A K_{OC} value can then be estimated by established correlations between retardation/mobility factors (R_F) and K_{OC} for reference substances. As mentioned above for OECD TG 121, the accuracy of the method is also affected by the choice of reference substances.

Similarly to the soil column leaching studies, these studies might underestimate adsorption due to difficulties in the exact determination of the relative rates of movement, handling of the soil, possible influence of the support material, and a probable non-equilibrium state for the test system (Mensink *et al*., 2008). Additional argumentation on the high uncertainty and potential underestimation of adsorption in soil TLC studies can be found in the EC (2002) opinion. Finally, application to volatile substances is problematic and any losses due to volatilisation need to be fully taken into account.

4.3.3.3.2. Other experimental information deriving a K_{OC} value

Field and lysimeter studies

The potential of substances for soil leaching to the groundwater may be provided by lysimeter and field studies. Verschoor *et al*. (2001) drafted some guidance on the interpretation and use of such studies for pesticidal-active substances. These studies usually resemble the environmental and field conditions better compared to laboratory studies. They are mostly performed under natural conditions, on a relatively large scale, and over longer periods of time. Moreover, they integrate a higher number of environmental processes and interactions than laboratory soil column leaching studies. Verschoor *et al*. (2001) and references therein reported an extensive list of quality parameters that need to be reported and met in order for a lysimeter or a field study to be regarded as reliable. These include the soil type/texture, information on the analytical method and leachate, meteorological data, mass balance, and other application-specific parameters. For the purpose of classification and labelling, their suitability, reliability and relevance would need to be demonstrated.

The likelihood of the test substance and its metabolites leaching to the groundwater from the soil is determined by the derivation of their concentrations in the groundwater/leachate and by comparing with the respective national regulatory criteria. Subsequently, the results from the lysimeter or field measurements are compared to those of a simulation model (for example, FOCUS PEARL, FOCUS PELMO, etc.) that allow an extrapolation to a wider range of relevant conditions (Leistra *et al*., 2001).

Inverse modelling techniques utilising the data from the field and leaching studies have been used for pesticides to refine input parameters such as K_{OC} and degradation half-lives of exposure models developed in the framework of FOCUS (Mertens *et al*., 2009, Sanco, 2014). These techniques entail entering the output from soil columns, lysimeter or field studies into an exposure model, the calibration of the model output with experimental data that is then used to calculate new values for the input parameters such as K_{OC}. Sanco (2014) details the use of inverse modelling procedures for leaching assessment of pesticidal-active substances and their metabolites to groundwater in the EU. Often, non-extractable residues are taken into account both in the degradation rate estimation and the sorption partition coefficient as degraded parent

substance. Such double-counting of the loss via the treatment of non-extractable residues data should be avoided in this type of modelling. Such modelling techniques consider the fate processes over longer time periods but require both *K*oc and $DegT_{50}$ to describe the solute transport modelling. For either of these parameters, values are needed either as starting points for the fitting of that parameter or as a fixed parameter while fitting the other parameter. Furthermore, inverse modelling can have the problem of non-uniqueness which refers to the fact that equally good fitting of the experimental data can be obtained with different parameter combinations and this needs to be considered by an appropriate assessment of the data (Sanco, 2014).

Both field leaching and lysimeter studies are application scenario-specific and introduce exposure considerations relevant to a local risk but not to an intrinsic hazard assessment. Lysimeter studies provide location-specific information which cannot alone represent the range of environmental conditions in the EU. The leaching and lysimeter studies are affected by the local environmental conditions (Hansen *et al*., 2000) and there is unclarity on whether they can sufficiently represent the conditions that need to be covered. The use of inverse modelling carries the cumulative uncertainty and assumptions of each individual model input parameter, as well as those of the associated experimental methods. Therefore, such results need to be carefully considered before assigning an appropriate weight within the overall WoE.

However lysimeter studies may be used for regulatory purposes (for example, CLP) in order to qualitatively identify additional transformation products that may not have been detected in a soil simulation test according to OECD TG 307 and that may leach to the groundwater (see also section 4.3.3.1.2.2). The current practice regarding pesticidal-active substance approvals according to Regulation 1107/2009[102] is that metabolites found in lysimeter studies at annual average concentrations exceeding 0.1 µg/L in the leachate are considered as transformation products, for which a groundwater risk assessment is to be performed.

OECD Guidance Document for the Performance of Outdoor Monolith Lysimeter Studies (No.22).

Monolith lysimeters have been used in research with crop protection products for years, as one of the tools for obtaining information on the fate and behaviour of a substance in an undisturbed soil under real world conditions[103]. With monolith lysimeters, mass fluxes of water and substances can be monitored and chemical distribution and transformation products can also be determined. As also for all other studies discussed in this Guidance, this method is applicable to substances for which an analytical method with suitable accuracy and sensitivity is available and resembles field conditions (local climatic conditions) closer than other laboratory studies. OECD Guidance Document No.22, proposes that for a better interpretation of results from such studies, "*it would be useful to conduct studies on adsorption/desorption or soil column leaching and on aerobic transformation in the same soil as found in the top layer of the lysimeter*". Consequently, the results need to be evaluated according to similar considerations regarding lysimeter and field studies in an overall WoE assessment.

4.3.3.3.3. Data from estimation methods (*e.g.* (Q)SARs) deriving a K_{OC} value

The predicted K_{OC} value of a test substance may be used for the purpose of classification and labelling. The conditions discussed earlier in the Guidance (4.3.3 (ii)) must be fulfilled, namely those related to the reliability and applicability domain, supported with adequate documentation.

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[102] Article 3(32) of Regulation (EC) No 1107/2009 defines that a metabolite "is deemed relevant if there is a reason to assume that it has intrinsic properties comparable to the parent substance in terms of its biological target activity, or that it poses a higher or comparable risk to organisms than the parent substance or that it has certain toxicological properties that are considered unacceptable."

[103] https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono%282000%298&doclanguage=en

When a measured *K*oc value of a test substance based on either OECD TG 106 or other experimental methods is not available, but a measured *K*ow (octanol-water partition coefficient) value of the test substance is available, the simplest and most widely occurring approach on estimating a K_{OC} value is based on the linear relationship between the *K*oc and the *K*ow. One of the first attempts to empirically regress this relationship is from Karickhoff (1979) who, based on experiments where K_{OC} values were measured for different soil organic contents and substances of different octanol-water partition coefficients, proposed the following empirical equation:

$K_{OC} = 0.41\ K_{OW}$

This equation is applicable to non-ionisable, non-surface active organic substances for which their environmental sorption is attributed practically entirely to organic matter, where the sorption mechanism is hydrophobic binding. In more recent years, more sophisticated models based on the linear regression between the two partition coefficients have been developed for a variety of substances (work of Abraham and colleagues, Sabljić *et al*., 1995, references in ECETOC, 2021). Computational methods have also been developed in the absence of available physicochemical data, namely by knowledge of only molecular structure. One example is the use of molecular connectivity indices (MCI) that are associating molecular structure information (for example, molecular size, volume, branching, etc.) to K_{OC} in terms of mathematical equations. Such *in silico* approaches of estimating organic carbon – water partition coefficient (ECB, 2003) include EPISuite[104] (US EPA 2012), the OECD QSAR Toolbox, OPERA[105], QSARINS[106] and several LFER models (for example, Bronner and Goss, 2011b). Further information on the experimental derivation of the octanol-water partition coefficient can be found in several related OECD guidelines.

Another approach refers to the use of the octanol-water distribution coefficient (D_{OW}) as a measure of the distribution of dissociated and non-dissociated species in octanol and water and as a function of pH. The D_{OW} can be derived using the K_{OW} and the dissociation constant (pK_a) (Neumann and Schliebner, 2019; Arp and Hale, 2023) based on the following equations:

$D_{OW} = (1/(1+10^{(pH - pKa)}))\ K_{OW}$ (for monoprotic acids)

$D_{OW} = (1 - 1/(1+10^{(pH - pKa)}))\ K_{OW}$ (for monoprotic bases)

Only in the absence of any other relevant information, may a D_{ow} be used instead of K_{OW} for predicting K_{OC} for ionisable substances by use of other *in silico* approaches ((Q)SARs), where available.

4.3.3.3.4. Monitoring data

The mere presence or absence of a substance in any given underground or surface water body cannot in itself demonstrate that a substance is mobile or not. The identification of any substance within the context of a monitoring campaign is dependent on a range of parameters, such as the proximity of the sampled area/point to emission sources and the quantity released in the environment, the route of entry into the environment, spatial and weather conditions (for example, geography/ topography, meteorology), its environmental fate (for example,

[104] https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface

[105] https://github.com/kmansouri/OPERA

[106] https://dunant.dista.uninsubria.it/qsar/?page_id=565

degradation and volatility), any transport and inter-media distribution processes, as well as on the analytical and sampling methods available. Nevertheless, in accordance with the assessment regarding Persistence and Bioaccumulation, the presence of a substance in a remote and pristine environment may be used within the overall WoE as an indication for mobility. Additionally, temporal trends highlighting potential increases or decreases over time within the same monitored media may prove increasingly important. In order to consider such data, there needs to be sufficient understanding of the substance distribution and transport behaviour and the uncertainties in the monitoring data must be adequately addressed (ECHA Guidance on IR&CSA, Chapter R.11.4.1.1.6).

Further details on the review of monitoring data and its implementation within the regulatory context of the Water Framework Directive (2000/60/EC) are provided in Guidance document No 7[107]. Concerning the conduct of groundwater monitoring studies for pesticide active substances and their metabolites in the context of Regulation (EC) 1107/2009, Gimsing *et al*. (2019) and EFSA (2023b) further elaborated on aspects such as the selection of the monitoring site, study design, time scales, treatment of positive and negative results, avoiding of contamination, as well as study documentation.

In all cases, an overall assessment of the relevance and reliability of any monitoring dataset should be conducted and included in the WoE reasoning.

4.3.3.3.5. Relevance of aged sorption data

The term aged sorption is used to describe the increased sorption (adsorption and absorption) of the substance to the soil over extended periods of time (weeks or months), as opposed to the much shorter time scales in a study performed according to OECD TG 106 or similar. Longer time exposures allow for the slow diffusion within the pores and channels of the solid or molecular diffusion in the macromolecular organic matter (ECETOC, 2021). Such approaches are often used in conjunction with equilibrium adsorption/desorption studies, in order to confirm the relevance of aged sorption if, for example, at least four of the aged sorption experiments showing evidence of aged sorption, according to the respective quality criteria (EFSA, 2015).

Recent regulatory and scientific progress has led to the publication of a guidance on the conduct, impact and use of aged sorption studies in the regulatory risk assessments of pesticides (Commission Guidance Document, 2021) that includes a comprehensive list of the uncertainties associated with the use of the aged sorption concept. Thus, K_{OC} values derived from such approaches should not be directly compared with the CLP M/vM criteria. Moreover, any potential influence of aging might be less relevant for low or non-adsorbing substances, due to the longer exposure time scales involved in such processes.

4.3.3.3.6. Considerations for ionisable substances

The following terminology will be used in this section:

"**Non-ionisable**" substances are substances that are not able to be ionised under relevant environmental conditions. Respectively, "**ionisable**" substances are substances that are able to be ionised (able to dissociate, forming ionic compounds) under relevant environmental conditions. **Anionic** substances are those substances that are in the anionic (negatively charged) form (in a percentage above 10%) and **cationic** substances are those substances that are in the cationic (positively charged) form (in a percentage above 10%), under relevant environmental conditions

[107] https://circabc.europa.eu/sd/a/63f7715f-0f45-4955-b7cb-58ca305e42a8/Guidance%20No%207%20-%20Monitoring%20%28WG%202.7%29.pdf

(any pH from 4 to 9). **Zwitterionic** substances are neutral substances that contain a positive and a negative charge, but will not be further expanded upon.

Ionisable substances need special scrutiny when measuring the *K*oc value in test systems due to the impact of the pH on their speciation. As defined in the M/vM criteria in CLP, it is necessary for the purpose of classification and labelling to derive the lowest *K*oc value within the environmental relevant pH range of 4 to 9. Specific considerations apply when, depending on the pH, a simple test substance can either occur in a deprotonated (negatively charged due to loss of H^+), protonated (positively charged due to take up of H^+) or neutral form, under relevant environmental conditions. A key indication of the form of the substance under relevant environmental conditions is the acid dissociation constant (K_a). For consistency, dissociation of bases is expressed using the dissociation constant of the conjugate acid. Pesticides are example substances that can often occur in an ionic form, with anionic pesticides in a rather basic soil assumed to have a lower *K*oc value and a lower potential to adsorb than neutral or protonated pesticides (RIVM, 2008).

Schaffer and Licha (2014) provided a simplified and general guideline for the identification of ionisable functional groups for more than 30 of the most frequently encountered ionisable compound classes, including their typical pK_a values (pK_a is the negative base-10 logarithm of the acid dissociation constant). Figure 4.3.1 visualises the species distribution for monoprotic substances in solution as a function of pH. Acidic substances (p*K*a acid = 4.5, Figure 4.3.1, A) will exist in the anionic form in a percentage above 1% when they are in a solution with a pH greater than pK_a - 2 (*i.e.* pH 2.5) and in a percentage above 99% at a pH greater than pK_a + 2 (*i.e.* pH 6.5). For basic substances (p*K*a base = 9.5, Figure 4.3.1, B), the cationic form will exist in a percentage above 1% when they are in a solution with a pH lower than pK_a + 2 (*i.e.* pH 11.5) and in a percentage above 99% at a pH lower than pK_a – 2 (*i.e.* pH 7.5). The estimation of the species distribution for compounds with more than one pK_a value is more complex and will not be further discussed in this guidance.

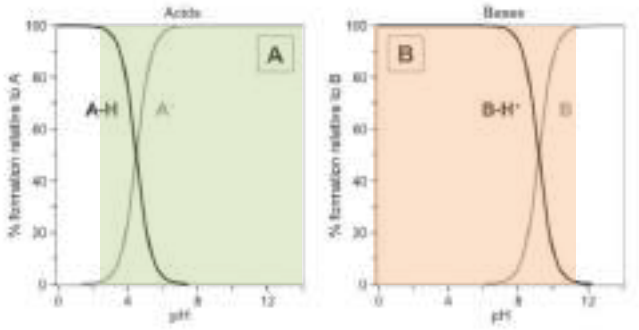

Figure 4.3.1. Visualisation of species distribution for monoprotic acidic and basic substances in solution as a function of pH as adapted from Schaffer and Licha (2014). The coloured areas cover the pH range at which the substances are present in the ionic form at > 1%. p*K*a acid = 4.5; p*K*a base=9.5

Relating to the mechanisms of adsorption/desorption of ionisable substances, extensive published literature exists that summarises the differences with organic non-ionisables, as well as alternative approaches to better assess their potential for adsorption (*e.g.* Arp and Hale, 2022; Sigmund *et al*., 2022; Henneberger and Goss, 2019; Droge and Goss, 2013; Bronner and Goss, 2011a; Mensink *et al*., 2008; Kah and Brown, 2007; Weber *et al*. 2004; Wauchope *et al.*, 2002). For neutral organic substances, soil organic matter is the key sorptive matrix (Mackay, 2001) and, therefore, as stated in section 4.3.3.3.3, adsorption/desorption could be reflected by the estimated *K*oc value derived from the *K*ow value. However, the potential for adsorption for charged substances (including various pesticides, pharmaceuticals, biocides but also industrial chemicals) is usually determined by multiple adsorption/desorption mechanisms (due to multiple matrix components such as clay and organic carbon), which cannot fully be reflected by the *K*ow value (partitioning between water and the octanol phase).

The publications mentioned above, highlight the interplay of complex interactions with the soil constituents and environmental variables (e.g. pH, ionic strength[108], dissolved organic matter, soil texture and mineral composition), other phases present (for example, coal, black carbon), non-linear sorption mechanisms and effects like aging and interface interactions that all need to be taken into account (Wauchope *et al.*, 2002, ECETOC, 2021).

Depending on these processes, substance speciation as a function of the soil pH must be considered in the assessment, as well as the different interaction types included, to the greatest degree possible. Adsorption studies on six acidic pesticides in nine soils revealed that the two strongest descriptors of the variability in adsorption were lipophilicity of the compound corrected for soil pH (Log D) and the soil organic carbon content (Kah and Brown, 2007). For cationic substances, there is evidence from the literature that the interactions underpinning their mobility may be even more complex than those for anionic substances (Kah and Brown, 2007). For example, it may in some cases, such as for soils with low organic matter content, be better characterised by adsorption to clay minerals than to soil organic matter (Sigmund *et al*., 2022, Droge and Goss, 2013, Weber *et al.*, 2004).

In general, the suitability of normalisation to soil organic carbon and, therefore, the use of octanol as a surrogate for sorption has been questioned for ionisable substances. Instead, different approaches have been proposed including the normalisation to clay content (Hermosin *et al.,* 2000) and to the estimated cation-exchange capacity (Droge and Goss, 2013), the development, validation and use of data-intensive poly parameter free energy relationships (PP-LFER) (Henneberger and Goss, 2019, Bronner and Goss, 2011b), as well as various experiments covering extended pH- and ionic strength-dependent sorption mechanisms of a wide array of soils and porewater chemistries (Sigmund *et al*., 2022, Arp and Hale, 2022). It needs to be noted that current PP-LFER approaches do not account for interactions such as electrostatic repulsion and attraction, charge-assisted H-bonding, cation bridging, etc. that may potentially be relevant for ionisable substances (Sigmund *et al*., 2022). At best, these data-intensive methods provide valuable insights into the sorption of a limited number of substances under specific soil and other environmental conditions, often containing a series of uncertainties and modelling assumptions, with limited validation datasets and with currently unaddressed complexities of extrapolating from small scales to the real hydrologic systems (Wauchope *et al*., 2002).

As can be understood from the above and is also acknowledged in the related scientific literature, none of the proposed alternatives to "K_{OC}-centric" sorption characterisation is currently available to be used for regulatory purposes to cover all types of ionic substances and interactions with soils. The currently proposed approaches lack harmonisation for uniform application by scientists and regulators, with no consensus having been built in agreeing on single sorption indices that can be derived under standardised experimental methods. Mechanistic information of the specific sorption mechanism can demonstrate further mobility potential of the substance in the environment and whether other than organic carbon normalisation is justified. The application and relevancy of such data to the mobility assessment can be justified on a case by case basis.

However, there is still an urgent need to generate information for ionisables that can be compared to the M/vM criteria within a hazard identification/ assessment context. Currently, recent literature still advocates the use of the organic carbon-water partition coefficient as derived from batch tests in a robust and conservative way, in order not to overestimate sorption (Arp and Hale, 2022; Sigmund *et al*. 2022). Such an approach is not context-specific as it does not take into account environmental and other exposure parameters. Some supportive evidence was provided by Wauchope *et al.* (2002) who reported relatively low variance between minimum and maximum experimental K_{OC} values for a high number of most commonly used pesticides.

[108] Ionic strength should however be representative of values derived from soil solutions in environment. According to Owsianiak *et al.* 2013 (table 1 in supportive document), effective ionic strength in pore water calculated for 760 soils range from 0.0011 to 0.4 M with a median value at 0.0065 M.

For **acidic substances** including, for example, carboxylic and sulfonic acids, mobility will be higher in the anionic form than in the neutral form due to their negative charge (soil is in most cases also negatively charged). In order to determine the mobility potential at all relevant conditions, testing of anionic substances needs to also include soils of high pH (when feasible, at a pH ≥ pK_a + 2) and low ionic strength (*i.e.* low ion concentration in solution). In such conditions, the anionic form dominates and the electrostatic repulsion with negatively charged soil moieties can increase mobility and the available cations for charge shielding and cation bridging are minimised (Sigmund *et al*. 2022). If the value of the soil pH is near the pK_a (pK_a-2 < pH < pK_a+2), then mobility will be sensitive to pH, as the anionic species concentration will vary as a function of the pH.

For **basic substances** including, for example, amines and amides, the adsorption behaviour could be more complex. As an example, at very low pH the electrostatic repulsion increases the mobility of the cationic forms. With increasing soil pH, the mobility will be minimal due to electrostatic attraction toward negatively charged soil moieties (Sigmund *et al*., 2022). At high pH (for example pH > pK_a +2), where the neutral form dominates, the mobility can increase due to a decrease of ionic interactions between the cationic base and the anionic surface charge of the soil (Sigmund *et al*., 2022). Thus, in order to determine the mobility potential at all relevant conditions, testing on cationic substances needs to also include soils of lower pH (when feasible, at a soil pH ≤ pK_a - 2). If the value of the soil pH is near the pK_a (pK_a-2 < pH < pK_a+2), then mobility will be sensitive to pH as the neutral species concentration will vary as a function of the pH. The selected soils should, thus, include soils of both low and high pH values, where both the charged and the neutral fractions can be studied.

In order to determine the mobility potential under all relevant conditions, it is recommended that testing for cationic substances should also take place in soils where sorption to clay (for example, illite, smectite) is not dominating, namely for soils of low clay content (for example below 10%). For these soils, with the caveats discussed above, the K_{OC} value is still considered appropriate provided that the organic carbon content is within the range given in Table 1 of OECD TG 106. The derivation of a clay- and/or CEC-normalised partition coefficient as well as their use for comparing with the CLP criteria may be considered in a future guidance update based on the scientific developments in the area.

When interpreting results from batch equilibrium adsorption/desorption studies (OECD TG 106) with ionisable substances, soil selection and characterisation are particularly important information to consider, as the soil pH defines the dominant species available in the test. Depending on the nature of the ionisable substance as described above, it is important to consider in the assessment whether the soils tested within a study included soil(s) in which the most mobile species were present, based on the soil pH. As recommended in the test protocol, soil pH should be measured in a 0.01 M of calcium chloride ($CaCl_2$) solution. Parameters such as the Cation Exchange Capacity, Anion Exchange Capacity as well as the clay content and mineralogy in the soil have been proposed to be reported together with organic carbon content and ionic strength for assessing the behaviour of such substances in the soil.

Regarding the interpretation of results for ionisables performed according to OECD TG 121 for a compound where at least 10% of the test compound will be dissociated within pH 4-9 (note, the respective OECD guideline mentions a pH range between 5.5 and 7.5), results from two tests should be available: one with the ionised form and one with the non-ionised form. The use of the appropriate buffer solutions and the suitability of the set of data for the reference ionisable substances needs to be carefully considered for assessing the reliability of the adsorption coefficient K_{OC} estimation.

Similarly, to the provisions above, the selected soils in a soil leaching experiment according to OECD TG 312 (Soil leaching columns) should also cover a wide range of pH, in order to evaluate the adsorption of ionisable and non-ionisable substances. The former needs to be considered only in the cases where the ionised form is present in at least 10% of the total amount of test substance within the environmentally relevant pH 4-9. In addition, as specified in the TG, at least 3 soils

should have a pH at which the test substance is in its mobile form. A suitable set of data for reference ionisable substances needs to be available for a reliable estimation of the adsorption coefficient K_{OC}. Similar principles apply for results on ionisable substances using the Soil TLC (Thin Layer Chromatography). The Table below provides a concise overview of the impact on the mobility of the acidic and basic ionisable substances, as a function of their dissociation constant (pK_a) and the pH.

Table 4.3.1 Dominant species and expected mobility of ionisable substances at soil pH values relative to the pKa of the substance (adapted from Wauchope et al., 2002)

	Dominant species* and mobility	
Soil pH	**Acids**	**Bases**
< pK_a - 2	XH (neutral) Behaves like non-ionisable substance.	$(XH)^+$ or X^+ (cation) Not mobile (clay surface and organic matter sorption)[109]
>pK_a - 2 and < pK_a + 2	X^-/XH ratio as a function of pH If the value of soil pH is near pKa mobility will be sensitive to pH. For acids mobility increases with increasing pH	$(XH)^+$/X or X^+/X(OH) as a function of pH If the value of soil pH is near pK_a mobility will be sensitive to pH.. For pH >> pKa the neutral species will be the predominant species and an intermediate mobility is expected.
> pK_a + 2	X^- (Anion) Highly mobile in soil.	X or X(OH) (neutral) Behaves like non-ionic substance.

*X^- refers for the anionic species, XH, X, XOH refers to neutral species, $(XH)^+$, X^+ refers to cationic species of the corresponding anionic or cationic substances.

[109] At low pH values, the electrostatic repulsion will increase the mobility of the cationic forms

4.3.3.4. Toxicity assessment

Commission Delegated Regulation (EU) 2023/707, Annex I: 4.3.2.3.3. and 4.4.2.3.3. The following information shall be considered for the assessment of T properties:

(a) results from long-term toxicity testing on aquatic invertebrates;

(b) results from long-term toxicity testing on fish;

(c) results from growth inhibition study on algae or aquatic plants;

(d) the substance meeting the criteria for classification as carcinogenic in Category 1A or 1B (assigned hazard statements: H350 or H350i), germ cell mutagenic in Category 1A or 1B (assigned hazard statement: H340), toxic for reproduction in Category 1A, 1B or 2 (assigned hazard statements: H360, H360F, H360D, H360FD, H360Fd, H360fD, H361, H361f, H361d or H361fd), specific target organ toxic after repeated dose in Category 1 or 2 (assigned hazard statements: H372 or H373);

(e) the substance meeting the criteria for classification as endocrine disruptor (Category 1) for human health or the environment (assigned hazard statements: EUH380 or EUH430);

(f) results from long-term toxicity testing on terrestrial organisms; invertebrates and plants;

(g) results from long-term toxicity testing on sediment organisms;

(h) results from long-term or reproductive toxicity testing on birds;

(i) other information, provided that its suitability and reliability can be reasonably demonstrated.

Annex I: 4.3.2.4.2. and 4.4.2.4.2. In applying the WoE determination, the following information, in addition to the information referred to in sections 4.3.2.3.3 and 4.4.2.3.3 shall be considered as part of the scientific assessment of the information relevant for the T properties:

Indication of T properties:

(i) Short-term aquatic toxicity (e.g. results from acute toxicity testing on invertebrates, algae or aquatic plants or fish, *in vitro* acute toxicity testing on fish cell line);
(ii) Other information provided that its suitability and reliability can be reasonably demonstrated.

A potential classification based on the study results from long-term toxicity testing on terrestrial and sediment organisms in the amended Annex I of CLP is new related to previous Toxicity assessments, as the ones under REACH Annex XIII. The following sections will present guidance on how information on terrestrial and sediment organisms can be assessed within the CLP context. In the absence of concrete, "real-life" examples of substances either classified or concluded as PBT/vPvB under REACH Article 57 (SVHC identification process) solely based on such test results, the current Guidance may need to be updated in the future based on the emergence of related cases proposed for harmonised classification. Similarly, in case of a potential future introduction of new hazard class(es)/criteria in CLP (or the UN GHS), a revisit of the described approach would be required.

4.3.3.4.1. Long-term aquatic toxicity

Section 4.1 and Annex I.3.2 of the current Guidance describes in detail the relevant experimental and other information that can be used to conclude on long-term aquatic toxicity, in the context of the assessment of aquatic hazards under CLP. However, despite the fact that the data used in the assessment of aquatic toxicity under hazardous to the aquatic environment (CLP Annex I, 4.1) and under PBT/vPvB and PMT/vPvM classification are the same, the regulatory criteria are not. Keeping this in mind, the ECHA Guidance on IR&CSA, Chapters R.7b and R.11 further detail the availability, applicability, adequacy (reliability and relevance) and other scientific and regulatory considerations for the use of the different test methods on long-term aquatic toxicity for substances of varying physico-chemical properties and regulatory uses. These considerations will not be repeated in the present Guidance.

Concerning long-term toxicity data for fish, for example, these Guidance documents elaborate further on exposure during relevant life-stages to regard the tests as long-term and describe in detail relevant considerations on the conduct and regulatory use of test methods OECD TG 210, 212, and 215. Aquatic invertebrates can be tested following OECD TG 211 (*Daphnia magna* Reproduction Test), whereas long-term effects on aquatic plants and algae can be investigated by a range of tests (for example, OECD TG 201 for freshwater alga and cyanobacteria, OECD TG 221 for *Lemna sp.* and OECD TG 238 and 239 for *Myriophyllum spicatum*).

Once reliable and relevant information is available resulting in a long-term NOEC or EC_{10} value in marine or freshwater organisms below the regulatory threshold of 0.01 mg/L, the substance can be concluded as fulfilling the CLP toxicity (T) criterion. In the presence of both long-term NOEC and EC_{10} for the same experimental study, CLP gives preference to EC_{10} (OECD, 2006 and current Guidance section 4.1).

4.3.3.4.2. Carcinogenicity (Carc. 1A or 1B)

A detailed description of the information considered relevant to conclude on the potential of a substance to fulfil the CLP criterion for carcinogenicity can be found in Part 3, section 3.6 of the CLP Guidance. A substance is considered as fulfilling the CLP toxicity (T) criterion if it can be classified in categories 1A or 1B for carcinogenicity (Carc. 1A or 1B), based on the criteria stipulated in section 3.6.2 of CLP Annex I.

4.3.3.4.3. Germ cell mutagenicity (Muta. 1A or 1B)

A detailed description of the information considered relevant to conclude on the potential of a substance to fulfil the CLP criterion for germ cell mutagenicity can be found in Part 3. section 3.5 of the CLP Guidance. A substance is considered as fulfilling the CLP toxicity (T) criterion if it can be classified in categories 1A or 1B for germ cell mutagenicity (Muta. 1A or 1B), based on the criteria stipulated in section 3.5.2 of CLP Annex I.

4.3.3.4.4. Toxic for reproduction (Repr. 1A, 1B or 2)

A detailed description of the information considered relevant to conclude on the potential of a substance to fulfil the CLP criterion for reproductive toxicity can be found in Part 3, section 3.7 of the CLP Guidance. A substance is considered as fulfilling the CLP toxicity (T) criterion if it can be classified in categories 1A, 1B or 2 for reproductive toxicity (Repr. 1A, 1B, or 2) based on the criteria stipulated in section 3.7.2 of CLP Annex I.

4.3.3.4.5. Specific target organ toxic after repeated dose (STOT RE 1 or 2)

A detailed description of the information considered relevant to conclude on the potential of a substance to fulfil the CLP criterion for specific target organ toxic after repeated exposure can be found in Part 3, section 3.9 of the CLP Guidance. A substance is considered as fulfilling the CLP toxicity (T) criterion if it can be classified in categories 1 or 2 for specific target organ toxic after repeated exposure (STOT RE 1 or 2) based on the criteria stipulated in section 3.9.2 of CLP Annex I.

4.3.3.4.6. Endocrine disruptor for Human Health (ED HH 1)

A detailed description of the information considered relevant to conclude on the potential of a substance to fulfil the CLP criterion for endocrine disruption for human health can be found in Part 3, section 3.11 of the CLP Guidance. A substance is considered as fulfilling the CLP toxicity (T) criterion if it can be classified in category 1 for endocrine disruption for human health (ED HH 1) based on the criteria stipulated in section 3.11.2 of CLP Annex I.

4.3.3.4.7. Endocrine disruptor for Environment (ED ENV 1)

A detailed description of the information considered relevant to conclude on the potential of a substance to fulfil the CLP criterion for endocrine disruption for the environment can be found in section 4.2 of this Guidance document. A substance is considered as fulfilling the CLP toxicity (T) criterion if it can be classified in category 1 for endocrine disruption for the environment (ED ENV 1) based on the criteria stipulated in section 4.2.2 of CLP Annex I.

4.3.3.4.8. Long-term terrestrial toxicity

Regarding highly adsorptive substances that are likely to be present in the terrestrial environment via inter-compartmental distribution processes or direct application (e.g. via sludge), effects on terrestrial organisms provide useful insights into the toxic potential of such substances. Under REACH, terrestrial toxicity testing usually refers to testing performed on terrestrial invertebrates (usually earthworms), microorganisms and terrestrial plants. Validated test methods are those according to OECD TG 222 (Earthworm Reproduction Test (*Eisenia fetida/Eisenia andrei*)), 220 (Enchytraeid Reproduction Test) and 232 (Collembolan Reproduction Test in Soil) for terrestrial invertebrates, OECD TG 216 (Soil Microorganisms: Nitrogen Transformation Test) and 217 (Soil Microorganisms: Carbon Transformation Test)[110] for soil microorganisms and OECD TG 208 (Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test), OECD TG 227 (Terrestrial Plant Test: Vegetative Vigour Test) and ISO 22030 (Soil Quality – Biological Methods – Chronic

[110] As indicated in OECD TGs 216 and 217, if agrochemicals are tested, two concentrations should be used. For non-agrochemicals, a geometric series of at least five concentrations must be used in order to cover the range needed to determine the ECx values.

toxicity in higher plants) for terrestrial plants. More details can be found in the ECHA Guidance on IR&CSA, Chapter R.7.11.

Additional terrestrial tests are mentioned under the PPPR, for example the predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil according to OECD TG 226. The Guidance for Biocidal Products Regulation Volume IV Part A: Information requirements (ECHA, 2022c) mentions ISO tests 16387, 11268-1, 11267 or OECD TG 226 for terrestrial invertebrates, ISO 14238:2012, BBA guideline Part VI, 1.1 or DIN EN ISO 23753-2 for soil microorganisms, as well as several test methods for honeybees (for example, OECD TG 213 and 214). Regarding honeybees and other pollinators, relevant tests include also ones performed according to OECD TG 245, 246 and 247 (guidance on the assessment of risks to bees from the use of plant protection products[111] and biocides[112]). These tests are both short- and long-term and are part of the "Additional Data Set" within the BPR context, meaning that they may be required for a certain biocidal product type, or for a certain use considering the likely exposure route, or depending on the properties of the substance. Information on non-target terrestrial arthropods is required when exposure is likely. Possible species of other non-target terrestrial arthropods to be tested in addition to honeybees are reported in the BPR Guidance, Volume IV. Part A, section 1.1.5.2. (ECHA, 2022c). Similar considerations are also relevant for the PPPR.

Considerations relative to birds are presented in section 4.3.3.4.10, whereas no further elaboration will be provided for other toxicity study information on mammals.

As for sediment organisms (see section 4.3.3.4.9), there are currently no concrete numerical threshold criteria in CLP for the direct comparison with results from long-term terrestrial toxicity studies (expressed as mg/kg dw). Spain has previously led a UN experts sub-committee panel on the Globally Harmonized System of Classification and Labelling of Chemicals (UNSCEGHS) and developed in 2006 a proposal on 'Classification criteria for the terrestrial environment' (UN, 2006). However, the criteria proposal has not been developed any further since. Additional efforts to define approaches of dealing with terrestrial toxicity data in the framework of PBT/vPvB assessment and classification and labelling have been made by JRC (2014) and, more recently, by the German UBA (2022). Further work on terrestrial toxicity is planned in the context of UN-GHS working groups. Once available, any relevant outcomes will be used for an update of this Guidance.

Until terrestrial hazard class(es) including threshold values are introduced in the regulatory framework, it is recommended that a similar approach is used for soil-living organisms as for sediment organisms by use of the Equilibrium Partitioning method (EPM). As such, results from long-term terrestrial toxicity studies are used to investigate whether the derived pelagic NOEC or EC_{10} can be compared to the T criterion of 0.01 mg/L of CLP Annex I, 4.3.2.1.3 (a) and 4.4.2.1.3 (a), by use of the following equation:

$$\boldsymbol{NOEC(EC_{10})_{porewater}} = \frac{\boldsymbol{NOEC(EC_{10})_{soil}}}{\boldsymbol{Kd_d}}$$

$NOEC(EC_{10})_{porewater}$: estimated NOEC ($EC_{10}$) for porewater exposure (mg/L)

K_d: adsorption coefficient (L/ kg dw)

$NOEC(EC_{10})_{soil}$: measured soil toxicity NOEC (EC_{10}) (mg/kg dw)

[111] https://www.efsa.europa.eu/en/efsajournal/pub/7989

[112] Guidance on the assessment of risks to bees from the use of biocides, 2024. https://echa.europa.eu/documents/10162/2324906/guidance_on_assessment_risks_to_bees_from_biocides_en.pdf/1fe886eb-0ba5-6d07-c06d-8a4823931a30?t=1707902780257

An EFSA scientific opinion (EFSA Journal 2009; 922, 1-90) based on a literature review confirmed that for soft-bodied soil organisms (earthworms, enchytraeids, nematodes) and plants in close contact with the soil solution, porewater mediated uptake of pesticides seems mainly responsible for the effects caused and would therefore be the relevant metric for effects assessment. However, toxic effects may also be exhibited via other mechanisms than porewater diffusion, whereas the assumption of linear correlation between NOEC(EC_{10})$_{porewater}$ and NOEC(EC_{10})$_{soil}$ may also introduce uncertainties. This could be the case, for example, for highly sorbing neutral organics or for substances with a corresponding adsorption or binding behaviour not predominantly driven by lipophilicity.

Preferably, porewater concentrations are based on well measured experimental porewater concentrations of the soil toxicity test. If these are not available, it might be possible to calculate a porewater concentration with a K_d value specific for the test soil. Otherwise, the K_d can be estimated from the generic K_{oc} as described in the section on mobility and the organic carbon content of the test soil (section 4.3.3.3.1). It should be noted that the uncertainty increases in this order of preference, and this has to be taken into account in the WoE.

The method should be applied with caution where relevant and justified, exercising expert judgement depending also on the availability of other information types. This approach, when applied to sediment organisms (section 4.3.3.4.9), has been shown to result in either an overestimation or underestimation of the toxicity to benthic organisms (Di Toro *et al*., 2005). For example, depending on the selection of soil parameters in the terrestrial toxicity test, the back calculation to aquatic organisms may not be adequate. Added uncertainty comes from the limited applicability domain of the EPM, namely that it is not applicable for ionizable substances and not reliable for substances with a log K_{OW} above 5. Finally, the EPM is not applicable to bees or non-target terrestrial arthropods. In all cases, this is envisaged to be the working approach until specific criteria are developed in the UN GHS level for toxicity to the terrestrial environment.

4.3.3.4.9. Long-term sediment toxicity

In cases where sediment effects assessment is necessary for substances that are known to be persistent in marine waters and may accumulate in sediments over time, tests on sediment organisms such as *Myriophyllum spicatum* (a submersed aquatic dicotyledon), *Chironomous sp*. (freshwater dipterans), or *Lumbriculus* (sediment-ingesting endobenthic aquatic oligochaetes) may provide useful information on the toxicity of the substance in the compartment in which it will be mainly found, namely sediment. Such validated test methods can, thus, be used for classification purposes and include OECD TG 239 (Water-Sediment *Myriophyllum spicatum* Toxicity Test) for *Myriophyllum* species, OECD TG 218 (Sediment-Water Chironomid Toxicity Test Using Spiked Sediment), 219 (Sediment-Water Chironomid Toxicity Test Using Spiked Water) or 233 (Sediment-Water Chironomid Life-Cycle Toxicity Test Using Spiked Water or Spiked Sediment) for Chironomids and OECD TG 225 (Sediment-Water *Lumbriculus* Toxicity Test Using Spiked Sediment) for *Lumbriculus*. It is hereby noted that in some cases analytical verification is made in the porewater, allowing expression of results directly in mg/L.

The choice of the test species depends on many factors, for example whether feeding on sediment particles takes place, for example, for strongly adsorbing or binding substances with a log K_{OW} above 5 (preference on *Lumbriculus variegatus*, *Tubifex tubifex*), whether there is a specific mode of action and/or sensitivity towards a given test organism, etc. (ECHA Guidance on IR&CSA, Chapter R.7.8.10.1). More details can be found in the ECHA Guidance on IR&CSA, Chapter R.7.8.9.1, including test methods according to ASTM, US-EPA and ISO test guidelines.

Currently, neither REACH Annex XIII nor CLP include a numerical threshold value to compare to the NOEC or EC_{10} value derived from a chronic sediment toxicity, for PBT and PMT assessment purposes. As described above, a proposed approach is the use of the EPM to estimate (no-effect) concentrations expressed in mass of test substance per volume of test medium (for example,

mg/L) from results of sediment toxicity test expressed in mass of test substance per mass of sediment (e.g. mg/kg of wet sediment). The estimated concentration (in mg/L) is then compared to the T criterion of 0.01 mg/L for toxicity in aquatic organisms. Further details on assumptions and considerations behind EPM are explained in ECHA Guidance on IR&CSA, Chapter R.10.5.2.1.

$$NOEC(EC_{10})_{porewater} = \frac{NOEC(EC_{10})_{sed}}{Kp_{susp}}$$

$NOEC(EC_{10})_{porewater}$: estimated NOEC (EC_{10}) for porewater exposure (mg/L)

Kp_{susp} : suspended solid-water partition coefficient (L/kg dw)

$NOEC(EC_{10})_{sed}$: measured sediment toxicity NOEC (EC_{10}) (mg/kg dw)

The suspended solid-water partition coefficient can be estimated from the K_{oc} of the substance as $Kp_{susp} = Foc_{susp} * K_{oc}$ where Foc_{susp} is the mass fraction of organic carbon in dry suspended matter.

The same considerations for the application of this approach as for terrestrial organisms (4.3.3.4.8) are also relevant for sediment organisms.

4.3.3.4.10. Long-term or reproductive toxicity in birds

Avian toxicity has been introduced in Annex X of the REACH Regulation to account for secondary poisoning risks to predators following chronic exposure to a substance via the fish (aquatic food chain) and earthworm (terrestrial food chain) (ECHA Guidance on IR&CSA, Chapter R.7.10.16). The standard tests typically measure lethal effects from either short- or medium-term exposures and/or chronic lethal and reproductive effects of long-term exposures. The exposures are expressed in terms of either a concentration or a dose. Longer-term exposure is preferred, as few (if any) scenarios are likely to lead to acute poisoning risks for birds, and evidence from pesticides (Regulation EC No 1107/2009) suggests that chronic effects cannot be reliably extrapolated or inferred from acute toxicity data (ECHA Guidance on IR&CSA, Chapter R.7.10.17).

Table R.7.10—4 from ECHA Guidance on IR&CSA provides an analytical summary of existing and proposed standardised avian toxicity tests. Additionally, *in vitro* approaches for birds are also currently under investigation, for example, Ball and Lavado (2021) who examined the use, limitations, and applications of avian cell-based models in an ecotoxicological context. Under the BPR, effects on birds based on OECD TGs 205, 206 and 223 have been required, if birds are likely to be exposed. Under the PPPR, a test for effects on reproduction in birds is currently requested in the pesticidal risk assessment, if birds are likely to be exposed. One standard study is usually requested, conducted in accordance with OECD TG 206 or USEPA OCSPP 850.2300 (EFSA 2023a).

ECHA Guidance on IR&CSA, Chapters R.7c and R.11 further clearly indicate that any results from reprotoxicity studies or other chronic data on birds (including from valid QSAR models) cannot be used on their own for direct comparison with the T criteria in REACH Annex XIII, in the absence of an agreed regulatory threshold value. This is also relevant for the assessment under CLP. Moreover, there are uncertainties relating to lack of data in the literature, too few species tested in the laboratory, different sensitivities between industrial chemicals and pesticides, interspecies differences, uncertain extrapolation to field conditions, etc. Thus, any such data can be used within the WoE determination to conclude on the toxicity of a substance, with a NOEC value below 30 mg/kg food considered as a strong indicator of fulfilment of the (T) criterion (ECHA Guidance on IR&CSA, Chapter R.7.10.16.2). In order to normalise between different bird species and sizes, as well as to account for any avoidance behaviour, a unit conversion of the test concentrations to dietary doses (in mg/kg body weight/day) by use of information on daily food consumption and body weight should also be performed and reported.

4.3.3.4.11. Other suitable and reliable information

REACH Annex XIII, section 3.1.3 considers short-term aquatic toxicity in accordance with section 9.1 of Annex VII and section 9.1.3 of Annex VIII as information relevant for the screening of the (T) property in PBT assessment. section 4.1 and Annex I.3.1 of the current Guidance provide details on the experimental and other information relating to acute aquatic toxicity and its use to conclude for aquatic acute classification purposes. These principles relating to the availability and assessment of such studies also apply when considering short-term aquatic toxicity as part of the different regulatory context of PBT/PMT assessment. Information from *in vitro* studies might also be considered in a WoE approach provided that they fulfil certain data quality requirements and comply with the REACH Annex XI criteria. These quality aspects are further detailed in ECHA Guidance on IR&CSA, Chapters R.7.8.3.1 and R.7.8.4.1 (R.7b), where the availability and applicability of such *in vitro* methods is further explained. As an example of a recently validated TG using fish cells, OECD TG 249 is currently available for rainbow trout gill cell lines (Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay).

In general, in the absence of long-term or chronic aquatic toxicity data that can be directly compared with the CLP criteria (see section 4.3.3.4.1), acute/short-term aquatic toxicity data may be used as an indication that the substance may fulfil the T criterion (R.11.2.2), also depending on the availability and quality of all other relevant information. When acute/short-term aquatic toxicity data show that the substance is very acutely toxic (L(E)C_{50} below 0.01 mg/L), a definitive conclusion can be drawn that the substance fulfils the (T) criterion. In cases of less acute aquatic toxic substances, results from such studies may likely not provide a true measure of the intrinsic aquatic toxicity of the substance (ECHA Guidance on IR&CSA, Chapter R.7.8.2).

In addition to data from standard toxicity tests, data from reliable non-standard tests and non-testing methods indicative of T properties may also be used if available. These data should be particularly assessed for their reliability, adequacy, relevance, and completeness (see *Chapter R.4* of the ECHA Guidance on IR&CSA). Additionally, the use of reliable (Q)SAR predictions, as well as adequately documented and justified read-across and/or grouping approaches is possible and assessed using expert judgement, on a case-by-case basis. For example, ECOSAR[113], KAshinhou Tool for Ecotoxicity (KATE)[114], iSafeRat®[115] may be used for predicting both short- and long-term aquatic toxicity. PETROTOX (Redman *et al*., 2017) has been developed to address petroleum substances based on substance composition (see ECHA Guidance on IR&CSA, Chapters R.11 and review published in 2012[116] for further details). The related provision in the CLP for the use of such data is "*other information, provided that its suitability and reliability can be reasonably demonstrated*". More information is included in section 4.3.4. Similarly, other information from toxicological studies may be relevant (for example, for allergens, neuro/immune toxicants, etc.) and needs to be considered.

[113] https://www.epa.gov/tsca-screening-tools/ecological-structure-activity-relationships-ecosar-predictive-model

[114] https://kate.nies.go.jp/

[115] https://www.kreatis.eu/isaferat_page

[116] https://echa.europa.eu/documents/10162/17221/review_environmental_physicochemical_methodol_en.pdf (last accessed: November 2023)

4.3.4. Application of the WoE to conclude on PBT/vPvB properties for classification and labelling

Commission Delegated Regulation (EU) 2023/707, Annex I: 4.3.2.3. Basis of classification

For the classification of PBT substances and vPvB substances, a WoE determination using expert judgement shall be applied, by comparing all relevant and available information listed in section 4.3.2.3 with the criteria set out in sections 4.3.2.1 and 4.3.2.2. That WoE shall be applied in particular where the criteria set out in sections 4.3.2.1 and 4.3.2.2 cannot be applied directly to the available information.

The information used for the purposes of assessment of the PBT/vPvB properties shall be based on data obtained under relevant conditions.

The identification shall also take account of the PBT/vPvB properties of relevant constituents, additives or impurities of a substance and relevant transformation or degradation products.

This hazard class (Persistent, Bioaccumulative and Toxic (PBT) or Very Persistent, Very Bioaccumulative (vPvB) properties) shall apply to all organic substances, including organo-metals.

The PBT/vPvB assessment must consider each property, namely persistence, bioaccumulation and toxicity against each respective criterion (P or vP, B or vB, and T) following the provisions and considerations described in section 4.3.3 of this Guidance. In CLP (green text above), the decision on whether classification in the PBT/vPvB hazard class is warranted is based on a WoE determination using expert judgement. WoE shall be applied in particular where the criteria set out in sections 4.3.2.1, 4.3.2.2 and 4.3.2.4 cannot be applied directly based on the available information. The following paragraphs will expand on some general principles of the WoE, with property-specific considerations further elaborated on after the current general principles section.

The following general principles are broadly adapted from OECD report No. 311 on the Guiding Principles and Key Elements for Establishing a Weight of Evidence for Chemical Assessment (OECD, 2019)[117]. Additionally, ECHA has developed a template and background document intended to be used in human health and environmental hazard assessments under REACH and CLP, in order to harmonise the use of WoE and uncertainty assessment, increase transparency in regulatory decision making and facilitate the integration and use of alternative methods and all available information[118]. Similarly, EFSA (2017) has issued a Guidance on the use of the WoE approach in scientific assessments that can also be consulted[119]. Thus, the individual steps in the general scheme are not explained in detail, but only the high-level/ key issues are highlighted. The general scheme can be seen below.

[117] https://www.oecd-ilibrary.org/docserver/f11597f6-en.pdf?expires=1706878428&id=id&accname=guest&checksum=8553021B60DFC988DB5EB50AC48B78C1

[118] https://echa.europa.eu/support/guidance-on-reach-and-clp-implementation/formats

[119] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2017.4971

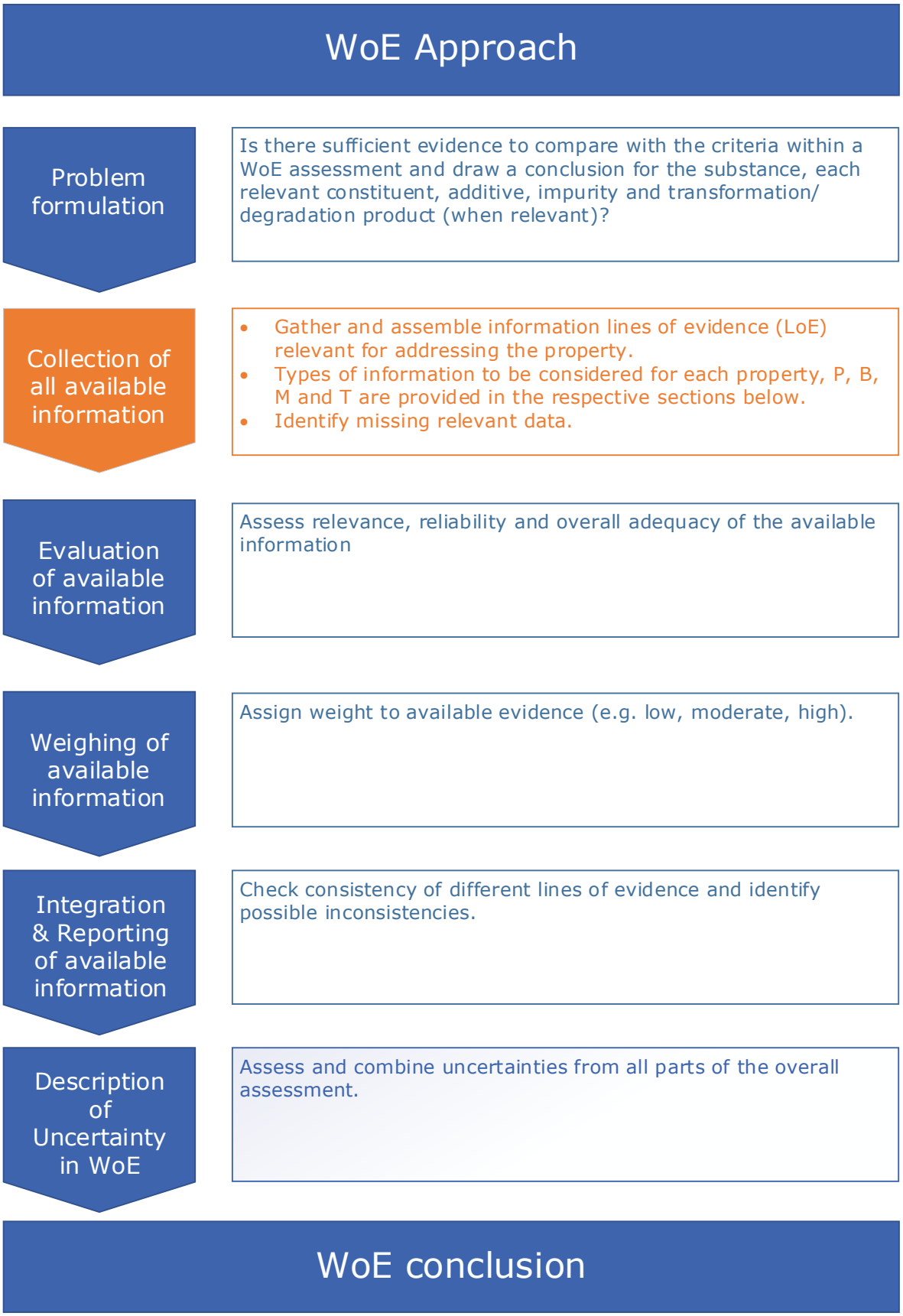

Figure 4.3.2 General scheme for the WoE approach (adapted from OECD, 2019)

Problem formulation: aim of the WoE approach

The first step of establishing the WoE approach is by defining the scope and goals of the assessment, namely whether there is sufficient evidence to support the conclusion that the CLP criteria for P or vP, B or vB, and T are or are not met. This refers to the substance, but also each relevant constituent, additive, impurity and transformation/ degradation product.

Collection and documentation of all information

CLP refers to the comparison of all relevant and available information with the criteria, particularly in cases where these criteria cannot be applied directly to the available information (Article 9). The assembled information must be put together into "lines of evidence (LoE)" that can be used to address the question(s) posed in the problem formulation step. It is very important to be transparent on how the information has been assembled and presented, which will also facilitate the easier identification of missing or uncertain evidence. The assembled information will need to cover different types of evidence, both direct and indirect, from different sources.

The collection and documentation refer to all available information (note that new data generation is only permitted as a last resort under CLP Article 8). Available information has been described comprehensively earlier in this Guidance and includes experimental and non-experimental information, *in vivo*, *in vitro* and *in silico* methods, monitoring and modelling data, results from studies from structurally similar substances, etc. sections 4.3.3.1, 4.3.3.2, 4.3.3.4 have already addressed the use of non-standard tests. Such test results can be considered within the WoE if deemed relevant, reliable and equivalent to other standardised methods, as well as the relevance of evidence from read-across, QSARs and monitoring data, for each individual property (P/B/T).

Evaluation of all available information

Chapter R.4 of the ECHA Guidance on IR&CSA provides guidance on how to evaluate available information under REACH and CLP, including an assessment of the adequacy, relevance and reliability of the available information as defined by Klimisch *et al.* (1997).

The relevance of a piece of information/LoE can be defined as the extent to which it is appropriate for a particular property (see *Chapter R.4* of the ECHA Guidance on IR&CSA). For example, whether the tested substance is representative of the one being assessed, whether the test species and test design are appropriate for the property being investigated, whether the appropriate dose and route of exposure are used, whether critical parameters influencing the property have been considered, etc. The current Guidance elaborates further in detail on several elements to establish the relevance of the provided information, both at a general level (for example, as in bullet point (ii) in section 4.3.3) and at an individual property and study level (see sections 4.3.3.1-4.3.3.4).

The reliability of test data is the inherent quality of a test relating to test methodology and the way that the performance and results of a test are described (see *Chapter R.4* of the ECHA Guidance on IR&CSA). Klimisch *et al.* (1997) developed a scoring system to assess the reliability of data, particularly from (eco)toxicological studies that may be extended also to physico-chemical and environmental fate studies. More recently, Moermond *et al*. (2016) developed the CRED (Criteria for Reporting and Evaluating Ecotoxicity Data) evaluation method that includes a set of 20 reliability and 13 relevance criteria, accompanied by extensive guidance. Detailed recommendations on how such studies can be reported have also been developed, to improve transparency and consistency on how ecotoxicological information is presented. In analogy to the Klimisch scoring, 4 reliability categories are also proposed within the CRED framework.

For (Q)SAR predictions and results based on multiple predictions, the Klimisch criteria are not applicable but a scoring system based on the OECD (Q)SAR Assessment Framework (OECD, 2023)

has been implemented in OECD harmonised templates (OHT) and consequently IUCLID and is recommended to be used as follows:

- (Q)SAR result with low uncertainty (Reliability 1);
- (Q)SAR result with medium uncertainty (Reliability 2);
- (Q)SAR result with high uncertainty (Reliability 3);
- (Q)SAR results with limited documentation/justification, but validity of model and of prediction considered adequate based on a generally acknowledged source (Reliability 2 or 3);
- (Q)SAR result with limited documentation/justification (Reliability 4).

When more than one relevant and reliable (see text above and section 4.3.3 (i) for definitions) experimental study result (value) is available for the same property, in most cases the most conservative value is used in order to achieve the regulatory protection goals and to account for any uncertainties of the test method and different experimental conditions.

However, there may be exceptional situations where it is appropriate to combine a sufficient number of study results from the same study type generated under similar conditions to generate a statistically robust representative value (e.g. suitable mean) for comparison with the CLP criteria. In such cases, statistical combination of the appropriate study results may be possible, provided that it is scientifically robust and properly substantiated. The statistical distribution of the data should be considered when selecting the representative value. A geometric mean is generally recommended when the data follow a log-normal distribution, whereas the arithmetic mean is generally recommended for data that follow a normal distribution. Martinez and Bartholomew (2017) give detailed guidance on estimation of an appropriate mean value.

As an example, the current CLP Guidance already provides some advice on this concerning aquatic hazards classification (section 4.1.3.2.4.3 for aquatic toxicity, Annex III.4.1 for aquatic bioaccumulation). Similarly, the ECHA Guidance on IR&CSA, Chapter R.11.4.1.1.3 provides some guidance on combining experimental study results from simulation degradation tests for the same compartment and conducted under similar test conditions (for example, temperature, pH, organic carbon content, microbial biomass, test design, etc.).

The above refers to the combination of values from experimental studies performed under similar conditions, for example for the same environmental compartment, species, life cycle/stage, test design, etc. It is not recommended that values from different study types (for example, an experimental fish BCF, a mussel BCF and a (Q)SAR prediction for BCF or laboratory simulation and field degradation studies test data) are combined. More detailed, property-specific information on the specific conditions for which combination of study results may be allowed can be found below and in section 4.3.5.

Expert judgement on the conditions that need to be met in order to be able to combine values for a property will always be needed. The reliability and relevance of all similar study results and their statistical distribution should be considered. An outlier is an observation that appears to deviate markedly from other observations in the sample and may be detected by visual inspection or standard statistical techniques and reasons for any outliers should also be considered. Such considerations should be adequately documented and reported and the scientific assessment will be conducted on a case-by-case basis. In all cases, any such combined representative value should be considered with all other relevant and reliable information available in the overall WoE to compare with the respective CLP criteria.

Weighing of available information

All available relevant information should be considered together and appropriate weight should be given. A more quantitative (for example, by use of numerical scoring systems) or a more qualitative (for example, high, medium, low weighing) approach to the WoE may be taken by the expert. OECD (2019) proposes ways of how available information may be weighed.

Integration and reporting of the available information

Important elements for the integration of the different LoE comprise the quality of the data, the variability and/or consistency of the results, the nature and severity of effects, the relevance of the information, the presence of any biases and outliers. The outcome of this integration will be based on expert judgement to structure the available information in order to come to an overall conclusion that can be used for classification purposes. This outcome will, in turn, be based on the availability and quality of the different LoE, as well as on substance-specific considerations. SCHEER (2018) provides further details on the process of integration of lines of evidence to determine the relative support for hypotheses or answering a question. Again, transparent documentation/articulation of the outcome is of utmost importance.

Description of uncertainty

Uncertainty refers both to any limitations and shortcomings of the available information (e.g. data paucity, study quality and reliability issues, incomplete documentation, question marks on study relevance, use of unvalidated or difficult to reproduce approaches, analytical difficulties based on substance property specific considerations, etc.) and to the level of regulatory acceptance that may impact the conclusion of the WoE.

Limitations of the available information must be identified and, to the degree possible, addressed by use of either more qualitative or more quantitative approaches, for example with the use of probabilistic analysis techniques. Qualitative approaches entail the identification of uncertainties in the hazard assessment and their grouping in sub-categories (high-medium-low, acceptable-unacceptable, etc.). The use of more statistically robust techniques is further detailed in OECD (2019) and ECHA Guidance on IR&CSA, Chapter R.19: Uncertainty analysis. Caution should be taken on the approaches detailed in the ECHA Guidance R.19, as they refer more to risk assessment and not to a solely hazard identification/assessment context as the one in CLP. Their use will also depend on the availability of adequate data and its statistical distribution patterns. In all cases, the uncertainty of each information source and the overall uncertainty need to be evaluated and transparently documented.

Concerning the level of uncertainty acceptance, a connection with the protection goal must be established, namely the high level of protection of human health and the environment, for CLP. Ultimately, as already reported in footnote 43, the Court of Justice has confirmed that in case of remaining uncertainty on the existence and extents of risks, appropriate measures should be taken "*in order to prevent certain potential risks for public health, safety and the environment without having to wait until the reality and seriousness of those risks become fully apparent*" (Cases C-65/21 P and C-73/21 P to C-75/21 P quoted earlier above, paragraphs 95-99), in line with the precautionary principle.

WoE conclusion

Separate conclusions are required for both PBT and vPvB, as well as for each of the P, B and T properties, based on the overall WoE. The need for explicit separate conclusions on the individual properties is also due the fact that meeting the criteria for two of the criteria for being PBT leads to the substance being considered as a "Candidate for Substitution (CfS)" under the BPR (Article 10(1)(d)) and PPPR (Annex II, 4), if applicable. These Regulations also define further the regulatory implications for CfS substances.

In order for the PBT or vPvB criteria to be fulfilled, all respective criteria must be met for the same substance or at least one (but always the same one for all properties) individual constituent, impurity, additive or transformation/degradation product, if applicable. The criteria for (v)P, (v)B and T referred to in Annex I of CLP, 4.3 do not all have to be met in the same test compartment *i.e.* aquatic, soil or sediment, as the General Court of the European Court of Justice has unequivocally ruled in two judgements[120]. The outcomes of the application of the WoE to conclude on the individual PBT/vPvB properties can be that the substance fulfils the P/vP/B/vB/T criteria or not.

It is very important to also provide clarification/justification why a substance does not meet the P/vP/B/vB/T criteria, in line with the current approach of ECHA's Risk Assessment Committee (RAC) where the opinion documents[121] contain justifications for a substance not meeting the classification criteria. Further elaborations on these are given in CLP Article 40. Knowledge of the reasons for the different conclusions constitutes invaluable information for both regulators and data holders and increases the transparency of the regulatory outcome, as well as the legal robustness of the conclusion.

The WoE determination is not a mechanism to justify disregarding valid test data and it is not a means to average results from different sources. ECHA Guidance on IR&CSA, Chapter R.11.4.1.1.1 contains more information on specific WoE considerations including the preference for experimental results from reliable studies that can directly be compared to the criteria and their higher relevance over "screening-type" information (for example, Chapters R.11.2.1 and R.11.4.1). This does not mean that all other types of information are not taken into consideration. One example of this preference refers explicitly to the results from reliable degradation simulation studies and the fact that, in their presence, a detailed analysis of the reasons of any potential inconsistencies with the outcomes of studies with lower weight is not necessary (ECHA Guidance on IR&CSA, Chapter R.11.4.1.1.1), as long as all available reliable information is considered within the WoE. This was confirmed in a recent ruling of the General Court of the European Court of Justice (Case T-177/19, see footnote 60).

Benchmarking

Benchmarking can also be used as part of the WoE and associates the fate or behaviour of a substance to that of a similar/comparable benchmark, well-described chemical (Adolfsson-Erici *et al.*, 2012). The comparability refers to the test conditions/set-up, on which test organisms the available data refers to, as well as the data analysis and interpretation. More details have been included in the relevant parts of this Guidance, as well as in ECHA Guidance on IR&CSA, Chapter R.11.4.1. One of the important aspects highlighted therein refers to the reporting of concentrations also based on a molar basis (for example, mmol/L), for a better application of benchmarking to assess toxicity. In this way, comparison of toxicity on molar basis prevents bias from molecular weight differences. This could be especially useful for multiconstituent and halogenated substances.

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[120] See judgment of 30 June 2021, Global Silicones Council and others v. Commission, T-226/18, not published, EU:T:2021:403, paragraphs 129 to 133 (see https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:62018TJ0226) and judgment of 30 June 2021, Global Silicones Council and others v. ECHA, not published, EU:T:2021:404, paragraphs 107 to 110 (see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX:62018TJ0519). Both cases have been appealed by Global Silicones Council in Cases C-558/21 P and C-559/21 P respectively. These appeals were dismissed by the Court of Justice on 9 November 2023.

[121] https://echa.europa.eu/registry-of-clh-intentions-until-outcome/-/dislist/name/-/ecNumber/-/casNumber/-/dte_receiptFrom/-/dte_receiptTo/-/prc_public_status/Opinion+Adopted/dte_withdrawnFrom/-/dte_withdrawnTo/-/sbm_expected_submissionFrom/-/sbm_expected_submissionTo/-/dte_finalise_deadlineFrom/-/dte_finalise_deadlineTo/-/haz_addional_hazard/-/lec_submitter/-/dte_assessmentFrom/-/dte_assessmentTo/-/prc_regulatory_programme/-/

4.3.4.1. Persistence

The P/vP assessment shall reach one of the following conclusions for each relevant constituent, additive, impurity and transformation/degradation product: P, vP or criteria not met (Figure 4.3.3). When a conclusion that the criteria are not met is reached, it should be further justified whether this is based on conclusive data, inconclusive data or lack of data. Inconclusive data refers to, for example, shortcomings in the provided information, uncertainties in the performance of the study(ies) and their underlying assumptions, contradictory evidence, incomplete documentation, paucity of data, lack of statistical analysis, severe deviations from the test protocols, etc. Lack of data refers to a complete absence of any reliable data.

Section 4.3.3.1 of the current Guidance described the relevant experimental and computational information that may be provided as part of the WoE determination on Persistence. The decision scheme needs to be followed on the available information, in order to come to a robust conclusion on whether the CLP criteria for Persistent and/or very Persistent are fulfilled (Figure 4.3.3).

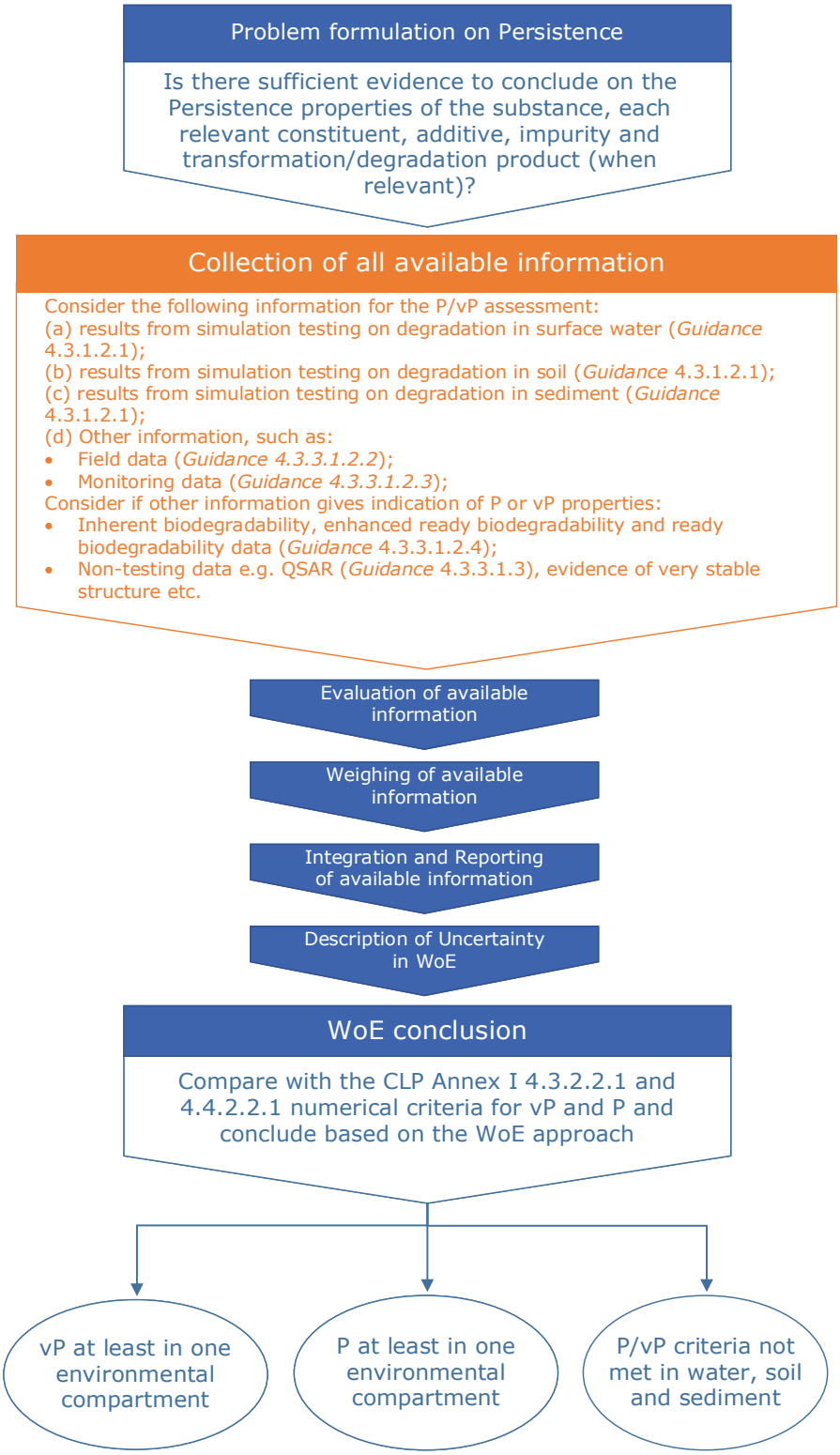

Figure 4.3.3 WoE approach for concluding on the persistence properties of the substance, each relevant constituent, additive, impurity and transformation/degradation product (when relevant). Regarding the available information, the relevant sections of the Guidance are indicated in brackets.

When deciding if a substance fulfils the P or vP criteria, its transformation/degradation potential in the surface water, sediment and soil should be considered. The WoE approach should address whether there is sufficient evidence to support the conclusion that the substance, each relevant constituent, additive, impurity and transformation/degradation product (when relevant):

- fulfils the P/vP criteria for degradation half-life in water, sediment or soil;
- in the absence of information to derive numerical degradation half-life values, is there sufficient evidence to support the P or vP conclusion in surface water, sediment or soil?

All available data should be considered as part of the WoE assessment leading to P or vP conclusion or a conclusion that the criteria are not met. When the vP criterion is met, then also the P criterion is met. P or vP conclusion reached for one environmental compartment is enough to consider that the substance meets the P or vP criteria. Concluding for one environmental compartment does not exclude the possibility that the substance might be also P or vP in other compartments. In some cases, WoE may lead to P/vP conclusion without specifying the compartment[122]. In order to conclude that a substance does not meet the P criteria, it must be demonstrated that the criteria are not met in surface water, sediment and soil.

Existing experimental data which can be directly compared with the criteria (see also sections 4.3.3.1.2.1 and 4.3.3.1.2.2).

More weight should be assigned to the results of the degradation simulation studies than those from screening-level studies in the WoE assessment. Degradation half-life (DegT50) obtained from a simulation degradation test in water, sediment or soil, conducted in relevant conditions can be directly (numerically) compared against the respective persistence criterion of CLP to determine whether the P or vP criteria are met or not. In sediment simulation tests (OECD TG 308), where DegT50 is reported separately for water, sediment and whole system, whole system half-lives obtained is preferred for comparison with the P/vP criteria for the sediment compartment. The same applies also for DegT50 values in soil, if a DegT50 in porewater has been estimated.

The reference temperature for providing DegT50 results on simulation tests or field degradation tests is 12°C for fresh or estuarine water, soil and fresh or estuarine water sediment environments and 9°C for marine water or sediment environments. P or vP conclusion reached in one of the environmental compartments is enough to consider that the substance meets the P or vP criteria. For example, the substance would be P or vP if criteria are met only for water but not for soil or sediment. In order to conclude a substance conclusively not P it must be demonstrated that the substance is not P in all of the environmental compartments listed in CLP Annex I, section 4.3.2.1.1, 4.4.2.1.1, 4.3.2.2.1 and 4.4.2.2.1. In general, results of a single simulation degradation study demonstrating not P in one compartment cannot be directly extrapolated to other non-tested environmental compartments. In some cases, extrapolation between compartments may be possible provided that results/bridging is supported by proper justification.

If a study has not been conducted in relevant conditions, for example if much higher suspended solids concentration than allowed in OECD TG 309 was used or sediment stratification was disturbed in OECD TG 308 study, DegT50 values obtained in such conditions may overestimate the degradation rate. Such DegT50 values can be used in a WoE assessment but their relevance should be considered with care.

Degradation half-lives derived from tests conducted solely under fully anaerobic test conditions are considered not to be especially relevant for the P assessment as permanently anaerobic soil or sediment systems are not common in the EU. Nevertheless, if anaerobic degradation data are

[122] Examples of this type of P/vP conclusions can be found e.g. in SVHC support documents for 2,4,6-tri-tert-butylphenol (EC 211-989-5), "DECHLORANE PLUS"TM (EC 236-948-9) and Perfluoroheptanoic acid and its salts.

available, they may be used as part of a WoE approach. The difference in degradation rates and pathways between aerobic and anaerobic conditions could sometimes provide important insights into the P assessment. Generally, it would be expected that an anaerobic degradation half-life would be greater than an aerobic degradation half-life where the main route of degradation is aerobic, namely if there is no oxygen, degradation will be hindered. However, care should be taken where the data in anaerobic sediment test show fast degradation of a substance. In such case, OECD TG 308 may overestimate the degradation rate of some substances in the aerobic environment. This has been shown for example with nitro- containing substances, like musk xylene[123].

In the presence of a reliable DegT50 obtained from simulation degradation test or field study, it is not necessary to analyse in detail the reasons for potentially inconsistent outcomes of the screening tests. The outcomes of a reliable and relevant simulation degradation or field study, have higher weight in the WoE than screening studies (ECHA Guidance on IR&CSA, Chapter R.11 provides further details on the WoE assessment).

With regard to persistence, it is insufficient to consider a dissipation half-life (DT50) alone, where this may simply represent removal from the test system or the transfer of a substance from one environmental compartment to another (e.g. from the water phase to the sediment or vice versa). If transfer processes have occurred simultaneously with degradation, the DT50 value is not representative of the DegT50 value (CLP Annex I, 4.3 and 4.4) and thus may only serve as supporting information in the assessment. Where primary degradation is observed, it is necessary to identify the transformation/degradation products and to assess whether they possess PBT/vPvB properties. All relevant transformation/degradation products should be considered in the assessment (See section 4.3.3 (v) of this Guidance).

Field studies, provided that their suitability and reliability can be reasonably demonstrated by also taking uncertainties in deriving field DegT50 into account, may be used as assessment information (CLP Annex I: 4.3.2.3.1. and 4.4.2.3.1.). However, when DegT50 derived from field studies are compared to the P/vP criteria, uncertainties related to the role of other dissipation processes such as volatilisation, leaching, etc. on the estimated DegT50 must be carefully considered (see also section 4.3.3.1.2.2 of this Guidance). Influence of dissipation processes in the derivation of the field DegT50 is difficult to quantify and thus in many cases lowers the reliability of the estimated degradation half-lives.

If pH dependency of degradation (e.g. ionisable substances) leads to different degradation rates between acidic and alkaline conditions within the environmentally relevant pH range, the most conservative DegT50 value should always be used when compared to the CLP Annex I criteria.

Monitoring studies

In addition to the simulation and field test data, existing monitoring data should be carefully examined. Monitoring studies, provided that their suitability and reliability can be reasonably demonstrated, may be used as assessment information (CLP Annex I: 4.3.2.3.1. and 4.4.2.3.1. and see section 4.3.3.1.2.3 of this Guidance). However, mere presence of a substance in one or more environmental compartment(s) cannot on its own demonstrate persistence, because presence in the environment is also dependent on a range of factors other than degradation rates, such as emission and distribution rates. Therefore, monitoring data should always be considered together with such related factors. For example, if monitoring data show that a substance is present in remote areas (namely, long distances from populated areas and known point sources, such as the Arctic sea or sub-Arctic/Arctic lakes in Scandinavia), it may be possible to conclude a substance as P or vP (this is especially the case for non-mobile substances) (ECHA Guidance on

[123] SVHC support document (EC 201-329-4) https://echa.europa.eu/documents/10162/909dd42e-2554-4f59-911a-729a2da1d529

IR&CSA, Chapter R.11). Monitoring data obtained in areas closer to the sources may also be useful for P/vP assessment as one line of evidence for supporting the conclusions. Also, significant concentrations of the substance in higher levels of the food chain may indicate high persistence (beside a potential to bioaccumulate).

Other information on persistence

The conclusion that a substance is not P/vP can be based on screening level information (including enhanced ready biodegradability tests) provided that, taking into account all available information in line with the Annex I of CLP, 4.3.3.2., there is no other evidence indicative of persistence in any of the relevant environmental compartments. In general, screening level information (including enhanced ready biodegradability tests) has lower weight than simulation and field studies in the WoE assessment in concluding a substance as P/vP. In some exceptional cases, if scientifically justified and supported by other available information, it is in principle possible to draw P/vP conclusion based on screening information. For example, if based on the structure of the substance (e.g. perfluorinated substances with covalent C-F bonds) it is known to be resistant towards degradation based on scientific evidence, screening level information would be adequate to conclude a substance as P/vP (unless other evidence indicates non-persistence).

If supported by other available weight of evidence, lack of or low mineralisation (<20% degradation) in an inherent biodegradability test (OECD TG 302) may provide sufficient information to confirm that the P-criteria are fulfilled for the purpose of persistence assessment. Additional lines of evidence information may, for example, consist of (Q)SAR predictions, consistency in the lack of degradation in other screening studies, or non-standard degradation studies[124]. Additionally, in specific cases it may be possible to conclude that the vP-criteria are fulfilled with such results if there is additional specific information supporting the conclusion (*e.g.* specific stability of the chemical bonds as described above). For example, low mineralisation in inherent degradation test supported with (Q)SAR predictions, monitoring data and non-standard studies has been used to conclude substances as vP[125,126]. However, it should be noted that lack of mineralization in inherent degradation test does not equate to lack of primary degradation (see also section 4.3.3.1.2.4).

The degradation half-lives obtained in an abiotic hydrolysis test can be used only as supporting information as abiotic degradation is primary degradation, and careful consideration is needed to address the potential formation of stable degradation products with PBT/vPvB properties. Abiotic hydrolysis data always need to be considered in connection with the other properties, such as partitioning properties and the knowledge on the abiotic and biotic degradation pathways. Similarly, data derived from other abiotic studies (e.g. photodegradation) should be considered as supporting information only in persistence assessment. Due to the large variation in the light available in different environmental compartments, the use of photolysis data is not generally recognised for persistence assessment. This is discussed in more details in the ECHA Guidance on IR&CSA, Chapter R.7b.

Results obtained from well-developed and reliable biodegradation (Q)SAR models can be used as part of the scientific assessment of the information relevant for the P, vP properties in the WoE. For this purpose, it is recommended to use, for example, combined results from three estimation models in the EPI Suite™ (US EPA, 2012; R.11). Acceptable (Q)SAR predictions can be used furthermore to support grouping or read-across assessment (see also section 4.3.3.1.3 of this Guidance). Degradation half-lives based on QSAR models using data from ready biodegradation

[124] SVHC Support Document - 2,4,6-tri-tert-butylphenol

[125] SVHC Support Document - BIS(2-ETHYLHEXYL) TETRABROMOPHTHALATE COVERING ANY OF THE INDIVIDUAL ISOMERS AND/OR COMBINATIONS THEREOF

[126] SVHC SUPPORT DOCUMENT - BTBPE

tests should only be used as supporting information in the assessment as derived half-life values are only based on screening level information and not on data obtained in relevant conditions to derive a degradation half-life.

The persistence assessment tool (PAT)[127] is a software tool designed for the recording and the evaluation of various lines of evidence. There is limited experience on its applicability in regulatory use. If this tool is used for recording the available information, assessment should follow advice provided in the relevant sections of this Guidance and the ECHA Guidance on IR&CSA, Chapters R.11 and R.7b.

Multiple experimental degradation study results

Where more than one relevant and reliable experimental degradation study result (value) resulting in DegT50 is available for the same environmental compartment[128], in most cases the most conservative value is used in order to achieve the regulatory protection goals and to account for any uncertainties of the test method and differing experimental conditions.

Combining experimental study results

DegT50 data from different environmental compartments should not be combined for PBT/vPvB classification purposes because each of the environmental compartments have compartment-specific DegT50 criteria. However, there may be exceptional situations where it would be appropriate to combine a sufficient number of DegT50 values for the same environmental compartment to generate a statistically robust representative value for comparison with the CLP criteria. However, combining all available DegT50 values is generally not appropriate.

Some existing guidance on generating representative DegT50 values is provided for example in the ECHA Guidance on IR&CSA, Chapter R.11.4.1.1.3. This Guidance provides a possibility to combine more than four experimental study results conducted on the same environmental compartment (water/soil/sediment) under similar test conditions (for example, temperature, pH, organic carbon content, microbial biomass, test design, etc.).

When comparing to CLP Annex I DegT50 criteria, combining DegT50 results from several degradation (e.g. simulation) studies with the same environmental compartment and study type, with similar test conditions, design and degradation kinetics (for example, SFO kinetics or biphasic kinetics), can be considered. DegT50 data from different environmental compartments should not be combined. The statistical distribution of the data should be considered when selecting the approach. Any data outliers should be assessed and removed from the data set if appropriate (see also section 4.3.4 "Evaluation of all available information").

Only test results from the same environmental compartment corresponding to similar test conditions (e.g. laboratory or field, aerobic or anaerobic, marine or fresh water) can be compared. The similarity of the tests results to be combined should be scientifically justified by considering whether the test conditions and/or characteristics of the test media (e.g. temperature, pH, organic carbon content, microbial biomass, source of the test media etc.) significantly influence the degradation potential of the substance. Differences in incubation temperatures can be compensated by normalising the DegT50 values to relevant reference temperature (for example, DegT50 at 12 °C).

In all cases, the approach used to generate a representative DegT50 value should be well justified and documented and should be supported by the WoE analysis. This should include a discussion

[127] Persistence Assessment Tool (PAT)

[128] The same environmental compartment refers to either marine water, fresh water, estuarine water, marine sediment, fresh water sediment, estuarine water sediment or soil.

of any outliers. In particular, the representativeness of the test conditions should be carefully assessed for each value. Particular scrutiny should be given if results from the tests are close to the P or vP threshold.

4.3.4.2. Bioaccumulation

The B/vB assessment shall reach one of the following conclusions for each relevant constituent, additive, impurity and transformation/degradation product: B, vB, or criteria not met. The latter conclusion is based on conclusive data, inconclusive data or lack of data. Section 4.3.3.2 of the current Guidance document describes the relevant experimental and computational information that may be considered as part of the WoE determination on Bioaccumulation.

The decision scheme needs to be followed based on the available information, in order to come to a robust conclusion on whether the CLP criteria for Bioaccumulative and/or Very Bioaccumulative are fulfilled (Figure 4.3.4).

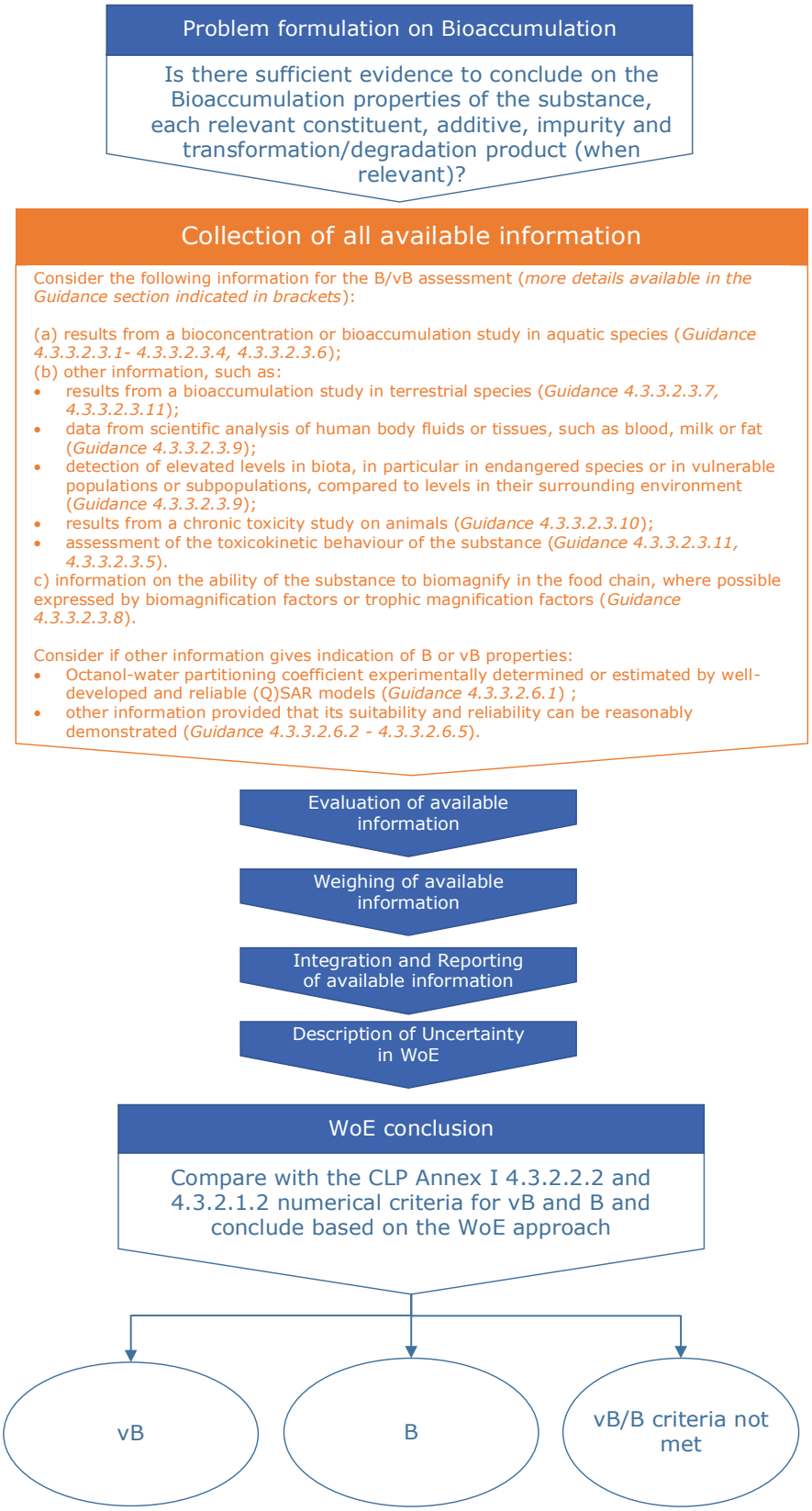

Figure 4.3.4 WoE approach for concluding on the bioaccumulation properties of the substance, each relevant constituent, additive, impurity and transformation/degradation product (when

relevant). Regarding the available information, the relevant sections of the Guidance are indicated in brackets.

When deciding if a substance fulfils the B or vB criteria, its bioaccumulation potential in the aquatic environment, the terrestrial environment, wildlife and humans is considered. The WoE approach should address whether there is sufficient evidence to support the conclusion that the substance, each relevant constituent, additive, impurity and transformation/degradation product (when relevant):

- fulfils the classification criteria in aquatic species
- bioaccumulates/biomagnifies in the aquatic environment, terrestrial environment, wildlife and humans.

All available data is to be considered as part of the WoE assessment. As such, a conclusion of B or vB based on either data for aquatic species (CLP Annex I, 4.3.2.3.2. (a)) or other available data (CLP Annex I, 4.3.2.3.2. (b)/(c)) is enough to consider that the substance meets the B or vB criteria. When the vB criterion is met, then also the B criterion is met. Similarly, a conclusion that the criteria are not met must also be based on all available data.

The results of reliable experimental *in vivo* bioaccumulation studies and field data are given more weight in the WoE assessment than the indicators of bioaccumulation based on physico-chemical properties and QSAR.

Existing experimental aquatic *in vivo* data which can be directly compared with the criteria (see also 4.3.3.2.3.1, 4.3.3.2.3.2, 4.3.3.2.3.3, and 4.3.3.2.3.4)

Each BCF study should be assessed in detail for its reliability and relevance considering the test substance, test design, exposure route, uptake and depuration periods, test species and age/life stage, test organism lipid content, test water (including pH, hardness and dissolved oxygen), test temperature, exposure concentration, analytical methods, need for growth correction and lipid normalisation and method of BCF calculation (steady-state or kinetic).

If there is a reliable aqueous bioaccumulation study available, such as an aqueous exposure fish OECD TG 305 study, or OECD TG 321 bioaccumulation study with *Hyalella azteca* or other aquatic invertebrate studies (e.g. mussels or oysters), the results can be directly compared against the respective bioaccumulation criterion of CLP to determine whether the B or vB criteria are met. The BCF should be growth corrected, if appropriate, then normalised to the appropriate lipid content for the organism (unless bioaccumulation is not driven by hydrophobicity).

The preferred endpoint from OECD TG 305 dietary exposure test is the BCF value estimated from the experimentally derived elimination rate constant, which can be directly compared to the numerical CLP criteria, unless it can be demonstrated that the uptake rate constant k_1 cannot be reliably estimated with the available methods. For very hydrophobic substances, k_1 estimates may become increasingly uncertain. In that case other methods (direct application of k_2, or using a correlation of dietary BMF and BCF results to interpolate other dietary BMF results) as described in OECD, 2017 should be used and the results assessed in a WoE approach.

Other *in vivo* data

Field data (see also 4.3.3.2.3.8)

Reliable information from field studies can be used to decide if the CLP B/vB criteria are fulfilled and should be given a high weight in a WoE approach where B/vB is indicated. A reliable field BMF >1 or field TMF >1 indicates that biomagnification of a substance occurs and can on its own be considered as a basis to conclude that a substance fulfils the B or vB criteria. However, absence of such a biomagnification potential cannot be used to conclude that these criteria are not fulfilled. This is because a field BMF only represents the degree of biomagnification in the specific

predator/prey relationship for which it was measured. A field TMF represents biomagnification in the specific food web studied. It is possible that biomagnification could still occur in another predator/prey relationship or food web.

Substances that partition into lipids should, as far as possible, be lipid normalised to account for differences in lipid content between prey and predator. It allows for a comparison of field BMF values in a direct and objective manner.

Field BAF values (based on reliable information) above 2000 or 5000 may indicate a B or vB concern, respectively, and should be considered as part of the WoE approach. For comparison of a fish field BAF with these thresholds, BAF values should be expressed on wet weight basis for whole body with a lipid content of 5%.

Detection of substances in wildlife and humans (see also 4.3.3.2.3.9)

The detection of a substance in wild biota (concentration or occurrence data) indicates that there had been exposure and that the substance can be taken up by organisms. Such information can be used to support the bioaccumulation assessment on a case by case basis. Together with the exposure level from the surrounding media and/or diet, concentrations in wild biota can be used as evidence for bioaccumulation. Furthermore, data from different time points as well as regions can give indications on temporal and spatial trends. Concentrations in biota increasing with age due to exposure and accumulation over a life-time, particularly in long-lived apex species (top predators), indicate an increased concern for bioaccumulation.

Information coming from scientific analysis of human body fluids or tissues, such as blood, milk, or fat can be used for the bioaccumulation assessment in a WoE approach.

Toxicokinetics data for mammals (see also 4.3.3.2.3.11)

If a whole-body, terminal elimination half-life is longer than 4 days in rat, and/or 50 days in humans, then this is an indication that the substance has vB properties. There may be exceptional cases where the derived elimination half-life threshold values in rats or humans cannot be used as an indicator of vB, for example where there is very low dietary absorption efficiency. Such cases require an individual assessment to determine whether the substance is vB or not.

If whole-body terminal elimination half-lives are between 2.5 and 4 days in rat, and/or 20 and 50 days in human, it is an indication that the substance has B properties.

In either case (B or vB), data indicating that the above thresholds are met should result in further consideration in a WoE assessment.

Other available data

Use of other available data is discussed in the respective sections of this Guidance:

- *In vitro* fish toxicokinetic tests (4.3.3.2.3.5)
- Bioaccumulation tests in sediment-dwelling species (4.3.3.2.3.6)
- Bioaccumulation tests in terrestrial (soil-dwelling) species (4.3.3.2.3.7)
- Field data – biomagnification in the food chain (4.3.3.2.3.8)
- Detection of substances in wildlife and humans (4.3.3.2.3.9)
- Chronic toxicity tests on animals (4.3.3.2.3.10)
- Octanol-water partitioning coefficient K_{OW} (4.3.3.2.6.1)
- Octanol-air partitioning coefficient K_{OA} (4.3.3.2.6.2)
- QSAR models to predict BCF (4.3.3.2.6.3)
- Biomimetic extraction procedures (4.3.3.2.6.4)

- Molecular size and octanol solubility (4.3.3.2.6.5)

Acceptable (Q)SAR predictions for Log K_{OW} and BCF can be used as part of the scientific assessment of the information relevant for the B, vB properties in the WoE determination.

A summary of the different indicative thresholds which can be used for assessing a range of parameters for bioaccumulation is provided in the Table below with a link to the respective section of this Guidance.

Table 4.3.2 Overview of indicative thresholds for WoE assessment

Parameter	Indicative threshold	Guidance section
Log K_{OW} (for aquatic organisms)	>4.5	4.3.3.2.6.1
Log K_{OA} *and* Log K_{OW} (for air breathing mammals)	>5 *and* >2	4.3.3.2.6.2
Field TMF	>1	4.3.3.2.3.8
Field BMF	>1	4.3.3.2.3.8
Field Fish BAF	>2000/5000	4.3.3.2.3.8
Human whole body terminal elimination half-life/days	20/50 days	4.3.3.2.3.11
Rat whole body terminal elimination half-life/days	2.5/4 days	4.3.3.2.3.11

The Bioaccumulation Assessment Tool (BAT), accompanied by guiding principles in the BAT manual (Armitage *et al*., 2021), is a tool that promotes standardised recording and evaluation of various lines of evidence related to the endpoint bioaccumulation. If this tool is used for recording the available information, assessment should follow advice provided in the relevant sections of this guidance and the ECHA Guidance on IR&CSA, Chapters R.11 and R.7c.

When integrating and weighing information between lines of evidence (*i.e.* same type of test or directed to the same endpoint), relevant and reliable evidence of bioaccumulation cannot be outweighed by information showing no bioaccumulation.

Multiple experimental study results for bioaccumulation

When more than one relevant and reliable experimental study result (value) is available, in most cases the most conservative value is used in order to achieve the regulatory protection goals and to account for any uncertainties of the test method and differing experimental conditions.

Combining experimental study results

BCF test results on different species, life stages or test conditions should not be combined for PBT/vPvB classification purposes. Different species can accumulate substances to a different degree because of differing physiological behaviour such as metabolic activity and ventilation rate (Wassenaar *et al.*, 2020). For BCF studies in fish, the size and age can affect the bioaccumulation potential. Test conditions such as TOC, temperature, pH, feeding rate and test concentration can also influence the measured BCF (Arnot and Gobas, 2006).

However, there may be exceptional situations where it is appropriate to combine a sufficient number of study results to generate a statistically robust representative value for the same species and life stage for comparison with the CLP criteria. For example, for BCF values derived from the same species and life stage, a suitable mean of the reliable BCF values may be used as the representative BCF value for that species, if the test conditions of the different studies are similar (for example regarding test concentration, pH, temperature, TOC, study design, feeding rate, etc.). The statistical distribution of the BCF data should be considered when selecting the approach. Some guidance on generating representative values for BCF is given in Annex III.4.1. There may be circumstances where a different approach is justified. The reason for any apparent BCF outliers should be assessed and it may be appropriate to remove them from the data set, for example if there was an experimental error.

Fish BCF studies may be performed at two or more exposure concentrations, and thus one BCF study could give several experimental results (BCF values), one for each tested concentration. It is not recommended to obtain a representative BCF value by averaging BCF values for different exposure concentrations obtained from a single study. This is because the BCF may vary with test concentration, for example due to saturation of metabolic mechanisms (See OECD, 2012 paragraphs 79, 80 and Annex 2 of OECD, 2017).

In all cases, the approach used to generate a representative value should be well justified and documented. This should include a discussion of any outliers. In particular, the representativeness of the test conditions should be carefully assessed for each value. Particular scrutiny should be given if test results are close to the B or vB threshold.

4.3.4.3. Toxicity

The T assessment shall reach one of the following conclusions described in the scheme (Figure 4.3.5) for each relevant constituent, additive, impurity and transformation/degradation product: T, or criteria not met. The latter conclusion is based on conclusive data, inconclusive data or lack of data.

Section 4.3.3.4 of the current Guidance document describes the relevant experimental and computational information that may be provided as part of the WoE determination on Toxicity. The decision scheme needs to be followed on the available information, in order to come to a robust conclusion on whether the CLP criteria for Toxicity are fulfilled (Figure 4.3.5).

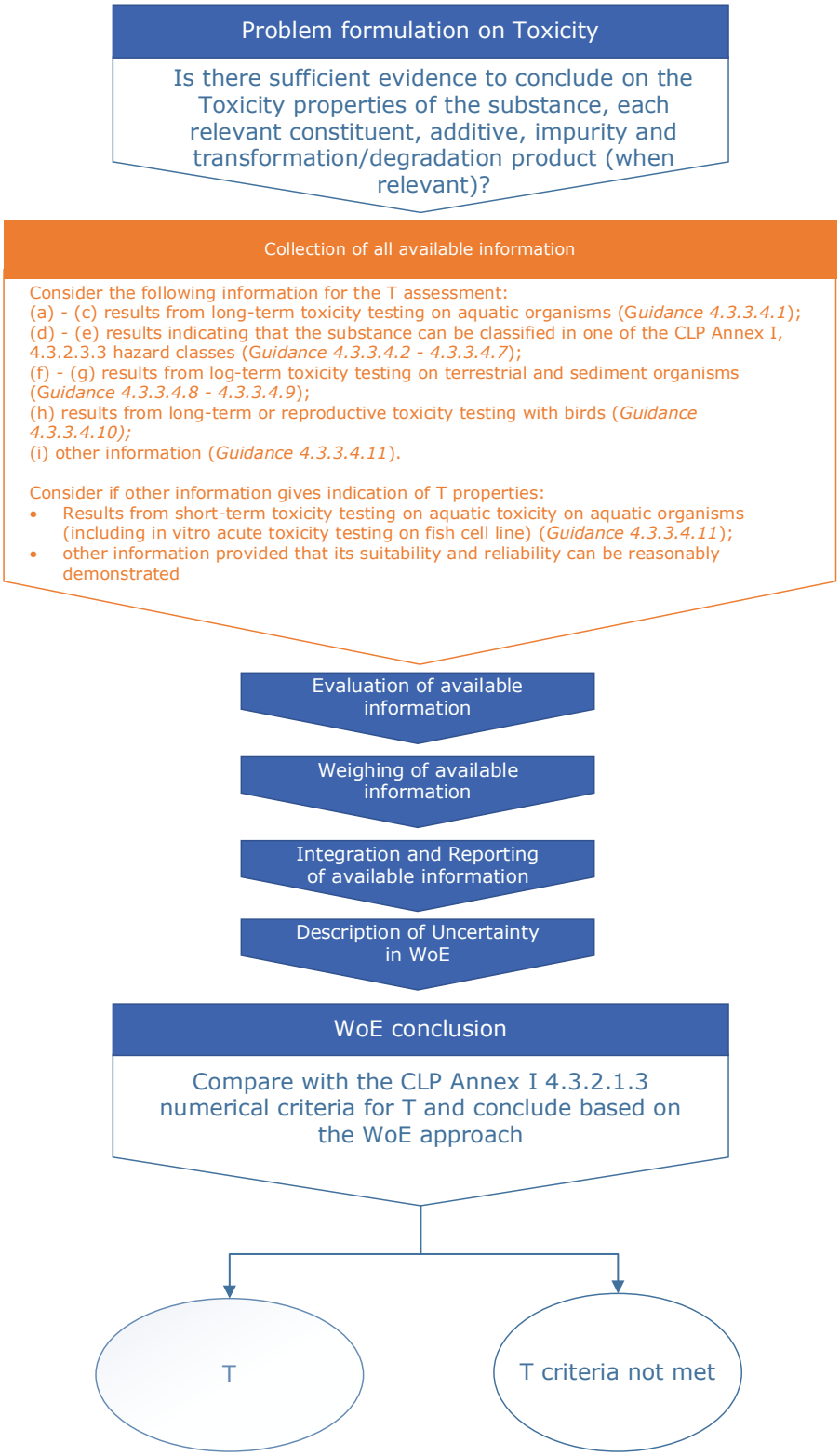

Figure 4.3.5 WoE approach for concluding on the toxicity properties of the substance, each relevant constituent, additive, impurity and transformation/degradation product (when relevant). Regarding the available information, the relevant sections of the Guidance are indicated in brackets.

As discussed in the introduction of section 4.3.4, results from studies that can directly be compared to the CLP criteria (CLP Annex I, 4.3.2.1.3 and 4.4.2.1.3 (a)-(d) and sections 4.3.3.4.1 - 4.3.3.4.7 of this Guidance) are to be given higher weight in the WoE assessment (Figure 4.3.5). As always, the studies must be reliable and conducted in relevant substance and testing conditions.

Concerning the results from long-term toxicity testing on terrestrial organisms and sediment, a case-by-case assessment of the study results including expert judgement should be performed. As discussed in sections 4.3.3.4.8 and 4.3.3.4.9, the equilibrium partitioning method (EPM) may be used to back-calculate a NOEC or EC_{10} value of an existing sediment or soil toxicity test to a corresponding aquatic NOEC or EC_{10}. This approach has uncertainties and the use of any such information needs to be treated with caution, depending on the presence of other information types. In cases where the available environmental database is limited exclusively to studies on terrestrial organisms and/or sediment, it is highly recommended that any proposal for harmonised classification is only submitted once information generation via different (REACH, PPPR, BPR or other) legislative contexts has been completed and/or if other, more conclusive, information relevant for classification becomes available. This is because direct generation of information cannot be triggered under CLP. It is worth noting that in case of future scientific and regulatory agreement on the introduction of additional numerical criteria for terrestrial organisms or sediments within UN GHS, this will need to be reflected in an updated CLP and Guidance.

Concerning the data for birds (section 4.3.3.4.10), they also cannot be directly, numerically compared with the T criteria in the absence of an agreed regulatory threshold value, but can be used in conjunction with other evidence of toxicity as part of a WoE determination. For PBT/vPvB assessment purposes under REACH, a NOEC value of below 30 mg/kg food in a long-term bird study was considered as a strong indicator for a substance possessing (T) properties (ECHA Guidance on IR&CSA, Chapter R.11.4.1.3.2).

Concerning the use of short-term aquatic toxicity study results, if such data show that the substance is very toxic (L/E)C_{50} < 0.01 mg/L, ECHA Guidance on IR&CSA, Chapter R.11.2.2), a conclusion may be drawn that the substance fulfils the Toxicity criteria, combined with all other available information. It is hereby noted that for certain lipophilic substances, acute toxicity may not occur at the limit of the water solubility of the substance (or the highest concentration) tested, but chronic toxicity may still be exhibited.

Other available convincing information that may be used is QSARs, read-across/ grouping approaches, data from mammalian studies and any other data with a suitability and reliability that can reasonably be demonstrated. Some (Q)SAR models predicting chronic and acute aquatic toxicity are currently available and further research on the (Q)SAR prediction of chronic toxicity may increase their predictive capacities. Therefore, at the current state of the art, (Q)SAR models generally seem not to be applicable for an unequivocal assessment of the T criterion (ECHA Guidance on IR&CSA, Chapter R.11.4.1.3.3). However, they may be used when they are applicable, in line with REACH Annex XI. Key considerations on important substance physical-chemical and environmental fate properties and any targeted modes of action introducing higher sensitivity to some species over others also need to be addressed, for example, for ionisable substances, as reported in several sections of the current Guidance.

In line with this Guidance (section 4.1.3.2.4.3), where more than one acceptable toxicity study result (value) is available for the same species, in most cases the most conservative (the one with the lowest L/EC_{50} or NOEC/EC_{10} value) may be used as the representative toxicity value for -those species. Effect concentrations for different species should not be aggregated but considered in a WoE approach. All the general principles on how to combine study results from similar study types must be followed, as reported in the introductory paragraphs of section 4.3.4 of the Guidance. As one example only, the current CLP Guidance already provides advice on the combination of aquatic toxicity study results in the context of aquatic hazards classification (section 4.1.3.2.4.3). The similarity of parameters such as species, life stage, pH, test

temperature, dissolved oxygen concentration, TOC, test design, duration, etc. must be considered before any such combination is to take place.

In case of very large data sets meeting the criteria for applying the Species Sensitivity Distribution (SSD) approach (see ECHA Guidance on IR&CSA, Chapter R.10) or other statistical and data combination techniques (e.g. HC_5 derivation, use of 10^{th} or 90^{th} percentiles, etc.) can be considered in order to estimate the aquatic toxicity reference value for classification (equivalent to using the lowest EC_{50} or NOEC), within the WoE.

In all cases, the approach should be well-justified and documented and should be supported by the WoE analysis, including a discussion of outlier results. In particular, the representativeness of the test conditions should be carefully assessed for each test result. Particular scrutiny should be given to results from tests close to the T threshold value.

4.3.4.4. Overall determination of PBT/vPvB classification

CLP Annex I, 4.3.2.4.1 states that "*the available results regardless of their individual conclusions shall be assembled together in a single WoE determination*". Therefore, on top of the conclusions drawn for the individual properties (P, B, vP, vB, T) that are also based on a WoE approach, the results must be assembled together in a single WoE determination. The assessment should also exhibit whether the relevant constituents, impurities, additives and transformation/degradation products possess PBT or vPvB properties or not (see bullet points (iv) and (v) in section 4.3.3). Such a conclusion may be based on relevant data for the main constituent of a mono-constituent substance, relevant data for a constituent (or group of constituents as in 4.3.3 (iv)) and/or relevant data for one or more relevant impurity, additive or transformation or degradation product of the substance fulfilling the PBT/vPvB criteria. In all cases, the main elements that need to be included within the WoE as analysed in the previous section 4.3.4, also apply for this concluding "single WoE determination".

Similarly, a conclusion that a substance and its relevant constituents, impurities, additives and transformation/degradation products does not meet all PBT/vPvB criteria is also based on the overall WoE. If any of the criteria P, B or T are not fulfilled, the substance is not classified as PBT. If any of the criteria vP or vB are not met, the substace is not classified as vPvB. A conclusion that a substance does not fulfil all PBT/vPvB criteria must be followed by a statement clarifying the reasons for this conclusion.

ECHA Guidance on IR&CSA, Chapter R.11.4.1.4 presents further details on the different conclusion types for PBT/vPvB assessment and the use of constituent data. The following Figure illustrates the decision scheme for concluding on the PBT/vPvB classification, once the assessment and conclusion for the individual properties has been finalised.

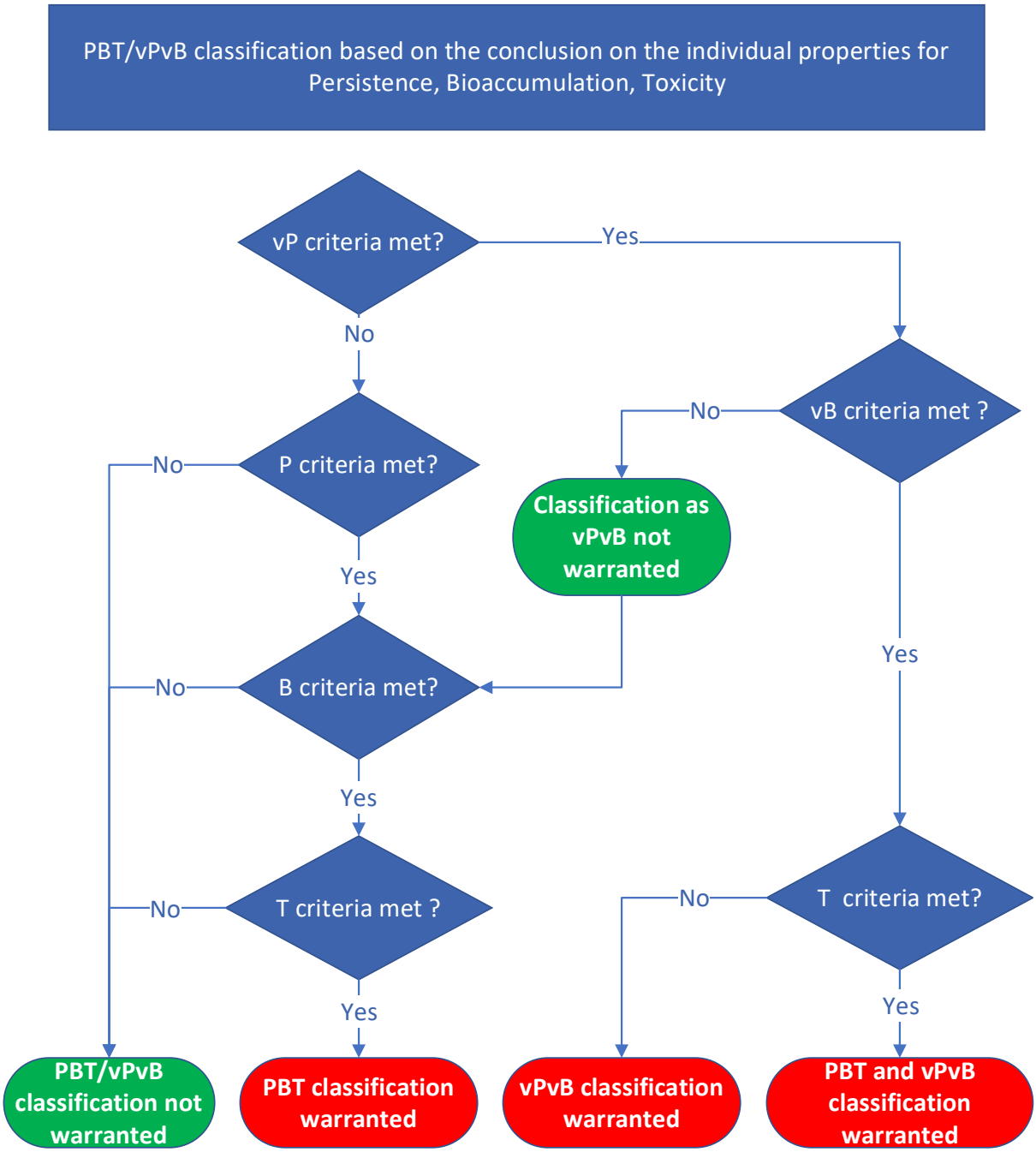

Figure 4.3.6 Decision scheme for concluding on PBT/vPvB classification

4.3.5. Application of the WoE to conclude on PMT/vPvM properties for classification and labelling

Commission Delegated Regulation (EU) 2023/707, Annex I: 4.4.2.3. Basis of classification

For the classification of PMT substances and vPvM substances, a WoE determination using expert judgment shall be applied, by comparing all relevant and available information listed in section 4.4.2.3 with the criteria set out in sections 4.4.2.1 and 4.4.2.2. That WoE shall be applied in particular where the criteria set out in sections 4.4.2.1 and 4.4.2.2 cannot be applied directly to the available information.

The information used for the purposes of assessment of the PMT/vPvM properties shall be based on data obtained under relevant conditions.

The identification shall also take account of the PMT/vPvM properties of relevant constituents, additives or impurities of a substance and relevant transformation or degradation products.

This hazard class (PMT and vPvM properties) shall apply to all organic substances, including organo-metals.

The exact same considerations detailed in the introduction of section 4.3.4 need also to be followed for the application of the WoE to conclude on PMT/vPvM properties. Very briefly, these refer, among others, to the need for separate conclusions for each property, the relevance and availability of the information, the fact that the criteria for P/vP, M/vM and T do not have to be met for the same environmental compartment, the higher weight placed on experimental studies that can directly be compared to the CLP criteria and the use of non-standard methods. Furthermore, in order for the PMT or vPvM criteria to be fulfilled, all respective criteria must be met for the same substance or at least one (but always the same one) individual constituent, impurity, additive or transformation/degradation product, if applicable.

As for PBTs/vPvBs, the conclusion of the application of the WoE on the individual PMT/vPvM properties can be that the substance fulfils the P/vP/M/vM/T criteria or not. The general principles of identification and assessment of hazard information for PMT/vPvM have already been presented in section 4.3.3.

For **Persistence and Toxicity,** see previous sections 4.3.4.1 and 4.3.4.3.

4.3.5.1. Mobility

The M/vM assessment shall reach one of the following conclusions described in the scheme (Figure 4.3.7) for each relevant constituent, additive, impurity and transformation/degradation product: M, vM or criteria not met. The latter conclusion is based on conclusive data, inconclusive data or lack of data. When the vM criterion is met, then also the M criterion is met.

Section 4.3.3.3 of this Guidance described the experimental and non-experimental methods that may be provided as part of the WoE determination on mobility. Briefly, test results according to OECD TG 106, sludge tests (ISO 18749 and OPPTS 835.1110), TG 121, TG 312, TLC studies and reliable QSAR methods have been described and important considerations and limitations on their use accounted for. Section 4.3.3.3.6 further presented key considerations for information provided for ionisables including recommendations on testing for K_{OC} derivation.

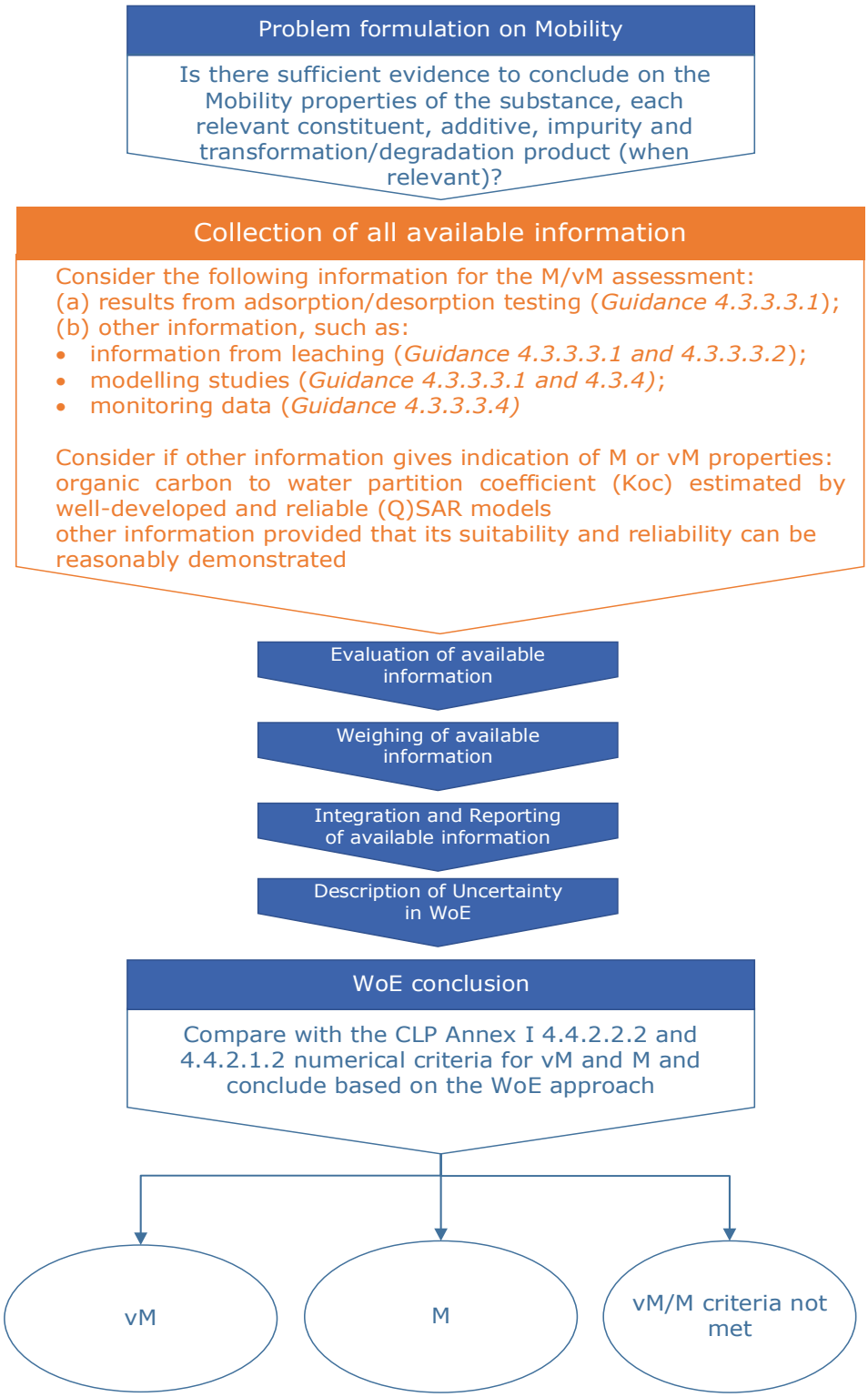

Figure 4.3.7 WoE approach for concluding on the mobility properties of the substance, each relevant constituent, additive, impurity and transformation/degradation product (when relevant). Regarding the available information, the relevant sections of the Guidance are indicated in brackets

Normally results from reliable experimental methods directly deriving a K_{OC} value are given higher weight in the WoE than for other lower weight information. From such methods, preference is placed into the one conducted according to OECD TG 106, as this is a standardised test method which provides information on the intrinsic property of the substance to partition in soils. Furthermore, it is applicable to both non-ionisable and ionisable substances and it includes testing on a wide range of different natural soils with varying soil types to cover the interactions of a given substance with naturally occurring soils.

Activated sludge studies (OPPTS 835.1110 and ISO 18749) are applicable for both non-ionisable and ionisable substances and may be compared to the CLP criteria. More specifically, for substances that can sorb to the soil only via hydrophobic interactions (non-ionisable substances), the derived log K_{OC} can be used for directly comparing with the CLP criteria within the WoE. For substances for which the sorption might also involve processes other than hydrophobic interactions (e.g. ionisable substances), activated sludge studies might be less relevant and, therefore, might be assigned a lower weight within the WoE (see also section 4.3.3.3.1, Studies on activated sewage sludge).

Test results from studies performed according to OECD TG 121, OECD TG 312 and soil thin and thick layer chromatography (TLC) can also provide relevant information, following the considerations of section 4.3.3.3.1 of the Guidance. Regulation (EU) No 283/2013 setting out the data requirements for active substance in pesticides pointed out that, where the batch equilibrium method cannot be applied due to fast degradation, methods such as studies with short equilibration times like the HPLC method shall be considered as an alternative (see point 7.1.3.1) referring on the use of OECD TG 121 in the related Commission Communication (2013/C 95/01). The same document (see point 7.1.4.1) refers also to the potential use of OECD TG 312 in conditions where the batch equilibrium method cannot be applied due to weak adsorption.

(Q)SARs and other estimation methods (Karickhoff equation) deriving a K_{OC} may also provide relevant information particularly for non-ionisable substances, as the sorption of such substances is dominated by the sorption to organic carbon. This is particularly the case for a substance with close structural analogues in the model's training set. However, such approaches are currently not adequately developed and validated for ionisable substances, and therefore their use for those substances is not recommended. It is expected that (Q)SAR approaches are more broadly used in the future, supported by the projected higher degree of generation of experimental data (for example, by use of OECD TG 106). For more details see also section 4.3.3.3.3.

Field and lysimeter studies, as well as results from aged sorption studies and modelling can be considered as part of the WoE assessment. However, the relevance of such approaches within a hazard assessment context should be carefully considered due to the uncertainties identified in sections 4.3.3.3.2 and 4.3.3.3.5. Such approaches have been used for the risk assessment of PPP substances and may also provide useful information on the mobility potential of a substance under specific test conditions. Additionally, results from such studies may be used for regulatory purposes in order to qualitatively identify additional transformation products which may be relevant for classification purpose (e.g. PMT/vPvM).

Information from monitoring studies and other approaches not leading to a numerical K_{OC} value may be considered, together with all other available information. Data from environmental monitoring must be treated with caution, as the absence of a chemical in a given aquatic medium may merely reflect site-specific, shortcomings in analytical methods, soil properties, environmental fate and/or exposure considerations rather than an intrinsic tendency of the chemical not to partition to water. Generally, less weight should be given to monitoring data close to point sources. When monitoring data, used as part of the WoE, show that a substance is present in groundwater or/and other relevant environmental compartments, it may be possible to conclude a substance as M or vM, within the considerations above and in section 4.3.3.3.4. Examples of supporting lines of evidence based on monitoring data that may be used as part of the WoE to fulfil the M/vM criteria are data on the long-range aquatic transport, the chemical presence in effluent water from sewage treatment plants (STPs) and/or the presence in drinking

water. More details on monitoring site characterisation and uncertainties related to false positives and negatives when conducting groundwater monitoring studies pursuant to the PPP regulation are given in Gimsing *et al*. (2019) and EFSA (2023b).

Combining multiple studies for mobility assessment

As already discussed in section 4.3.4, when more than one relevant and reliable experimental study result (value) is available for mobility, in most cases the most conservative of the relevant and reliable value is used, in order to achieve the regulatory protection goals of better protection of human health and the environment. Combining several relevant and reliable study results from the same test types generated under similar conditions must be justified to generate a statistically robust representative value (e.g. suitable mean) for comparison with the CLP criteria. Approaches for combining study results must be considered in a case-by-case basis by exercise of expert judgement but should generally follow the principles described in the following paragraphs.

Reliable experimental study results from the same study type may be combined. Study results from different study types (for example, one OECD TG 312 study, a field study, some (Q)SAR predictions and evidence from monitoring data) cannot be combined but will be considered within the WoE, as independent lines of evidence (LoE). The comparability/similarity of the study results to be combined should be scientifically justified by considering whether the test conditions of the study (e.g. temperature, pH, soil texture, soil organic matter content, soil organic carbon content, cation/anion exchange capacity and sludge composition, etc.) significantly influence the adsorption potential of the substance. Only results from studies in which the test conditions are not significantly influencing the sorption of the substance may be combined.

For substances for which sorption processes only involve hydrophobic interactions (e.g. non-ionisable substances) K_{OC} values generated under similar conditions could be combined. A sufficient number of study results is required in order to increase the statistical robustness of the combined value, and this number may vary depending on the availability and reliability of the relevant information. The use of arithmetic mean for the different derived Log K_{OC} values (or geometric mean for K_{OC}) is recommended, based on the expected distribution of such data. Regarding Tier 3 test results, K_{OC}* values must first be derived by use of the method detailed in section 4.3.3.3.1 of this Guidance (one value per test concentration). Tier 3 K_{OC}* values for the same soil may then be combined for the different aqueous concentrations. K_{OC} and K_{OC}* values cannot be considered (combined) together, as they originate from a different set of data (Tier 2 and Tier 3 data sets). Various other approaches may be used, if adequately justified, for aggregating the data and for investigating further the influence of various factors on sorption of the substance.

For substances for which the sorption might also involve processes other than hydrophobic interactions, data combination is generally not recommended. For example, for ionisable substances, the sorption potential of the substance is influenced by the test conditions (for example, pH and soil texture). In such cases, the lowest Log K_{OC} value for pHs between 4 and 9 should be compared with the CLP criteria (CLP Annex I, 4.4.2.1.2 and 4.4.2.2.2).

In all cases, the approach should be well justified and documented and should include a discussion of any outliers. In particular, the relevance of the test conditions should be carefully assessed for each value, with a particular scrutiny given to results from tests that are close to the M or vM threshold.

4.3.5.2. Overall determination of PMT/vPvM classification.

Similar considerations as the ones described at the end of section 4.3.4 also apply for concluding on the PMT/vPvM hazard class, where the concept of "the available results regardless of their individual conclusions shall be assembled together in a single WoE determination" (CLP Annex I,

4.4.2.4.1) also applies. Figure 4.3.8 illustrates the decision scheme for concluding on the PMT/vPvM classification.

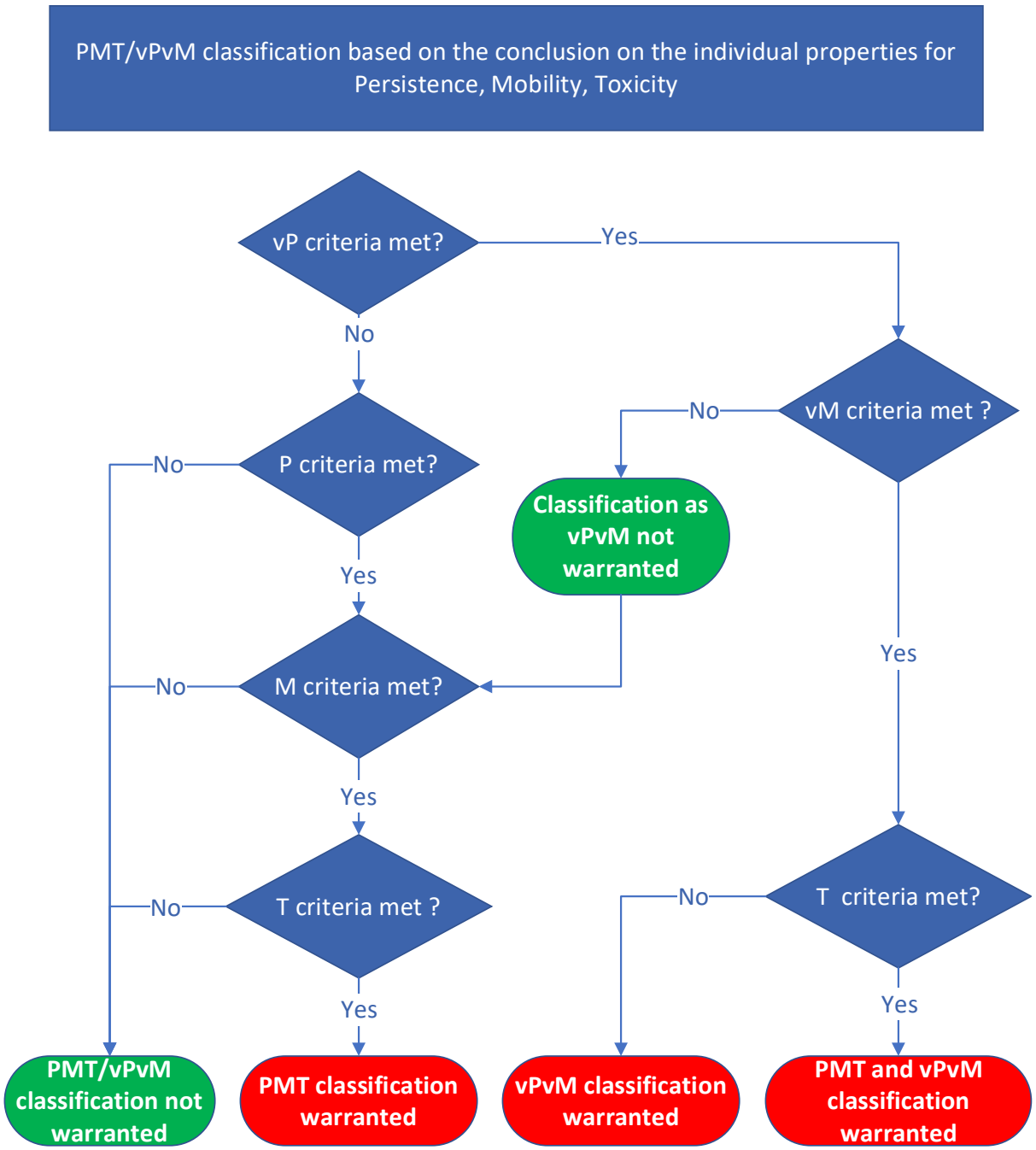

Figure 4.3.8 Decision scheme for concluding on PMT/vPvM classification

4.3.6. Classification criteria for PBT/vPvB and PMT/vPvM mixtures

Annex I: 4.3.3.1 and 4.4.3.1.

A mixture shall be classified respectively as a PBT or vPvB when at least one component contained in the mixture has been classified respectively as a PBT or vPvB and is present at or above 0,1 % (weight/weight).

A mixture shall be classified as a PMT or vPvM where at least one of its components has been classified as a PMT or vPvM and is present at or above 0,1 % (weight/weight).

Classification of mixtures shall be based on available information for the substances in the mixture and not for the mixture itself. This relates to the persistence, mobility and bioaccumulation properties referred to sections 4.3.2.3.1, 4.3.2.3.2, 4.4.2.3.1 and 4.4.2.3.2 of CLP Annex I, where the relevant available information for each of the known constituents in the substance shall be assessed[129]. Thus, when at least one of these components is present in the mixture in a concentration equal to or exceeding the generic concentration limit of ≥ 0.1% (w/w), the mixture shall be classified as PBT/vPvB or PMT/vPvM.

However, in certain cases, data on the mixture itself may also be relevant. This is the case in particular where that data demonstrates persistent, bioaccumulative and mobile properties, or where it supports data on the individual constituents. Therefore, it is appropriate that data on the whole mixture is used in those cases.

[129] https://data.consilium.europa.eu/doc/document/ST-16721-2023-REV-1/en/pdf. The current text of the provisional agreement from the tripartite negotiations may still change.

4.3.7. Hazard communication for PBT/vPvB and PMT/vPvM substances

4.3.7.1. Pictograms, signal words, hazard statements and precautionary statements

Annex I: 4.3.4. Label elements shall be used in accordance with Table 4.3.1 for substances or mixtures meeting the criteria for classification in this hazard class (PBT and vPvB properties).

Table 4.3.1.
Label elements for PBT and vPvB properties

	PBT	vPvB
Symbol/pictogram		
Signal word	Danger	Danger
Hazard Statement	EUH440: Accumulates in the environment and living organisms including in humans	EUH441: Strongly accumulates in the environment and living organisms including in humans
Precautionary Statement Prevention	P201 P202 P273	P201 P202 P273
Precautionary Statement Response	P391	P391
Precautionary Statement Disposal	P501	P501

Annex I: 4.4.4. Label elements shall be used in accordance with Table 4.4.1 for substances or mixtures meeting the criteria for classification in this hazard class (PMT and vPvM properties)

Table 4.4.1.
Label elements for PMT and vPvM properties

	PMT	vPvM
Symbol/pictogram		
Signal word	Danger	Danger
Hazard Statement	EUH450: Can cause long-lasting and diffuse contamination of water resources	EUH451: Can cause very long-lasting and diffuse contamination of water resources
Precautionary Statement Prevention	P201 P202 P273	P201 P202 P273
Precautionary Statement Response	P391	P391
Precautionary Statement Disposal	P501	P501

A pictogram is currently not applicable for these two new hazard classes, as explained in recital (17) of Regulation 2023/707, and may be introduced at a later stage if adopted in the context of the UN GHS. When included in GHS but not yet implemented in CLP, it is strongly recommended to be used. The wording of the Precautionary Statements is found in CLP, Annex IV, Part 2.

Further explanations on the precautionary statements can be found in Annex IV of CLP.

4.3.7.2. Additional labelling provisions

There are no additional labelling provisions for substances and mixtures classified as PBT/vPvB and PMT/vPvM in CLP.

4.3.8. Examples PBT/vPvB and PMT/vPvM substances

The following section includes selected examples of substances that may or may not be classified as ones with PBT/vPvB and/or PMT/vPvM properties. As, at the time of publication of this Guidance, there is not any experience gained on dealing with such hazard classes under CLP, these examples broadly refer to substance properties similar to the ones of substances that have already been identified as SVHCs (PBT/vPvB/ELoC) under REACH. The Guidance will be updated with more elaborative examples, also for PMT/vPvM substances, once more experience is gained.

In the meantime, very important reference material can be found in the following link that refers to the Candidate List of substances of very high concern for Authorisation[130], part of which comprises substances identified as PBTs and/or vPvBs under REACH (namely, meeting the REACH Article 57(d) and (e) criteria). Finally, it is noted that one additional example substance refers to the only (thus far) non-approval decision taken by the European Commission for a pesticidal active substance, due to its PBT and vPvB properties. This example and the full targeted hazard assessment conducted by EFSA will not be reproduced in the current document, as the conclusion document on the pesticide peer review is already publicly available[131].

It should be noted that the decision on classification is influenced by the strength of the overall evidence and should be decided on a case-by-case basis. If the evaluation shows that the criteria are fulfilled, a classification as PBT/vPvB and/or PMT/vPvM should be assigned. For the labelling elements, see section 4.3.7 of this Guidance.

List of examples included in this section:

- 4.3.8.1. Example A: Substance meeting the CLP classification criteria for PBT and vPvB, based on the overall WoE;
- 4.3.8.2. Example B: Substance meeting the CLP classification criteria for vPvB, based on constituent data and on the overall WoE;
- 4.3.8.3. Example C: Substance meeting the CLP classification criteria for PMT and vPvM, based on the overall WoE.

For each example substance, a table of all relevant data elements is presented, followed by relevant elements regarding the PBT/PMT hazard assessment, a section showing the PBT/PMT classification, a section with the reasoning behind the conclusions, and finally a table presenting the applicable labelling elements. This structure is identical to the one followed for aquatic classification (section 4.1.3.4 of this CLP Guidance) and is not indicative of the order of such information that may be presented in a potential proposal for harmonised classification and labelling.

[130] https://echa.europa.eu/candidate-list-table

[131] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5085

4.3.8.1. Example A: Substance meeting the new CLP classification criteria for PBT and vPvB, based on the overall WoE

DATA ELEMENTS	Value	Test method / remarks
Physico-chemical properties and environmental fate		
Vapour pressure	2.0 10^{-5} Pa	OECD TG 104, Klimisch (1)
Water solubility	0.25 mg/L	WATERNTv1.01; WSKOW v.1.41; (Q)SAR prediction with low uncertainty, Reliability (1)
log octanol/water partition coefficient (log K_{OW})	6.3 (at 23°C)	(Q)SAR prediction with low uncertainty, Reliability (1)
log organic carbon/water partition coefficient (log K_{OC})	4.7	KOCWIN v 2.00 (EPI Suite 4.11)) – QSAR prediction with medium uncertainty, Reliability (2)
Degradation		
Ready biodegradability	2% in 28d	OECD TG 301C, Klimisch (1)
Simulation studies in water-sediment	$DT_{50,wat}$: 4-12d $DegT_{50,sed}$: 30-250d $DegT_{50,whole}$: > 180d	OECD TG 308 (for analogue substance in pond and river systems). Test conducted at 12°C. Klimisch (1)
Hydrolysis	$T_{1/2}$ = 350d	OECD TG 111, Klimisch (2)
Field degradation in soil	DT_{50}: 70-190d	Field study, several analogues
Monitoring studies	Presence in soils	For both substance A and analogues
QSARs	Slow degradation	BIOWIN 2, 3 and 6 predictions, (Q)SAR predictions with low uncertainty, Reliability (2)
Bioaccumulation		
Bioconcentration in fish (BCF), normalised to 5% lipid [no growth correction reported]	6 000-12 000	OECD TG 305, Klimisch (2)
Aquatic Toxicity		
Crustacea *Daphnia magna*:	3 mg/L (48h EC_{50})	OECD TG 202, Klimisch (1)

Algae/aquatic plants *Desmodesmus subspicatus*:	0.75 mg/L (72h E_rC_{50})	OECD TG 201, Klimisch (1)
Crustacea *Daphnia magna*	0.45 mg/L (21d NOEC)	OECD TG 211, Klimisch (1)
Other Toxicity		
STOT RE2 criteria met		

Hazard assessment elements:

Physico-chemical properties:

- Substance A is poorly water soluble and strongly sorbing to solid matrices (log K_{OW} = 6.3, log K_{OC} = 4.7). No information on dissociation.

Degradation:

- Hydrolysis data indicate long abiotic degradation half-lives;
- During a reliable ready biodegradation study, substance A was shown to be non-readily biodegradable (2% degradation after 28d);
- No simulation study is available for the substance. Water-sediment and soil field studies are available for analogue substances showing very slow degradation in solid matrices. The whole system degradation half-life was above 180 d. Faster dissipation was exhibited for the water-phase in the water-sediment simulation test according to OECD TG 308;
- Several monitoring studies are available to indicate the presence of substance A and other structurally similar substances in sediments, many years after cessation of environmental releases.

Bioaccumulation:

- One reliable bioconcentration study on fish is available that derived high BCF values, indicating high potential for bioaccumulation. This is supported by a log K_{OW} value of 6.3.

Toxicity:

- Substance A meets the criteria for classification as STOT RE 2 as defined in the CLP Regulation. Available aquatic toxicity data indicate toxicity values below 1 mg/L for both acute and chronic toxicity.

Mobility:

- One QSAR prediction deriving a log K_{OC} value greater than 4. There is some evidence from the water solubility and other experimental fate information that Substance A may have a preferential partition to solid phases.

Classification (pursuant to CLP Annex I, 4.3 and 4.4):

Persistent, Bioaccumulative and Toxic (PBT) properties: CLP Annex I, 4.3 **criteria met**

Very Persistent, Very Bioaccumulative (vPvB) properties: CLP Annex I, 4.3 **criteria met**

Persistent, Mobile and Toxic (PMT) properties: CLP Annex I, 4.4 **criteria not met**

Very Persistent, Very Mobile (vPvM) properties: CLP Annex I, 4.4 **criteria not met**

Reasoning:

Persistence (the lines of evidence are sorted based on their respective weight from high to low weight):

- a water-sediment simulation study on an analogue substance. The read-across approach has been properly documented and the argumentation for its use (mainly very high structural similarity) is acceptable. The analogue substance was shown to dissipate fast from the water phase to the sediment, where the degradation half-lives in both systems were above 180d, which exceed the regulatory threshold value for being very persistent in sediment (high weight);
- a soil field dissipation study on a very closely structurally similar substance, with dissipation half-lives as high as 190 days. Again, the read-across was comprehensively assessed and was deemed acceptable (high weight);
- additional information from several monitoring studies for substance A and other structurally similar substances indicating long-term presence in sediments (medium weight);
- acceptable (Q)SAR predictions appropriate for the structure of substance A indicating slow environmental degradation (medium weight).
- hydrolysis data indicating slow abiotic degradation rates (medium weight);
- a ready biodegradation study that suggests that substance A is not subject to biodegradation (2% after 28 days) (low weight);

Thus, it can be concluded that substance A fulfils the CLP Annex I, 4.3.2.1.1 and 4.4.2.1.1 (and also REACH Annex XIII 1.1.1.) **P**- and **vP**- criteria.

- Bioaccumulation:

In a BCF study on fish according to OECD TG 305, lipid-normalized BCF values of 6 000 – 12 000 were found (high weight). As the study was protocol-compliant and was deemed scientifically reliable, it can be concluded that substance A fulfils the CLP Annex I, 4.3.2.1.2 and 4.3.2.2.2 (and also REACH Annex XIII 1.1.2.) **B**- and **vB**- criteria.

- Toxicity:

Substance A fulfils the criteria for classification as STOT RE 2 as defined in CLP Regulation Annex I, 3.9. Therefore, it can be concluded that substance A fulfils the CLP Annex I, 4.3.2.1.3 and 4.4.2.1.3 (c) (and also REACH Annex XIII 1.1.3.) **T** criteria.

- Mobility:

In the absence of a log K_{OC} value below the regulatory threshold values of 2 and 3, it can be concluded that Substance A does not fulfil the CLP Annex I, 4.4.2.1.2. and 4.4.2.2.2 **M** and **vM** criteria.

Label elements based on the classification:

Element	Resulting Labelling Elements (code)
GHS Pictogram	-
Signal Word	Dgr
Hazard Statement	EUH441[132]
Precautionary statement(s)	P201, P202, P273, P391, P501

[132] In line with Annex III to Regulation (EC) No 1272/2008 Part 1 point (c) "if the hazard statement EUH441 "Strongly accumulates in the environment and living organisms including in humans" is assigned, the statement EUH440 "Accumulates in the environment and living organisms including in humans" may be omitted

4.3.8.2. Example B: Substance meeting the new CLP classification criteria for vPvB, based on constituent data and on the overall WoE

Substance B is a UVCB and its constituent X is present in the substance at ≥ 0.1 % w/w.

Data for Constituent X:

DATA ELEMENTS: Constituent X	Value	Test method / remarks
Physico-chemical properties and environmental fate		
Vapour pressure	-	-
Water solubility	0.06; 0.58; 1.24 mg/L	WATERNTv1.01; WSKOW v.1.41 (EPI Suite v4.11); QSAR prediction with medium uncertainty, Reliability (2) experimental value in Episuite
log octanol/water partition coefficient (log K_{OW})	5.5	KOWWIN v1.68; QSAR prediction with low uncertainty, Reliability (1)
log organic carbon/water partition coefficient (log K_{OC}) pKa	5.3; 4.8 not ionisable	KOCWIN v2.00 (EPI Suite v4.11) MCI method; *K*ow method - QSAR prediction with low uncertainty, Reliability (1) based on chemical structure
Degradation		
Hydrolysis	not expected	based on chemical structure
Phototransformation in air	$DegT_{50}$ 14 hours	AOP v1.92
Phototransformation in water	no significant decrease in concentration after 29 days	Klimisch (4), Only brief study summary available
Simulation studies in water; OECD TG 309 (study performed at 12°C)	$DegT_{50}$ >60 days	Klimisch (2)
Simulation study in seawater	Primary $DegT_{50}$ >182 days at 20 °C	Klimisch (4), raw data not available, used as supporting information
BIOWIN 2 & 3 predictions	slow degradation	(Q)SAR result with medium uncertainty, Reliability (2)

BIOWIN 3 & 6 predictions	slow degradation	(Q)SAR result with medium uncertainty Reliability (2)
Bioaccumulation		
Bioconcentration in fish, *O. mykiss* (BCF_{KgL})	12 993	Klimisch (2), similar to OECD TG 305
BCF_{SSL} (5% lipid), *Cyprinus carpio*	1900 ± 300; 1100± 200	Klimisch (4), concentrations in fish not reported
BCF_K, *Lepomis macrochirus*	8148	Klimisch (4), no information on lipid content or fish growth
Dietary BMFgL (5% lipid), *Oncorhynchus mykiss*	0.2	Klimisch (2), depuration half life 8.1 days; estimated BCF_L 3513-7694 using method 1 of OECD TG 305 (Uptake rate constant estimation method: Use of models to estimate k1, combined with dietary k2 to provide BCF)
BCF (QSAR estimate)	2041; 1146	EPISUITE BCFBAF v3.01 (regression; Arnot-Gobas), (Q)SAR results with medium uncertainty, Reliability (2)
Toxicity		
Crustacea *Daphnia magna*:	48h EC50 0.045 mg/L	Klimisch (4), OECD TG 202
Algae	72h NOECr 1.4 mg/L	Klimisch (4), OECD TG 201
Fish *Oryzias latipes*	21-day LC50 0.025 mg/L	Klimisch (4), OECD TG 204
Fish *Oryzias latipes*	96-hour LC50 0.12 mg/L (95 % confidence interval: 0.053 – 0.27 mg/L).	Klimisch (4), OECD TG 203
Fish *Oryzias latipes*	41d NOEC: 11 µg/L	Klimisch (4), OECD TG 210
No relevant human health data	-	-

Data for the whole substance, Substance B:

DATA ELEMENTS: Substance B	Value	Test method / remarks
Physico-chemical properties and environmental fate (Substance B)		
Vapour pressure	0.002 hPa at 20 °C	calculated from experimental data at higher temperature using the Antoine equation, Klimisch (2)
Water solubility	0.061 mg/L at 20 °C	OECD TG 105, Klimisch (2)
log octanol/water partition coefficient (log K_{OW})	5.3 – 6.5 at 20 °C	OECD TG 117, Klimisch (2)
log organic carbon/water partition coefficient (log K_{OC})	4.2 – 6.1 at 20 °C	OECD TG 121, Klimisch (2)
Degradation (Substance B)		
Hydrolysis	not expected	based on structure
Ready biodegradability	14% biodegradation in 35 days (CO_2 evolution)	OECD TG 301B, Klimisch (2)
Soil simulation study; similar to OECD TG 307 (temperature corrected to 12°C)	DegT_{50} >218 days	no measurement of NER but study considered reliable and relevant for P assessment, Klimisch (2)
Bioaccumulation (Substance B)		
Bioconcentration in fish (BCF)	-	-
Toxicity (Substance B)		
Crustacea *Daphnia magna*:	EC_{50} > 0.069 mg/L, NOEC 0.008 mg/L	OECD TG 202. Klimisch (2)
Crustacea *Daphnia magna*	21 d NOELR for reproduction < 1.0 mg/L	OECD TG 211, Klimisch (4), WAF
No relevant human health classification	-	

Hazard assessment elements:

Physico-chemical properties:

- Constituent X is poorly water soluble, lipophilic and is not ionisable based on its chemical structure. It is present in Substance B in the concentration range 0.2-2%.

Degradation:

- Constituent X is not expected to hydrolyse based on its chemical structure. There is no ready biodegradability study on Constituent X but Biowin 2, 3 and 6 predictions indicate slow degradation. A ready biodegradability study (Klimisch 2) on Substance B reached 14% biodegradation in 35 days.
- A reliable (Klimisch 2) simulation test in river water is available for Constituent X showing that it meets the vP criteria, DegT50 > 60 days at temperature 12 °C. This is supported by a study in seawater performed at 20°C giving primary DegT50 >182 days. The primary DegT50 corrected to a temperature of 9°C would be even longer. The reliability of this study could not be assigned due to missing information (Klimisch 4).
- No monitoring studies are available for Constituent X or Substance B.

Bioaccumulation:

- One reliable (Klimisch 2) fish BCF study (BCF_{KgL} = 12993) and one reliable fish dietary study (Klimisch 2) (DietaryBMFgL (5% lipid) = 0.2, estimated BCF_{L} = 3513-7694) are available for Constituent X performed on *Oncorhynchus mykiss*. BCF QSAR predictions point to a BCF around 2000. The QSAR result is considered to have medium uncertainty, Reliability (2). Two other BCF studies are of unassignable reliability, one pointing to vB and the other pointing to not vB/borderline B. These two studies are given lower weight since their reliability cannot be verified.

Toxicity:

- Substance B is not classified for human health. There is no human health data available for Constituent X. The available aquatic toxicity studies for Constituent X are all of unassignable reliability (Klimisch 4) due to missing information. A long-term *Daphnia* study using WAF of unassignable reliability (Klimisch 4) on Substance B gives a NOELR for reproduction of < 1.0 mg/L. It is not clear which constituents contributed to the toxicity. There is insufficient information to conclude that Constituent X fulfils the T criterion.

Mobility:

- (Q)SAR predictions of the Log *Koc* for Constituent X are 5.3 and 4.8. The molecular weight of Constituent X and structural fragments fall within the range of the training set, and similar substances in the training set are predicted well. The QSAR results are considered to have low uncertainty, Reliability (1). Constituent X is not expected to be ionisable based on its chemical structure so influence of pH does not need to be taken into account for the mobility assessment.
- Experimental information for Substance B (OECD TG 121, reliability 4) gives a measured

log K_{OC} of 4.2-6.1. None of the constituents of Substance B are expected to be ionisable based on their chemical structures.

Classification (pursuant to CLP Annex I, 4.3 and 4.4):

Persistent, Bioaccumulative and Toxic (PBT) properties: CLP Annex I, 4.3 **criteria not met**

Very Persistent, Very Bioaccumulative (vPvB) properties: CLP Annex I, 4.3 **criteria met**

Persistent, Mobile and Toxic (PMT) properties: CLP Annex I, 4.4 **criteria not met**

Very Persistent, Very Mobile (vPvM) properties: CLP Annex I, 4.4 **criteria not met**

Reasoning:

Persistence (the lines of evidence are sorted based on their respective weight from high to low weight):

- A reliable simulation test in river water performed at 12°C is available for Constituent X showing that it meets the vP criteria in water, DegT50 > 60 days at temperature 12 °C. This value exceeds the P and vP criteria and the study is given high weight;
- A simulation study in seawater on Constituent X gave primary DegT50 >182 days at 20 °C. The reliability of this study could not be assigned due to missing information but it supports the P and vP conclusion (low weight);
- Biowin 2, 3 and 6 QSAR predictions suggest that Constituent X has slow degradation. Currently there is no universally accepted definition of model domain for the Biowin models, however, the molecular weight is within the training set range for Constituent X, the BIOWIN models recognise the fragments of the constituent X, the training set data included similar substances and the QSAR predictions are considered to have medium uncertainty, Reliability (2). This information is given low weight;
- A ready biodegradation study on the whole substance suggests that some constituents of the substance are not subject to biodegradation (14% after 35 days) (low weight as this does not bring information specifically for Constituent X).

Thus, it can be concluded that Constituent X fulfils the CLP Annex I, 4.3.2.1.1. (and also REACH Annex XIII 1.1.1.) **P**- and **vP**- criteria. Since Constituent X is present in the UVCB Substance at ≥0.1%, Substance B also fulfils the P and vP criteria in accordance with CLP.

Bioaccumulation (the lines of evidence are sorted based on their respective weight from high to

low weight):

- In a reliable (Klimisch 2) fish bioaccumulation study performed using a method similar to OECD TG 305 a lipid-normalised, growth-corrected kinetic fish BCF of 12 993 was measured in *Oncorhynchus mykiss* for Constituent X (high weight), indicating that the vB classification criterion is fulfilled;

- A reliable Klimisch (2) dietary fish bioaccumulation study in *Oncorhynchus mykiss* gave a dietary BMFgL (5% lipid) of 0.2 and depuration half-life of 8.1 days; the estimated BCF_L using method 1 of OECD TG 305 (Uptake rate constant estimation method: Use of models to estimate k1, combined with dietary k2 to provide BCF) is 3513-7694, which exceeds the B criterion and partly the vB criterion (medium weight);

- Two other experimental fish bioaccumulation studies are given low weight due to missing study information: BCF_{SSL} (5% lipid), *Cyprinus carpio* = 1900 ± 300; 1100± 200 and BCF_K, *Lepomis macrochirus* = 8148

- QSAR predictions using EPISUITE BCFBAF v3.01 (regression; Arnot-Gobas) of medium uncertainty, Reliability (2) give BCFs of 2041 and 1146 with one prediction exceeding the B criterion (low weight).

It can be concluded that Constituent X fulfils the CLP Annex I, 4.3.2.1.2. (and also REACH Annex XIII 1.1.2.) **B**- and **vB**- criteria. Since Constituent X is present in the UVCB Substance at ≥0.1%, Substance B also fulfils the B and vB criteria in accordance with CLP.

Toxicity:

Neither Substance B nor its Constituent X meet the classification criteria for human health. There are insufficient reliable data on aquatic toxicity. It is not possible to conclude whether the T criteria are met.

Mobility (the lines of evidence are sorted based on their respective weight from high to low weight):

- Experimental information for Substance B (OECD TG 121, reliability 2) gives a measured log K_{OC} above 3 (4.2-6.1) indicating that the M and vM criteria are not fulfilled (high weight). None of the constituents of Substance B are expected to be ionisable based on their chemical structures;

- Two QSAR predictions for Constituent X with low uncertainty, Reliability (1) predict *Log* K_{OC} values above 3 (5.3; 4.8)(medium weight). Based on its chemical structure, Constituent X is not expected to ionise so pH should not influence the K_{OC} value.

In the absence of a log K_{OC} value below the regulatory threshold values of 2 and 3 for any of the constituents of Substance B, including Constituent X, it can be concluded that Substance B and Constituent X do not fulfil the CLP Annex I, 4.4.2.1.2. and 4.4.2.2.2 M and vM criteria.

Label elements based on the classification:

Element	Resulting Labelling Elements (code)
GHS Pictogram	-
Signal Word	Dgr
Hazard Statement	EUH441
Precautionary statement(s)	P201, P202, P273, P391, P501

4.3.8.3. Example C: Substance meeting the new CLP classification criteria for PMT and vPvM, based on the overall WoE

DATA ELEMENTS	Value	Test method / remarks
Physico-chemical properties and environmental fate		
Vapour pressure	3.5 10^{-6} Pa	
Water solubility	2.3 g/L	EU Method A.6, Klimisch (1)
log octanol/water partition coefficient (log K_{OW})	-1.4	ACD/ Labs, QSAR prediction with medium uncertainty, Reliability (2)
log organic carbon/water partition coefficient (log K_{OC})	1.5 1.1 0.9 1.2; 1.8 1.4 3.2	KOCWIN v2.00, QSAR prediction with medium uncertainty, Reliability (2)[133] Extrapolation from log K_{OW} OECD TG 106 (pHs 4.5-7.5), Klimisch (1) FOOTPRINT Pesticides Properties Database, experimental information CompTox Chemicals Dashboard Experimental study, non-ionic species
pK_a	7.1	Substance is in the anionic state
Degradation		
Ready biodegradability	3% in 28 days	OECD TG 301C, Klimisch (1)
Simulation studies in surface water	>80 days	OECD TG 309, Klimisch (1)
Biodegradation in soil	> 3 years	ECETOC, non-standard study
Abiotic degradation	Negligible degradation by hydrolysis and photodegradation	Experimental studies
Bioaccumulation		
Bioconcentration in fish (BCF)	<10	OECD TG 305C , Klimisch (1)

[133] The substance is ionisable and, therefore, the Koc prediction should be investigated whether it considers ionisation.

Bioconcentration in fish (BCF)	<1	Non-standard study, Klimisch (2)
Bioconcentration in fish (BCF)	<0.5	Non-standard study, Klimisch (4)
Aquatic Toxicity		
Short and long-term fish	> 10 mg/L	Several OECD test protocols, Klimisch (1) and (2)
Short and long-term aquatic invertebrates	> 100 mg/L	Several OECD test protocols, Klimisch (1) and (2)
Algae and aquatic plants	> 100 mg/L	Several OECD test protocols, Klimisch (1) and (2)
Other Toxicity		
STOT RE 1 (H372) criteria met Carc 1B (H350) criteria met		
Other Information		
Monitoring studies	Presence in drinking and groundwater, rivers and lakes	
Modelling studies (CTD)	>2 000 km atmospheric transport potential	OECD Tool
Modelling studies (STP)	>98% in water phase for a municipal STP	SimpleTreat
Modelling studies (STP)	>90% partitioning to water	Mackay Level I; Mackay Level III

Hazard assessment elements:

Physico-chemical properties:

- Substance C is very water soluble, not volatile and with very low adsorption potential. It can be found also at an ionised state, under relevant environmental conditions.

Degradation:

- Evidence from both abiotic degradation experimental studies (hydrolysis and photodegradation) indicates that it abiotically degrades very slowly;
- One ready biodegradability (OECD TG 301C) and one surface water simulation test (OECD TG 309) provided very low biotic degradation rates;

- The same conclusion is confirmed by both field (chemical presence in several biological wastewater treatment plants, WWTP) and modelling data and compartmental distribution) after cessation of environmental releases;
- Results from inherent biodegradability studies performed according to OECD TG 302B revealed <15% degradation after 28 days of incubation.

Bioaccumulation:

- One experimental study with no reporting limitations (indicated that substance C is not bioaccumulative to fish);
- The same conclusion also confirmed by two non-standard studies;
- Non-standard study on terrestrial bioaccumulation is available;
- Indication from the octanol-water partition coefficient (=-1.4) of low biomagnification potential. Caution is advised on the use of such results due to the applicability of such models for ionisables.

Mobility:

- Substance C has high water solubility;
- Experimental information (OECD TG 106) that log K_{OC} is below 1;
- Several computational studies all estimated log K_{OC} values below 2 (caution due to their applicability for ionisables);
- Field evidence that Substance C is present in several different water bodies in high concentrations;
- Modelling evidence that Substance C partitions to water, does not volatilise and is slowly degraded;
- The low calculated Henry's law constant (=4.6 10^{-7} Pa*m^3/ mol, calculated as the ratio of the vapour pressure and water solubility, with a molecular weight of 300 g/mol) also provides additional evidence for low volatility from water bodies;
- Atmospheric transport over thousands of kilometres is predicted by modelling techniques.

Toxicity:

- Substance C has a harmonised classification as STOT RE 1 (H372);
- Substance C has a harmonised classification as Carc 1B (H350);
- Substance C has low aquatic toxicity.

Classification (pursuant to CLP Annex I, 4.3 and 4.4):

Persistent, Bioaccumulative and Toxic (PBT) properties: CLP Annex I, 4.3 **criteria not met**

Very Persistent, Very Bioaccumulative (vPvB) properties: CLP Annex I, 4.3 **criteria not met**

Persistent, Mobile and Toxic (PMT) properties: CLP Annex I, 4.4 **criteria met**

Very Persistent, Very Mobile (vPvM) properties: CLP Annex I, **4.4 criteria met**

Reasoning:

Persistence (the lines of evidence are sorted based on their respective weight from high to low weight):

In the surface water simulation study according to OECD TG 309, the degradation half-life in surface water was higher than 60 days (high weight) Moreover, a degradation half-life of more than 3 years was estimated for soil (medium weight, non-standard study), whilst experimental studies on abiotic degradation (medium weight), ready biodegradability (low weight) and monitoring (low weight) also support the conclusion that Substance C fulfils the CLP Annex I, 4.3.2.1.1 and 4.4.2.1.1 **P** criteria, as well as the CLP Annex I, 4.3.2.2.1 and 4.4.2.2.1 **vP** criteria.

Bioaccumulation:

The available data include one experimental BCF study on fish (high weight), a non-standard study on terrestrial bioaccumulation and two non-standard bioconcentration fish studies (medium and low weight), as well as indication from the octanol-water partition coefficient (low weight) support the conclusion that Substance C does not meet the CLP Annex I, 4.3.2.1.2. **B** criteria or the CLP Annex I, 4.3.2.2.2. **vB** criteria.

Mobility:

Results from several experimental and computational models have generated log K_{OC} values below 2 (high and medium weight). For the non-ionic species of substance C, a log K_{OC} of 3.2 was derived (medium weight). Furthermore, it has high water solubility and low volatilisation from water potential (H = 2 10^{-7} Pa*m^3/ mol) (low weight). Monitoring data reveal the substance's wide presence in different water bodies with concentrations up to 5 µg/L in groundwater and other surface water bodies (low weight). Distribution modelling computations also confirm its affinity to water bodies and slow environmental degradation (low weight). Finally, there is evidence that Substance C is not likely to be efficiently removed by adsorption to organic materials in sewage treatment plants (WWTP) or in drinking water production (low weight). In summary, Substance C can be concluded to fulfil the CLP Annex I, 4.4.2.1.2 and 4.4.2.2.2 criteria for **M** and **vM**.

Toxicity:

Substance C fulfils the CLP Annex I, 4.3.2.1.3 and 4.4.2.1.3 **T** criteria, as it has a harmonised classification as STOT RE 1 and Carc 1B.

Label elements based on the classification:

Element	Resulting Labelling Elements (code)
GHS Pictogram	-

Signal Word	Dgr
Hazard Statement	EUH451[134]
Precautionary statement(s)	P201, P202, P273, P391, P501

[134] In line with Annex III to Regulation (EC) No 1272/2008 Part 1 point (d) "if the hazard statement EUH451 "Can cause very long-lasting and diffuse contamination of water resources" is assigned, the statement EUH450 "Can cause long-lasting and diffuse contamination of water resources" may be omitted.

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5. PART 5: ADDITIONAL HAZARDS

5.1. HAZARDOUS TO THE OZONE LAYER

The criteria chapter for classification and labelling of substances and mixtures hazardous to the ozone layer are short and the need for guidance is limited to the actual ODP-value that would trigger classification for a substance.

Annex I:

5.1.2 Classification criteria for substances

5.1.2.1. A substance shall be classified as Hazardous to the Ozone Layer (Category 1) if the available evidence concerning its properties and its predicted or observed environmental fate and behaviour indicate that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer.

5.1.3 Classification criteria for mixtures

5.1.3.1. Mixtures shall be classified as Hazardous to the Ozone Layer (Category 1) on the basis of the individual concentration of the substance(s) contained therein that are also classified as Hazardous to the Ozone Layer (Category 1), in accordance with Table 5.1.

Any substances having an Ozone Depleting Potential (ODP) greater or equal to the lowest ODP (*i.e.* 0.005) of the substances currently listed in Annex I to Regulation (EC) No 1005/2009[135] should be classified as hazardous to the ozone layer (category 1).

[135] Regulation (EC) No 1005/2009 of the European Parliament and of the Council of 16 September 2009 on substances that deplete the ozone layer.

ANNEXES

I ANNEX I: AQUATIC TOXICITY

I.1 Introduction

The basis for the identification of a hazard to the aquatic environment for a substance is the aquatic toxicity of that substance. Classification is predicated on having toxicity data for fish, crustacea, and algae/aquatic plant available. These taxa are generally accepted as representative of aquatic fauna and flora for hazard identification. Data on these particular taxa are more likely to be found because of this general acceptance by regulatory authorities and the chemical industry. Other information on the degradation and bioaccumulation behaviour is used to better delineate the aquatic hazard. This section describes the appropriate tests for ecotoxicity, provides some basic concepts in evaluating the data and using combinations of testing results for classification. Further detailed guidance is given in IR&CSA Chapter R.7b, sections R.7.8.3 – R.7.8.5).

I.2 Description of tests

For classifying substances in the harmonised system, freshwater and marine species toxicity data can be considered as equivalent data. It should be noted that some types of substances, e.g. ionisable organic chemicals or organometallic substances may express different toxicities in freshwater and marine environments. Since the purpose of classification is to characterise hazard in the aquatic environment, the result showing the highest toxicity should normally be chosen. However, there are circumstances where a weight of evidence approach is appropriate.

The criteria for determining aquatic hazards should be test method neutral, allowing different approaches as long as they are scientifically sound and validated according to international procedures and criteria already referred to in existing systems for the hazard of concern and produce mutually acceptable data. Where valid data are available from non-standard testing and from non-testing methods, these shall be considered in classification provided they fulfil the requirements specified in section 1 of Annex XI to the REACH Regulation (EC) No 1907/2006.

According to GHS, 2011, fourth revised edition, section 4.1.1.3:*"Acute toxicity would normally be determined using a fish 96 hour LC_{50} (OECD Test Guideline 203 or equivalent), a crustacea species 48 hour EC_{50} (OECD Test Guideline 202 or equivalent) and/or an algal species 72 or 96 hour EC_{50} (OECD Test Guideline 201 or equivalent). These species are considered as surrogate for all aquatic organisms and data on other species such as the duckweed Lemna may also be considered if the test methodology is suitable."*

Chronic testing involves an exposure that covers a significant period of time when compared to the organism´s life cycle. The term can signify periods from days to a year, or more depending on the reproductive cycle of the aquatic organism. Chronic tests can be done to assess certain information relating to growth, survival, reproduction and development.

According to GHS, 2011, fourth revised edition, section 4.1.1.3:*"Chronic toxicity data are less available than acute data and the range of testing procedures less standardised. Data generated according to the OECD Test Guidelines 210 (Fish Early Life Stage), 202 Part 2 or 211 (Daphnia Reproduction) and 201 (Algal Growth Inhibition) or equivalent can be accepted (see also Annex 9, para. A9.3.3.2). Other validated and internationally accepted tests could also be used. The NOECs or other equivalent EC_x should be used."*

It should be noted that several of the test guidelines cited as example tests for potential consideration for classification purposes have been revised or updated. Such revisions may lead to minor modifications of test conditions or even introduction of the potential use of additional

effects endpoints depending on the regulatory context. Therefore, the expert group that developed the harmonised criteria for classification intended some flexibility in test duration and/or species and number of animals used.

Guidelines for conducting acceptable tests with fish, crustacea, and algae can be found from many sources (*e.g.* Test Methods Regulation 440/2008; OECD e.g. the OECD monograph No.11, Detailed Review Paper on Aquatic Toxicity Testing for Industrial Chemicals and Pesticides, 1999; EPA, 1996; ASTM, 1999; ISO standards; EU standards).

Some general guidance on commonly faced issues with aquatic toxicity testing:

- Evidence should be provided that test concentrations/dose levels and number of concentrations are known and where possible evidence should be provided that concentrations have been maintained throughout the duration of the test. Therefore, mean measured concentrations are preferred over nominal (non-measured) concentrations. If mean measured concentrations are not within ±20% of nominal concentrations, effect values should be related to mean measured concentrations. Where test concentrations remain within ±20% of nominal, mean measured or nominal concentrations can be used, following expert judgement on a case-by-case basis. For flow-through studies the arithmetic mean of measured concentrations should be calculated. For static or semi-static tests the geometric mean of measured concentrations should be calculated (see IR&CSA Chapter R.7b, Appendix R.7.8—1). In some cases where only nominal concentrations are provided, expert judgement may be required to decide whether test concentrations are likely to have been maintained (Note, Nominal testing results on metals or metal compounds (those relevant for assessment under Annex IV according to Annex IV.1) cannot be used for hazard classification as further specified in Annex IV, In contrast, only nominal concentrations (from loading rates) can be used for multi-constituent substances tested using WAF approaches, see Annex I.4.5);
- Some substances, depending on their specific mode of action may produce toxic effects at time points earlier than those typically used in any given test guideline. In such cases, caution should be exercised and evaluation on a case-by-case basis is required. If effect durations less than those indicated in the test guideline are to be accepted for classification purposes, the full test duration should be reported and the respective test guideline validity criteria for that end point must be fulfilled at the time to be used;
- Regarding rapidly degrading substances, studies conducted under flow-through and/or semi-static (usually after 24 hours) conditions are preferred in order to maintain test concentration during the duration of the study. See OECD Guidance 23 (OECD 2019) for further information.
- There are instances where the only data available may lack test details described above. In such cases, expert judgement should be used to determine the suitability of the test for use in classification and the reliability of the endpoint values. For example, there should be enough information to determine whether the test conditions are suitable for the organisms being tested, the viability of the test organisms, demonstration of exposure of the test organisms, a suitable concentration range, and a clear dose response relationship for endpoints of interest. Tests where such information is not available may not be suitable for classification and labelling.

I.2.1 Fish tests

I.2.1.1 Acute testing

Acute tests are generally performed with young juveniles 0.1 – 5 g in size for a period of 96 hours. The observational endpoint in these tests is mortality. Fish larger than this size range and/or durations shorter than 96 hours are generally less sensitive. However, for classification, they could be used if no acceptable data with the smaller fish for 96 hours are available or the results

of these tests with different size fish or test durations would influence classification in a more hazardous category. Tests consistent with OECD TG 203 (Fish 96 hour LC_{50}) or equivalent should be used for classification.

The Fish Embryo Acute Toxicity (FET) Test (OECD TG 236) was adopted on the 26th July 2013 and its potential to fulfil the REACH information requirement on acute fish toxicity has been the subject of an ECHA commissioned study (May 2015, http://echa.europa.eu/ publications/technical-scientific-reports), which highlighted certain regulatory limitations in its use and thus concluded that the FET test cannot be considered as stand-alone information for addressing the information requirement for acute fish toxicity under the REACH Regulation. Based on current knowledge, ECHA considers that OECD TG 236 might best be used within a Weight of Evidence (WoE) approach together with other independent, adequate, relevant, and reliable sources of information for classification and labelling. However, if the only available information on the fish toxicity of a substance is the FET, the test can be used for classification, even if no other information for weight of evidence is available (https://www.echa.europa.eu/documents/10162/22931011/non_animal_approcches_en.pdf/87ebb68f-2038-f597-fc33-f4003e9e7d7d).

With regards to *in vitro* test methods, as new non-animal alternatives are developed (e.g. the fish cell line assay, OECD TG 249, adopted 2021), it may be possible to use these, as outlined for FET, to assess acute toxicity to fish provided that they fulfil relevant data quality requirements (IR&CSA Chapter R.7b, section R.7.8.4.1).

I.2.1.2 Chronic testing

Chronic or long-term tests with fish can be initiated with fertilized eggs, embryos, juveniles, or reproductively active adults. Durations can vary widely depending on the test purpose (anywhere from 7 days to over 200 days). Observational endpoints can include hatching success, growth (length and weight changes), spawning success, and survival. Tests consistent with OECD Test Guideline 210 (Fish Early-life Stage Toxicity Test, FELS), US EPA 850.1500 (the fish life-cycle test), or equivalent can be used in the classification scheme. Currently, other relevant tests include OECD TG 212 (Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages) and OECD TG 215 (Fish, Juvenile Growth Test).

OECD TG 210 (FELS) is widely accepted as a predictor of chronic toxicity and is used as such for purposes of classification in the harmonised system, particularly as fish early-life stage toxicity data are much more available than fish life cycle or reproduction studies. Technically, OECD TG 210 is not a 'chronic' test, but a sub-chronic test on sensitive life stages.

However, it is preferred as it is considered to be more sensitive than OECD TG 212 or OECD TG 215, covering several life stages of the fish from the newly fertilized egg, through hatch to early stages of growth (IR&CSA Chapter R.7b, section R.7.8.4.1). Moreover, the FELS toxicity test is preferable for examining the potential toxic effects of substances which are, for example: expected to cause effects over a longer exposure period, which require a longer exposure period of time to reach steady state (in non flow-through test systems), substances that have a latency of effects, or a longer period to onset of maximum (often sub-lethal) effect. Regarding OECD TG 212, care should be taken when interpreting data from this test to ensure that the test substance is not too lipophilic (e.g. Log K_{ow} > 4), that there is no evidence of endocrine disrupting properties, or other specific modes of action[136]. For further guidance on the use of OECD TG 212 and information on the animal welfare issues related to this test, consult OECD 171 (Fish Toxicity

[136] Following amendments to the REACH annexes (Reg (EU) 2022/477 of 24 March 2022) OECD TG 212 has been deleted as a new information requirement due to animal welfare concerns. However, an existing such test can be considered to fulfil the LT toxicity data requirement for fish at Annex IX.

Testing Framework). However, smaller differences in sensitivity between OECD TG 212 and 215 would be expected for chemicals with a non-specific, narcotic mode of action (Kristensen, 1990).

OECD TG 215 measures the growth of juvenile fish over a fixed period of time and it is considered a sensitive indicator of toxicity for the growth endpoint. Although it does not cover all the sensitive points in the fish life cycle, as would normally be required of a chronic toxicity test such as the FELS test, it can be used, for example, when growth inhibition is the most relevant effect endpoint in fish.

Where it has been determined that a substance has endocrine disrupting properties, tests such as the following may be available:

OECD TG 234 (Fish Sexual Development Test adopted on July 28th 2011) is in principle an enhancement of OECD TG 210, where the exposure is continued until the fish are sexually differentiated, *i.e.* about 60 days post-hatch for Japanese medaka (*Oryzias latipes*), the three-spined stickleback and zebrafish (the exposure period can be shorter or longer for other species that are validated in the future), and endocrine-sensitive endpoints are added. OECD TG 234 currently states that a minimum of three test concentrations should be used, with five test concentrations recommended if the data are to be used for risk assessment. The use of five test concentrations is recommended in order to derive a more precise NOEC/LOEC or ECx value. It should be noted that vitellogenin (VTG) level is affected by exogenous oestrogen stimulation and cannot be used alone for aquatic hazard identification as an endpoint on its own. Rather, it can be used as part of a WoE approach when considered with other data on relevant related effects (*e.g.* this endpoint in combination with sex ratio endpoint can demonstrate the endocrine mode of action of the test substance).

OECD TG 240 (adopted 28th July 2015) is an extended one generation test using Japanese medaka running until hatching in the F2 generation. This test provides standard long-term toxicity endpoints (hatching and larval survival, growth *i.e.*, length and body weight, etc.) but also has secondary effect endpoints for endocrine disruption. For the latter (vitellogenin etc.) similar considerations as outlined for OECD TG 234 apply.

Regarding OECD TG 204 (Fish, Prolonged Toxicity Test: 14-day Study), OECD has deleted the test guideline on 2nd April 2014. It is noted that, according to the Guidance on Information Requirements and Chemical Safety Assessment (IR&CSA Chapter R.7b, section R.7.8.4.1), "*tests performed according to OECD 204 or similar guidelines cannot be considered suitable long-term tests. They are, in effect, prolonged acute studies with fish mortality as the major endpoint examined*".

I.2.2 Tests with Aquatic Crustacea

I.2.2.1 Acute testing

Acute tests with crustacea generally begin with first instar juveniles. For daphnids, test duration of 48 hours is used. For other crustacea, such as mysids or others, duration of 96 hours is typical. The observational endpoint is mortality or immobilisation as a surrogate to mortality. Immobilisation is defined as unresponsive to gentle prodding. Tests consistent with OECD TG 202 (*Daphnia* acute) or USA-EPA OPPTS 850.1035 (Mysid acute toxicity) or their equivalents should be used for classification.

Although crustacea are the preferred group (*Daphnia magna* being the most common test species), other aquatic invertebrates can be considered on a case-by-case basis. As such, toxicity tests using aquatic insects (*e.g.* mayfly larvae , chironomids [OECD TG 235]) and molluscs can be acceptable for hazard classification, providing they are relevant and reliable. Where a substance has an intended target invertebrate group (e.g. insecticides), or a group of particular sensitivity, data from such a group or species may be available in addition to crustacea (*e.g.* daphnids).

I.2.2.2 Chronic testing

Chronic tests with crustacea also generally begin with first instar juveniles and continue through maturation and reproduction. Observational endpoints include time to first brood, number of offspring produced per female, growth, and survival. For daphnids, in particular *Daphnia magna*, 21 days is sufficient for maturation and the production of at least 3 broods, while for mysids 28 days is necessary, and *Ceriodaphnia dubia* produces 3 broods within 7 days (Connors *et al*., 2022). It is recommended that tests consistent with OECD TG 211 (*Daphnia* reproduction), US-EPA 850.1350 (Mysid chronic), ISO 20665 (chronic toxicity to *Ceriodaphnia dubia*), or their equivalents be used in the classification scheme. Older data according to OECD TG 202 part II could in principle still be available for use pending a thorough assessment.

Data from invertebrate species other than the crustacea mentioned can be used, following the considerations outlined above in Annex I.2.2.1. Concerning the sediment-dwelling larvae of the freshwater dipteran *Chironomus* sp. (OECD TG 218/219), data from such tests can be used provided it can be demonstrated that exposure to the test material is through the water phase and not via sediment. For most substances, uptake from water (bioconcentration, defined as the net result of uptake, transformation, and elimination of a substance in an organism due to waterborne exposure) is believed to be the predominant route of exposure for aquatic organisms. For organic substances and metals, pore water is one of the primary exposure routes for benthic organisms (Di Toro *et al.*, 1991; Ankley *et al.*, 1991). However, for highly lipophilic compounds or other substances that adsorb to particles (e.g. metals), uptake from food or sediment may contribute to the overall exposure, depending on the living and feeding strategy of the exposed organisms. The importance of dietary exposure relative to water exposure as a cause of toxicity is currently not fully understood.

In summary, factors that influence adsorption and thus distribution between sediment and water also influence toxicity to aquatic (pelagic and benthic) species. Generally, the substance must not partition to sediments to any great extent. The low sorption potential of the substance must be demonstrated, and the test design should use the water-spiking method. This may indicate that the organisms are exposed to the substance primarily via the water. When considering the life-stage used in an available test, *Chironomus* spend their most sensitive larval stage (first instar) free swimming in the water phase and will therefore only be exposed via the water in this stage. Other life-stages will involve the sediment so interpretation of results may be less straight-forward. Generally, where sediment is included in the test system, it must be clear what caused the observed toxicity, with regards to the exposure route. Test system equilibrium should favour exposure from the water phase. If it cannot be ruled out that the test organisms were exposed to the substance or metabolites adsorbed to the sediment particle surfaces, the test may not be relevant for classification purposes.

I.2.3 Algae / other aquatic plant tests

I.2.3.1 Tests with algae

Algae are cultured and exposed to the test substance in a nutrient-enriched medium. Tests consistent with OECD TG 201 (Algal growth inhibition) should be used. Standard test methods employ a cell density in the inoculum in order to ensure exponential growth through the test, usually 3 to 4 days duration. Annex 2 of OECD TG 201 (Algae) and IR&CSA R.7b report some of the species shown to be generally suitable for the testing and include green algae, diatoms, and cyanobacteria.

The algal growth inhibition test is a short-term test that provides both acute and chronic endpoints. The EC_{50} is treated as an acute value for classification purposes. Classification shall be based on both, the algal growth rate reduction endpoint, E_rC_{50} [= EC_{50} (growth rate)] and NOE_rC [= NOEC (growth rate)] or EC_x value, provided that the control growth is exponential (greater

than a factor of 16) (where available, EC_{10} is preferred over NOEC, see 4.1.3.1.1). This endpoint is preferred because it is not dependent on the test design, whereas the endpoint biomass (growth) inhibition (E_bC_{50}) depends on both, growth rate of the test species as well as test duration and other elements of test design.

In circumstances where the basis of the EC_{50} is not specified and no E_rC_{50} is recorded, classification shall be based on the lowest EC_{50} available. Where the algal toxicity E_rC_{50} falls more than 100 times below the next most sensitive species and results in a classification based solely on this effect, consideration should be given to whether this toxicity is representative of the toxicity to aquatic plants. Where it can be shown that this is not the case, professional judgment should be used in deciding if classification should be applied.

I.2.3.2 Tests with aquatic macrophytes

The most commonly used vascular plants for aquatic toxicity tests are duckweeds (*Lemna gibba* and *L. minor*). The tests last for up to 14 days and are performed in nutrient enriched media similar to that used for algae but may be increased in strength. The observational endpoint is based on change in the number of fronds produced. Tests consistent with OECD TG 221 on *Lemna* and US-EPA 850.4400 (aquatic plant toxicity, *Lemna*) and OECD TG 238/239 (*Myriophyllum spicatum*) should be used. The observational endpoint for *Myriophyllum spicatum* is based on the vegetative growth. Note that for tests using sediment (*e.g.* OECD TG 239), TG considerations regarding sediment should be followed and exposure should be from the water.

The respective macrophyte TGs are considered to provide both acute and chronic endpoints over the experimental time periods specified.

I.3 Aquatic toxicity concepts

This section addresses the use of acute and chronic toxicity data in classification, and special considerations for exposure regimes, algal toxicity testing, and use of (Q)SARs.

I.3.1 Acute toxicity

Acute toxicity for classification purposes refers to the intrinsic property of a substance to be harmful to an organism in a short-term exposure to that substance. Acute toxicity is generally expressed in terms of a concentration which is lethal to 50 % of the test organisms (lethal concentration, LC_{50}), causes a measurable adverse effect to 50 % of the test organisms (e.g. immobilisation of daphnids, EC_{50}), or leads to a 50 % reduction in test (treated) organism responses from control (untreated) organism responses (e.g. growth rate in algae, ErC_{50}).

Acute aquatic toxicity is normally determined using a fish 96 hour LC_{50}, a crustacea species 48 hour EC_{50}, an algal species 72 or 96 hour EC_{50} and/or aquatic plants 7 days EC_{50}. These species cover a range of trophic levels and taxa and are considered as surrogate for all aquatic organisms. Data on other species shall also be considered if the test methodology is suitable. Since the purpose of classification is to characterise hazard in the aquatic environment, the result showing the highest toxicity should be chosen. However, there are circumstances when a weight of evidence approach is appropriate.

Substances with an acute toxicity determined to be less than 1 mg/L are generally recognised as being very toxic. The handling, use, or discharge into the environment of these substances poses a high degree of hazard and they are classified in category Acute 1. When classifying substances as Acute 1, it is necessary at the same time to indicate an appropriate Multiplying factor, M-factor. M-factors are derived using the endpoint value used to derive the hazard category (see section 3).

I.3.2 Chronic toxicity

Chronic toxicity, for classification purposes, refers to the intrinsic property of a substance to cause adverse effects to aquatic organisms during exposures which are determined in relation to the life-cycle of the organism. Such chronic effects usually include a range of sublethal endpoints and are generally expressed in terms of a No Observed Effect Concentration (NOEC), or an equivalent EC_x. Observable endpoints typically include survival, growth and/or reproduction. Chronic toxicity exposure durations can vary widely depending on the test endpoint measured and test species used.

For long-term hazard classification, a differentiation is made between rapidly degradable and non-rapidly degradable substances. Substances that rapidly degrade are classified in category Chronic 1 when the chronic toxicity NOEC or EC_x is determined to be ≤ 0.01 mg/L. Rapidly degradable substances with a chronic toxicity NOEC or EC_x between 0.01 and 0.1 mg/L are classified in category Chronic 2. Rapidly degradable substances with a chronic toxicity NOEC or EC_x between 0.1 and 1.0 mg/L are classified in category Chronic 3. Finally, those rapidly degraded substances with chronic toxicity NOECs or EC_xs over 1.0 mg/L are not classifiable for long-term hazard in any of the categories Chronic 1, 2 or 3.

For substances that do not rapidly degrade, based on data or by default (*i.e.*, default applies in cases where no information on rapid degradation is available), two chronic categories are used: category Chronic 1 if the chronic toxicity NOEC or EC_x is determined to be ≤ 0.1 mg/L and category Chronic 2 if the chronic toxicity NOEC or EC_x is determined to be between 0.1 and 1.0 mg/L. Finally, those not rapidly degraded substances with chronic toxicity NOECs or EC_xs over 1.0 mg/L are not classifiable for long-term hazard in any of the categories Chronic 1 or 2. Note, category Chronic 3 does not apply to non-rapidly degraded substances.

When classifying substances as Chronic 1, it is necessary at the same time to indicate an appropriate M-factor. M-factors are derived using the endpoint value used to derive the hazard category (see section 3).

In the absence of chronic toxicity data, chronic hazard is identified by appropriate combinations of acute toxicity, lack of degradability, and/or the potential or actual bioaccumulation. However, where adequate chronic toxicity data exist (see section 4.1.3.3.1), this shall be used in preference over the classification based on the combination of acute toxicity with degradability and/or bioaccumulation. In this context, the following general approach should be used.

a. If adequate chronic toxicity data are available for all three trophic levels, this is used directly to determine an appropriate long-term hazard category.

   In cases where chronic toxicity data are available for all three trophic levels, but data are absent for the most acutely sensitive species, the chronic toxicity dataset may not be considered adequate (see section 4.1.3.3.1). In such cases, it may be appropriate to derive a classification using available acute toxicity data for the most acutely sensitive species following the procedure outlined below (b);

b. If adequate chronic toxicity data are available for one or two trophic levels, it should be examined if acute toxicity data are available for the other trophic level(s). A potential classification is made for the trophic level(s) with chronic toxicity data and compared with that made using the acute toxicity data for the other trophic level(s). The final classification shall be made according to the most stringent outcome (Figure 4.1.1 and Table 4.1.0(b)(iii) of Annex I to CLP);

c. In order to remove or downgrade a chronic aquatic classification, using chronic toxicity data, it must be demonstrated that the NOEC(s) (or equivalent EC_x) used would be suitable to remove or downgrade the concern for all taxa which resulted in classification based on acute data in combination with degradability, and/or bioaccumulation. This can often be achieved by using a long-term NOEC or EC_x for the most sensitive species identified by the acute toxicity. Thus, if a long-term hazard classification has been based on a fish acute

LC_{50}, it would generally not be possible to remove or downgrade this classification using a long-term NOEC or EC_x from an invertebrate toxicity test. In this case, the NOEC or EC_x would normally need to be derived from a long-term fish test of the same species or one of equivalent or greater sensitivity. Equally, if classification has resulted from the acute toxicity of more than one taxonomic group, it is likely that NOECs or EC_xs from each taxonomic group will be needed. In case of classification of a substance as Chronic 4, sufficient evidence should be provided that the NOEC or EC_x or equivalent EC_x for each taxonomic group is greater than 1 mg/L or greater than the water solubility of the substances under consideration.

I.3.3 Exposure regimes

Three types of exposure conditions are employed in both acute and chronic tests and in both freshwater and saltwater media: static, static-renewal (semi-static), and flow-through. The choice for which test type to use usually depends on test substance characteristics, test duration, test species, and regulatory requirements.

I.3.4 Test media for algae and Lemna

Algal and *Lemna* tests are performed in nutrient-enriched media and use of one common constituent, EDTA, or other chelators, should be considered carefully. When testing the toxicity of organic chemicals, trace amounts of a chelator like EDTA are needed to complex micronutrients in the culture medium; if omitted, growth can be significantly reduced and compromise test utility. However, chelators can reduce the observed toxicity of metal test substances. Therefore, for metal compounds, it is desirable that data from tests with high concentration of chelators and/or tests with stoichiometric excess of chelator relative to iron be critically evaluated. Free chelator may mask heavy metal toxicity considerably, in particular with strong chelators like EDTA (see Annex IV to this guidance on Metals and inorganic metal compounds). However, in the absence of available iron in the medium the growth of algae and *Lemna* can become iron limited, and consequently data from tests with no or with reduced iron and EDTA should be treated with caution.

I.3.5 Use of substance categorisation (read-across and grouping) and (Q)SARs for classification and labelling

See section 1.4 of Part 1 of this guidance series.

I.4 Substances which are difficult to test

The following paragraphs provide some detailed guidance on some of these problems of interpretation. In doing so it should be remembered that this is guidance and rigid rules cannot be applied. The nature of many of the difficulties means that expert judgement must always be applied both in determining whether there is sufficient information in a test for a judgement to be made on its validity, and also whether a toxicity level can be determined that is suitable for use in applying the classification criteria. Metals present their own set of difficulties and are discussed separately (see Annex IV on metals).

For classification of organic compounds, it is desirable to have stabilised and analytically measured test concentrations. Although measured concentrations are preferred, classification may, under certain circumstances, be based on studies where nominal concentrations are the only valid data available.

If the material is likely to substantially degrade or otherwise be lost from the water column, care must be taken in data interpretation and classification should be done taking into account the loss of the toxicant during the test, if relevant and possible. In cases where loss of test material may occur, actual test concentrations are likely to be less than the nominal or expected test

concentrations and under-represent the actual toxicity. In circumstances where a substance is known to be difficult to test, expert judgement is needed to determine the acceptability of such tests for use in classification. Similarly, caution is also needed when deriving appropriate M-factors.

I.4.1 Unstable substances

While testing procedures which minimise the impacts of instability in the test media should ideally have been deployed, in practice, in certain tests, it can be almost impossible to maintain a constant concentration throughout the test. Common causes of lack of constant exposure concentration during the test include but are not limited to: oxidation, hydrolysis, photodegradation, volatilisation, sorption, and biodegradation. While the latter forms of degradation can be more readily controlled, such controls may be absent in much existing testing. Nevertheless, for some testing, particularly acute and chronic fish toxicity testing, a choice of exposure regimes is available to help minimise losses due to instability, and this should be taken into account in deciding on the test data validity.

Where instability is a factor in determining the level of exposure during a test, an essential prerequisite for data interpretation is the existence of measured exposure concentrations at suitable time points throughout the test. In the absence of analytically measured concentrations at least at the start and end of the test, no valid interpretation can be made, and the test should be considered as invalid for classification purposes. Where measured data are available, a number of practical guidelines can be considered:

a. where measured data are available for the start and end of the test (as is normal for the acute *Daphnia* and algal tests), the L(E)C_{50}, for classification purposes, may be calculated based on the geometric mean concentration of the start and end of test. Where concentrations at the end of the test are below the analytical detection limit, such concentrations shall be considered to be half of that detection limit;

b. where measured data are available at the start and end of media renewal periods (as may be available for the semi-static tests), the geometric mean for each renewal period should be calculated, and the mean exposure over the whole exposure period calculated from these data;

c. where the toxicity can be attributed to a degradation breakdown product, and the concentrations of this product are known, the L(E)C_{50} for classification purposes may be calculated based on the geometric mean of the degradation product concentration, back calculated to the parent substance;

d. similar principles may be applied to measured data in chronic toxicity testing.

I.4.2 Poorly soluble substances

These substances, usually taken to be those with a solubility in water of < 1 mg/L, are frequently difficult to dissolve in the test media, and the dissolved concentrations will often prove difficult to measure at the low concentrations anticipated. For many substances, the true solubility in the test media will be unknown and will often be recorded as below the detection limit in purified water. Nevertheless such substances can show toxicity and, where no toxicity is found, expert judgement must be applied to whether the result can be considered valid for classification. Judgement should err on the side of caution and should not underestimate the hazard. Here, OECD Guidance 23 (OECD 2019) provides guidance on how to proceed.

Ideally, tests using appropriate dissolution techniques and with accurately measured concentrations within the range of water solubility should be used. Where such test data are available, they should be used in preference to other data. It is normal, however, particularly when considering older data, to find substances with toxicity levels recorded in excess of the water solubility, or where the dissolved levels are below the detection limit of the analytical method. Thus, in both circumstances, it is not possible to verify the actual exposure

concentrations using measured data. Where these are the only data available on which to classify, some practical rules can be considered by way of general guidance:

a. where the acute toxicity is recorded at levels in excess of the water solubility, the L(E)C_{50} for classification purposes may be considered to be equal to or below the measured water solubility. In making this decision, due attention should be paid to the possibility that the excess undissolved substance may have given rise to physical effects on the test organisms. Where this is considered the likely cause of the effects observed, the test should be considered as invalid for classification purposes;

b. where no acute toxicity is recorded at levels in excess of the water solubility, the L(E)C_{50} for classification purposes may be considered to be greater than the measured water solubility. In such circumstances, consideration should be given to whether the category Chronic 4 should apply. In making a decision that the substance shows no acute toxicity, due account should be taken of the techniques used to achieve the maximum dissolved concentrations. Where these are not considered as adequate, the test should be considered as invalid for classification purposes;

c. where the water solubility is below the detection limit of the analytical method for a substance, and acute toxicity is recorded, the L(E)C_{50} for classification purposes may be considered to be below the analytical detection limit. Where no toxicity is observed, the L(E)C_{50} for classification purposes, may be considered to be greater than the water solubility. Due consideration should also be given to the quality criteria mentioned above;

d. where chronic toxicity data are available, the same general rules should apply. In principle, only data showing no observed effect concentrations at levels above the water solubility limit, or greater than 1 mg/L need be considered. Again, where these data cannot be validated by analytically verified/ measured concentrations, the techniques used to achieve the maximum dissolved concentrations must be considered as appropriate.

I.4.3 Other factors contributing to concentration loss

A number of other factors can also contribute to losses of test material from solution and, while some can be avoided by correct study design, interpretation of data where these factors have contributed will be necessary.

a. sedimentation: this can occur during a test for a number of reasons. A common explanation is that the substance has not truly dissolved despite the apparent absence of particulates, and agglomeration occurs during the test leading to precipitation. In these circumstances, the L(E)C_{50} for classification purposes, may be considered to be based on the end of test concentrations. Equally, precipitation can occur through reaction with the media. This is considered under instability above;

b. adsorption: this can occur for substances of high adsorption characteristics such as high Log K_{oc}/log K_{ow} substances or some substances with a permanent charge (such as some cationic surfactants). Where this occurs, the loss of concentration is usually rapid, and exposure may best be characterised by the end of test concentrations;

c. bioaccumulation: losses may occur due to the bioaccumulation of a substance into the test organisms. This may be particularly important where the water solubility is low and log K_{ow} correspondingly high. The L(E)C_{50} for classification purposes, may be calculated based on the geometric mean of the start and end of test concentrations.

I.4.4 Perturbation of the test media

Strong acids and bases may exert their toxicity through extreme pH. However, changes of the pH in aquatic systems are normally prevented by buffer systems in the test medium. If no data are available on a salt, the salt should generally be classified in the same way as the anion or cation, *i.e.*, as the ion that receives the most stringent classification. If the effect concentration is related

to only one of the ions, the classification of the salt should take the molecular weight difference into consideration by correcting the effect concentration by multiplying with the ratio: MW_{salt}/MW_{ion}.

Polymers are typically not available in aquatic systems. Dispersible polymers and other high molecular mass materials can perturb the test system and interfere with uptake of oxygen and give rise to mechanical or secondary effects. These factors need to be taken into account when considering data from these substances. Many polymers behave like complex substances, however, having a significant low molecular mass fraction which can leach from the bulk polymer. This is considered further below.

I.4.5 Complex substances

Complex substances (UVCBs and multi-constituent substances) (OECD series on testing and assessment Number 23, Guidance Document on Aquatic Toxicity Testing of Difficult to Test Substances and Mixtures) are characterised by a range of chemical structures, covering a wide range of water solubilities and other physico-chemical characteristics. In addition to water, equilibrium will be reached between the dissolved and undissolved fractions which will be characteristic of the loading of the substance. For this reason, such complex substances are usually tested as a WSF or WAF, and the $L(E)C_{50}$ recorded based on the loading or nominal concentrations. Analytical support data are not normally available since the dissolved fraction will itself be a complex mixture of components. The toxicity parameter is sometimes referred to as LL_{50}, related to the lethal loading level. This loading level from the WSF or WAF may be used directly in the classification criteria. Whilst taking a constituent based approach may be required for assessing rapid degradability and bioaccumulation, in the absence of adequate whole substance aquatic toxicity data the mixtures approaches (following figure 4.1.2 of CLP) can be followed.

Polymers represent a special kind of complex substance, requiring consideration of the polymer type and their dissolution/dispersal behaviour. Polymers may dissolve as such without change (true solubility related to particle size), be dispersible, or portions consisting of low molecular weight fractions may go into solution. In the latter case, in effect, the testing of a polymer is a test of the ability of low molecular mass material to leach from the bulk polymer, and whether this leachate is toxic. It can thus be considered in the same way as a complex mixture in that a loading of polymer can best characterise the resultant leachate, and hence the toxicity can be related to this loading.

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II ANNEX II: RAPID DEGRADATION

II.1 Introduction

Degradability is one of the important properties of substances that has an impact on the potential for substances to exert an aquatic hazard. Non-degradable substances will persist in the environment and may consequently have a potential for causing long-term adverse effects on biota. In contrast, degradable substances may be removed in the sewers, in sewage treatment plants or in the environment. It should be noted that data from degradability tests on mixtures are difficult or impossible to interpret and are therefore not used in classification and labelling.

Classification of substances is primarily based on their intrinsic properties. However, the degree of degradation depends not only on the intrinsic degradability or recalcitrance of the molecule, but also on the actual conditions in the receiving environmental compartment such as redox potential, pH, temperature, presence of suitable microorganisms, concentration of the substance and occurrence and concentration of other substrates. The interpretation of the degradation properties in an aquatic hazard classification context therefore requires detailed criteria which consider the intrinsic properties of the substance and the prevailing environmental conditions into a concluding statement on the potential for long-term adverse effects.

The term degradation is defined in section 4.1 of Annex I to CLP as 'the decomposition of organic molecules to smaller molecules and eventually to carbon dioxide, water and salts'. For inorganic compounds and metals, the concept of degradability has limited or no meaning. Rather the substance may be transformed by normal environmental processes to either increase or decrease the bioavailability of the toxic species. Therefore, the present section applies only to organic and organometal compounds. A separate section on the classification & labelling (C&L) of metals is provided in Part 4.4.1.5 and Annex IV to the CLP guidance.

Data on degradation properties of a substance may be available from standardised tests, or from other types of investigations, or they may be estimated from the structure of the molecules *i.e.* via SAR or (Q)SAR approaches. The interpretation of such degradation data for classification purposes often requires detailed evaluation of the (test) data. The use of biodegradation data for classification purposes is only applicable to substances. Biodegradation data on mixtures cannot be used as it does not provide a reliable indication of environmental fate (CLP Annex I, section 4.1.3.3.1).

II.2 Interpretation of degradability data

Based on the harmonised criteria (CLP I.4.1.2.9), guidance for interpretation of degradation data is presented below.

II.2.1 Ready biodegradability

Ready biodegradability is defined in the OECD Test Guidelines No. 301 methods A-F (OECD 1992), OECD TG 306 (marine water) and OECD TG 310 (OECD 2006). All organic substances that degrade to a level higher than the pass level in a standard OECD ready biodegradability or a similar test should be considered readily biodegradable, and consequently also rapidly degradable. Many test data found in the open literature, however, do not specify all of the conditions that should be evaluated to demonstrate whether or not the test fulfils the requirements of a ready biodegradability test. Expert judgement is therefore needed as regards the validity of the data before use for classification purposes.

Regarding marine data, OECD TG 306 series on Biodegradability in Seawater includes seawater variants of the Closed Bottle Test (OECD 301 D) and of the Modified OECD Screening Test (OECD 301 E). Degradation of substances in seawater has generally been found to be slower than that in freshwater tests inoculated with activated sludge or sewage effluent. This is also confirmed in

the research program conducted in CEFIC LRI ECO11 project, where it was demonstrated that both magnitude and variation in the bacterial diversity were higher in the following order for the different environmental sources: activated sludge > rivers > estuaries > sea water.

OECD test guideline 306 explicitly indicates that results of those tests (shake flask and closed bottle) "*are not to be taken as indications of ready biodegradability, but are to be used specifically for obtaining information about the biodegradability of chemicals in marine environments". Those tests "are not tests for ready biodegradability since no inoculum is added in addition to the microorganisms already present in the seawater. Neither do the tests simulate the marine environment since nutrients are added and the concentration of test substance is very much higher than would be present in the sea*". However, it is acknowledged that biodegradation in seawater is generally slower. Therefore >60% ThOD or >70% DOC removal in a Biodegradability in Seawater test (OECD 306) obtained after 28 day (Closed Bottle Method) or 60 day (Shake Flask Method) is indicative of potential for ultimate biodegradation in the marine environment and can also be regarded as a piece of evidence that the substance is likely to fulfil the criteria for ready biodegradability. For example, a positive OECD TG 306 test is regarded as an indication of rapid degradation for classification and labelling. However, if the ratio of inoculum to substrate in the test system is enhanced by increasing the concentration of microorganisms as it has been proposed recently in Ott *et al*. (2020), this also increases the degradation potential. In this case the test system does not resemble a pelagic water body anymore and is thus less stringent. This has consequences for interpretation of the so produced degradation data with respect to conclusion on ready biodegradation behaviour as such enhancements will render the test unsuitable for assessing ready biodegradability (rapid degradation) under CLP.

Before concluding on the ready biodegradability of a test substance, however, at least the following parameters should be considered.

II.2.1.1 Concentration of test substance

Relatively high concentrations of test substance are used in the OECD ready biodegradability tests (2-100 mg/L). Many substances may however be toxic to the inoculum at such high concentrations, resulting in a low degradation of the substances in these tests, although the substances might be rapidly degradable at lower non-toxic concentrations. A toxicity test with microorganisms (*e.g.* OECD TG 209), or inhibition of the inoculum observed with a positive control substance may demonstrate the toxicity of the test substance. Guidance on the selection of suitable microbial inhibition test methods is provided in IR&CSA Chapter R.7b, section R7.8.18. When it is likely that inhibition is the reason for a substance being not readily degradable, results from a test employing lower non-toxic concentrations of the test substance should be used when available.

II.2.1.2 Time window

The harmonised criteria include a general requirement for all of the ready biodegradability tests on achievement of the pass level within ten days of the onset of biodegradation. This is not in line with OECD TG 301 in which the 10-day time window applies to the OECD ready biodegradability tests except the MITI I test (OECD TG 301C). Furthermore, in the Closed Bottle test (OECD TG 301D), a 14-day window may be used instead when measurements have not been made after ten days or are not possible due to experimental design. However, occasionally only limited information is available for biodegradation tests. Thus, as a pragmatic approach, the percentage of degradation reached after 28 days may be used directly for assessment of ready biodegradability when no information on the 10-day time window is available. This should, however, only be accepted for existing experimental studies and test results where the 10-day window does not apply or is not available. Tests following current test guidelines should allow assessment of the 10-day window, where appropriate.

Where there is sufficient justification, the 10-day window condition may be waived for certain complex substances and the pass level is applied at 28 days. This applies to multi-constituent and

certain UVCB substances (such as oils and surfactants) consisting of structural similar constituents with different chain-lengths, degree and/or site of branching or stereoisomers, even in their most purified commercial forms. Testing of each individual constituent may be costly and impractical. If a test on such a complex substance is performed and it is anticipated that a sequential biodegradation of the individual constituents is taking place, then the 10-day window should not be applied to interpret the results of the test. A case by case evaluation should however take place on whether a biodegradability test on such a substance would give valuable information regarding its biodegradability as such *i.e.* regarding the degradability of all the constituents, or whether instead an investigation of the degradability of carefully selected individual constituents of the complex substance is required (OECD 2006).

II.2.2 BOD_5/COD

Information on the 5-day biochemical oxygen demand (BOD_5) will be used for classification purposes only when no other measured degradability data are available. Thus, priority is given to data from ready biodegradability tests and from simulation studies regarding degradability in the aquatic environment. Therefore, this test should not be performed anymore for assessment of the ready biodegradability of substances. Older test data may however be used when no other degradability data are available. For substances where the chemical structure is known, the theoretical oxygen demand (ThOD) can be calculated and this value should be used instead of the chemical oxygen demand (COD).

II.2.3 Other convincing scientific evidence

Rapid degradation in the aquatic environment may be demonstrated by other data than a ready biodegradability test, or a BOD_5/COD ratio. These may be data on biotic and/or abiotic degradation. Data on primary degradation can only be used where it is demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment.

The fulfilment of criterion (c) of paragraph 4.1.2.9.5 of CLP (other convincing scientific evidence) requires that the substance is degraded in the aquatic environment to a level of > 70 % within a 28-day period. If first-order kinetics are assumed, which is reasonable at the low substance concentrations prevailing in most aquatic environments, the degradation rate will be relatively constant for the 28-day period. Thus, the degradation requirement will be fulfilled with an average degradation rate constant, $k > -(\ln 0.3 - \ln 1)/28 = 0.043$ day^{-1}. This corresponds to a degradation half-life, $t_{½} < \ln 2/0.043 = 16$ days.

Moreover, as degradation processes are temperature dependent, this parameter should also be taken into account when assessing degradation in the environment. Data from studies employing environmentally realistic temperatures e.g. 5 – 25 °C should be used for the evaluation. When data from studies performed at different temperatures need to be compared, the traditional Q10 approach could be used, *i.e.* that the degradation rate is halved when the temperature decreases by 10°C.

The evaluation of data in the context of rapid degradation should be conducted on a case-by-case basis by expert judgement. However, guidance on the interpretation of various types of data that may be used for demonstrating a rapid degradation in the aquatic environment is given below. In general, only data from aquatic biodegradation simulation tests are considered directly applicable (*e.g.* OECD TG 309). However simulation test data from other environmental compartments could be considered as well (*e.g.* OECD TG 308), but such data require in general more scientific judgement before use.

II.2.3.1 Aquatic simulation tests

Aquatic simulation tests (*e.g.* Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test, OECD TG 309) are tests conducted in the laboratory, but simulating environmental

conditions and employing natural samples as inoculum. Results of aquatic simulation tests may be used directly for classification purposes, when realistic environmental conditions in surface waters are simulated, *i.e.*:

a. substance concentration that is realistic for the general aquatic environment (often in the low µg/L range);

b. inoculum from a relevant aquatic environment;

c. realistic concentration of inoculum (10^3-10^6 cells/mL);

d. realistic temperature e.g. 5 °C to 25 °C, with 12 °C being the average surface water temperature in the EU (see Table R.16-8 in IR&CSA Chapter R.16 "Environmental exposure estimation"), that can be considered under CLP;

e. ultimate degradation is determined *i.e.*, determination of the mineralisation rate or the individual degradation rates of the total biodegradation pathway.

II.2.3.2 Field investigations

Parallel to laboratory simulation tests are field investigations or mesocosm experiments. In such studies, fate and/or effects of chemicals in the environment or in environmental enclosures may be investigated. Fate data from such experiments can in principle be used for assessing the potential for a rapid degradation. This may, however, often be difficult, as it requires that ultimate degradation can be demonstrated. This may be documented by preparing mass balances showing that no non-degradable intermediates are formed, and which take the fractions into account that are removed from the aqueous system due to other processes such as sorption to sediment or volatilisation from the aquatic environment.

II.2.3.3 Monitoring data

Monitoring data may demonstrate the removal of contaminants from the aquatic environment. Such data are, however, very difficult to use for classification purposes, although detection of an anthropogenic substance in groundwater could be an indication that the substance is not rapidly degradable. The following aspects should be considered before use:

a. Is the removal a result of degradation, or is it a result of other processes such as dilution or distribution between compartments (sorption, volatilisation)?

b. Is formation of non-degradable intermediates excluded?

Only when it can be demonstrated that removal as a result of ultimate degradation fulfils the criteria for rapid degradability, can such data be considered for use for classification purposes. In general, monitoring data should only be used as supporting evidence for demonstration of either persistence in the aquatic environment, or of rapid degradation.

II.2.3.4 Inherent and Enhanced Ready Biodegradability tests

Substances that are degraded more than 70% in tests for inherent biodegradability (OECD TG 302) have the potential for ultimate biodegradation. However, because of the optimised conditions in these tests, the rapid biodegradability of inherently biodegradable substances in the environment cannot be assumed. The optimised conditions in inherent biodegradability tests stimulate adaptation of the microorganisms thus increasing the biodegradation potential, compared to natural environments. Therefore, positive results in general should not be interpreted as evidence for rapid degradation in the environment.

IR&CSA Chapters R.7b and R.11 refer in the context of persistence testing to a new category of tests, *i.e.* the 'enhanced ready (screening) biodegradability tests'. These are in essence ready biodegradability tests to which more flexibility is given to demonstrate the occurrence of degradation e.g. via prolonged testing times, larger test volumes, adaptation, etc. These methods are not yet validated and/or standardised for C&L.

II.2.3.5 Sewage treatment plant simulation tests

Results from tests simulating the conditions in a sewage treatment plant (STP) e.g. OECD TG 303 (Simulation Test - Aerobic Sewage Treatment -- A: Activated Sludge Units; B: Biofilms) and OECD TG 314 B (Simulation Tests to Assess the Biodegradability of Chemicals Discharged in Wastewater) cannot be used for assessing the degradation in the aquatic environment, for classification purposes. The main reasons for this are that the microbial biomass in a STP is significantly different from the biomass in the environment, that there is a considerably different composition of substrates, and that the presence of rapidly mineralised organic matter in wastewater may facilitate degradation of the test substance by co-metabolism.

II.2.3.6 Soil and sediment degradation data

It has been argued that for many non-sorptive substances comparable degradation rates are found in soil and in surface water. For sorptive substances, a lower degradation rate may generally be expected in soil than in water due to a lower bioavailability caused by sorption. Thus, when a substance has been shown to be degraded rapidly in a soil simulation study, it is most likely also rapidly degradable in the aquatic environment. It is therefore proposed that in the absence of aquatic degradation data an experimentally determined rapid degradation in soil is sufficient documentation for a rapid degradation in surface waters when:

a. no pre-exposure (pre-adaptation) of the soil microorganisms has taken place; and

b. an environmentally realistic concentration of substance is tested; and

c. the substance is ultimately degraded within 28 days with a half-life < 16 days corresponding to a degradation rate > 0.043 day^{-1} .

The same argumentation is considered valid for data on degradation in sediment under aerobic conditions.

II.2.3.7 Anaerobic degradation data

Data regarding anaerobic degradation (*e.g.* OECD TG 311) cannot be used to decide whether a substance should be regarded as rapidly degradable for classification purposes, because the aqueous phase of the aquatic environment is generally regarded as the aerobic compartment where the aquatic organisms, such as those of relevance for aquatic hazard classification, are found.

II.2.3.8 Hydrolysis

Data on hydrolysis e.g. OECD TG 111 might be considered for classification purposes only when the longest half-life $t_{½}$ determined within the pH range 4-9 is shorter than 16 days. However, hydrolysis is not an ultimate degradation and various intermediate degradation products may be formed, some of which may be only slowly degradable. Only when it can be satisfactorily demonstrated that the hydrolysis products formed do not fulfil the criteria for classification as hazardous for the aquatic environment, data from hydrolysis studies could be considered.

When a substance is quickly hydrolysed e.g. with $t_{½}$ < a few days, this process is a part of the degradation determined in biodegradation tests. Hydrolysis may be the initial transformation process in biodegradation.

II.2.3.9 Photochemical degradation

Information on photochemical degradation e.g. OECD TG 316 is difficult to use for classification purposes. The actual degree of photochemical degradation in the aquatic environment depends on local conditions e.g. water depth, suspended solids, turbidity as well as seasonal influences, and the hazard of the degradation products is usually not known. Probably only seldom will adequate information be available for a thorough evaluation based on photochemical degradation.

II.2.3.10 Estimation of degradation

Hydrolysis: Certain (Q)SARs have been developed for prediction of an approximate hydrolysis half-life, which should only be considered when no experimental data are available, or in a Weight of Evidence approach. However, a hydrolysis half-life can only be used with great care in relation to classification, because hydrolysis does not concern ultimate degradability (see 'Hydrolysis' of this section). Furthermore the (Q)SARs developed until now have a rather limited applicability and are only able to predict the potential for hydrolysis on a limited number of chemical classes (see also IR&CSA Chapter R.7b, section R.7.9.3.1).

Biodegradation: In general, no quantitative estimation method ((Q)SAR) for estimating the degree of biodegradability of organic substances is yet sufficiently accurate to unequivocally predict rapid degradation. However, results from such methods may be used to predict that a substance is not rapidly degradable or be used in a Weight of Evidence approach. For example, when in the Biodegradation Probability Program e.g. BIOWIN version 3.67, Syracuse Research Corporation the probability is < 0.5 estimated by the linear or non-linear methods, the substances should be regarded as not rapidly degradable (OECD, 1994; Pedersen *et al.*, 1995 & Langenberg *et al.*, 1996). Also other (Q)SAR methods may be used as well as expert judgement, for example, when degradation data for structurally analogue compounds are available, but such judgement should be conducted with great care. See also IR&CSA Chapter R.7b, section R.7.9.3.1.

In general, a (Q)SAR prediction that the substance is not rapidly degradable is considered a better justification for classification than application of a default classification, when no useful degradation data are available.

Degradation data from structurally related substances may provide evidence that a given substance displays very similar degradation properties. Such information may be employed in a read-across or weight of evidence approach for C&L.

II.2.3.11 Volatilisation

Chemicals may be removed from some aquatic environments by volatilisation. The intrinsic potential for volatilisation is determined by the Henry's Law constant (H) of the substance. Apart from the substance's physical-chemical properties, volatilisation from the aquatic environment is also highly dependent on the environmental conditions of the specific water body in question, such as the water depth, the gas exchange coefficients (depending on wind speed and water flow) and stratification of the water body. Because volatilisation only represents removal of a chemical from the water phase, and not degradation, the Henry's Law constant cannot be used for assessment of degradation in relation to aquatic hazard classification of substances (see also Pedersen *et al.*, 1995).

II.2.4 No degradation data available

When no useful data on degradability are available - either experimentally determined or estimated data - the substance should be regarded by default as not rapidly degradable.

II.3 General interpretation problems

II.3.1 Complex substances

The harmonised criteria for classification of chemicals as hazardous for the aquatic environment refer to single substances. However, complex substances also need to be considered for classification and labelling and these are normally considered as single substances in a regulatory context. Some complex substances such as multi-constituent substances of natural origin, chemicals that are produced or extracted from mineral oil or plant material, etc., are highly complex with a high number of constituents, many of which may be unidentified. The constituents of a complex substance can be highly varied and possess a wide range of physical-chemical

properties important for aquatic degradation assessment (*e.g.* water solubility, Log k_{ow}, adsorption characteristics, and volatility). Therefore, it may not be possible to make a degradation assessment using a test on the whole complex substance and data on known constituents will need to be considered to assess the whole substance. Occasionally, complex substances may be defined as a homologous series of substances within a certain range of carbon chain length and/or degree of substitution. When this is the case, no major difference in degradability is foreseen and the degree of degradability can be established from tests of the complex chemical. Occasionally, a borderline degradation is found because some of the individual constituents may be rapidly degradable and others may not be rapidly degradable. As above, this requires a detailed assessment of the degradability of the individual constituents in the complex substance. When the constituents that are not-rapidly-degradable constitute a significant part of the complex substance e.g. more than 20 %, or for a hazardous constituent, an even lower content, the substance should be regarded as not rapidly degradable.

II.3.2 Availability of the substance

The present standard methods for investigating degradability of substances are developed for readily soluble test compounds. However, many organic substances are only slightly soluble in water. As the standard tests (OECD TG 301 suite and 310) recommend 2-100 mg/L of the test substance, sufficient availability may not be reached for substances with low water solubility. In general, the DOC Die-Away test (OECD TG 301A) and the Modified OECD Screening test (OECD Test Guideline 301E) are less suitable for testing the biodegradability of poorly soluble substances since adsorption may be confused with degradation. In such cases, test adaptations may be considered with e.g. continuous mixing and/or an increased exposure time. Also tests with a special design, where concentrations of the test substance lower than the water solubility have been employed e.g. with radiolabelled test chemicals, could be relevant.

II.3.3 Test duration less than 28 days

Sometimes degradation is reported for tests terminated before the 28 day period specified in the standards e.g. the MITI, 1992. These data are of course directly applicable when a degradation greater than or equal to the pass level is obtained. When a lower degradation level is reached, the results need to be interpreted with caution. One possibility is that the duration of the test was too short and that the chemical structure would probably have been degraded in a 28-day biodegradability test. If substantial degradation occurs within a short time period, the situation may be compared with the criterion $BOD_5/COD \geq 0.5$ or with the requirements on degradation within the 10-day time window. In these cases, a substance may be considered readily degradable (and hence rapidly degradable), if:

a. the ultimate biodegradability exceeds 50 % within 5 days; or

b. the ultimate degradation rate constant in this period is greater than 0.1 day^{-1} corresponding to a half-life of 7 days.

These criteria are proposed in order to ensure that rapid mineralisation did occur, although the test was ended before 28 days and before the pass level was attained. Interpretation of test data that do not comply with the prescribed pass levels must be made with great caution. It is mandatory to consider whether a biodegradability result below the pass level was due to a partial degradation of the substance and not a complete mineralisation. If partial degradation is the probable explanation for the observed biodegradability, the substance should be considered not readily biodegradable.

II.3.4 Primary biodegradation

In some tests, only the disappearance of the parent compound *i.e.* primary degradation is determined for example by following the degradation by specific or group specific chemical analyses of the test substance. Data on primary biodegradability may be used for demonstrating

rapid degradability only when it can be satisfactorily demonstrated that the degradation products formed do not fulfil the criteria for classification as hazardous to the aquatic environment.

II.3.5 Conflicting results from screening tests

Where data from more than one experimental degradation study are available for the same substance, there is a possibility that the results may be conflicting. The IR&CSA Chapter R.7b, section R.7.9.4.1 indicates that ready biodegradability tests may sometime fail because of the stringent test conditions, positive test results should generally supersede negative test results in cases where good quality experimental studies are presented. However, when conflicting test results are reported, possible differences in the test conditions and design should be investigated. In particular the origin of the inoculum should be examined in order to verify whether or not there are differences in the adaptation of the inoculum which may explain the differences in the results (OECD, 2006b). Also, differing results always have to be assessed considering the test conditions, substance properties and reliability of the data. Good data reliability depends on the test method applied, statistical robustness of the study and its reporting which in turn depend on several factors, e.g. number of replicates, and number of controls.

The suitability of the inoculum for degrading the test substance depends on the presence and amount of competent degraders. When the inoculum is obtained from an environment that has previously been exposed to the test substance, the inoculum may be adapted as demonstrated by a degradation capacity greater than that of an inoculum from a non-exposed environment. As far as possible the inoculum must be sampled from an unexposed environment, but for substances that are used ubiquitously in high volumes and released widespread or more or less continuously, this may be difficult or impossible. When conflicting results are obtained, the origin and density of the inoculum should be checked in order to clarify whether or not differences in the adaptation of the microbial community may be the reason.

As mentioned above, many substances may be toxic or inhibitory to the inoculum at the relatively high concentrations tested in ready biodegradability tests. This is especially likely in the Modified MITI (I) test (OECD TG 301C) and the Manometric Respirometry test (OECD Test Guideline 301F) where high concentrations (100 mg/L) are prescribed, compared to the low test substance concentrations prescribed in the Closed Bottle test (OECD Test Guideline 301D) where 2-10 mg/L is used. The possibility of toxic effects may be evaluated by including a toxicity control in the ready biodegradability test or by comparing the test concentration with toxicity test data on microorganisms (for test methods see IR&CSA Chapter R.7b, section R.7.8.14) or by assessing whether there is evidence of inhibition of intrinsic respiration in the ready biodegradability test (*e.g.* lower respiration in the test item vessel compared to the blank inoculum may be indicative of an inhibitory effect).

Volatile substances should only be tested in closed systems, such as the Closed Bottle test (OECD Test Guideline 301D), the MITI I test (OECD Test Guideline 301C) the Manometric Respirometry test (OECD Test Guideline 301F), or OECD 310 (CO2 in sealed vessels – Headspace Test). Results from other tests should be evaluated carefully and only considered if it can be demonstrated, e.g. by mass balance estimates, that the removal of the test substance is not a result of volatilisation.

II.3.6 Variation in simulation test data

A number of simulation test data may be available for certain high priority chemicals. Often such data can provide a range of half-lives for various environmental media such as soil, sediment and/or surface water. The observed differences in half-lives from simulation tests performed on the same substance may reflect differences in test conditions, all of which may be environmentally relevant. A suitable conservative half-life, *i.e.* a realistic worst case of the observed range of half-lives from such investigations, should be selected for classification by employing a weight of evidence approach and taking the realism and relevance of the employed tests into account in relation to environmental conditions. In general, simulation test data of surface waters (*e.g.* OECD

TG 309) are preferred relative to aquatic sediment or soil simulation test data for the evaluation of rapid degradability in the aquatic environment.

II.4 Decision scheme

The following decision scheme may be used as a general guidance to facilitate decisions in relation to rapid degradability in the aquatic environment and classification of chemicals hazardous to the aquatic environment.

A substance is considered to be **not** rapidly degradable **unless** at least one of the following is fulfilled:

c. The substance is demonstrated to be readily biodegradable in a 28-day test for ready biodegradability. The pass level of the test (70 % DOC removal or 60 % theoretical oxygen demand) must be achieved within 10 days from the onset of biodegradation, if it is possible to evaluate this according to the available test data (the 10-day window condition may be waived for complex multi-component substances and the pass level applied at 28 days, as discussed in II.2.1.2). If this is not possible, then the pass level should be evaluated within a 14-day time window if possible, or after the end of the test; or

d. The substance is demonstrated to be ultimately degraded in a surface water simulation test with a half-life of < 16 days (corresponding to a degradation of > 70 % within 28 days); or

e. The substance is demonstrated to be primarily degraded biotically or abiotically e.g. via hydrolysis, in the aquatic environment with a half-life < 16 days (corresponding to a degradation of > 70 % within 28 days), and it can be demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment.

When these preferred data types are not available rapid degradation may be demonstrated if one of the following criteria is justified:

a. The substance is demonstrated to be ultimately degraded in an aquatic sediment or soil simulation test with a half-life of < 16 days (corresponding to a degradation of > 70 % within 28 days); or

b. In those cases where only BOD_5 and COD data are available, the ratio of BOD_5/COD is greater than or equal to 0.5. The same criterion applies to ready biodegradability tests of a shorter duration than 28 days, if the half-life furthermore is < 7 days; or

c. A weight of evidence approach based on read-across provides convincing evidence that a given substance is rapidly degradable.

If none of the above types of data are available, then the substance is considered as **not** rapidly degradable. This decision may be supported by fulfilment of at least one of the following criteria:

i. the substance is not inherently degradable in an inherent biodegradability test; or

ii. the substance is predicted to be slowly biodegradable by scientifically valid (Q)SARs, e.g. for the Biodegradation Probability Program, the score for rapid degradation (linear or non-linear model) < 0.5; or

iii. the substance is considered to be not rapidly degradable based on indirect evidence, such as knowledge from structurally similar substances; or

iv. no other data regarding degradability are available.

II.5 References

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III ANNEX III: BIOACCUMULATION

III.1 Introduction

Bioaccumulation of a substance by an organism is not in itself a hazard. However, the bioaccumulation of a substance should be considered in relation to the potential for that substance to exert long-term effects. Chemical concentration and accumulation may result in internal concentrations of a substance in an organism (body burden), which may or may not lead to toxic effects over long-term exposures. For most organic chemicals uptake from water (bioconcentration) is believed to be the predominant route of uptake. Only for very hydrophobic substances does uptake from food become important. The classification criteria use the bioconcentration factor (BCF) or, in the absence of it, the octanol/water partition coefficient (log K_{ow}) as the measure of the potential for bioaccumulation. For these reasons, the present guidance document mainly considers bioconcentration and does not discuss in detail uptake via food or other routes. However, the possibility to use information on the biomagnification factor (BMF) as supportive evidence for bioaccumulation of highly lipophilic substances may be taken into account on a case by case basis.

Classification of a substance is primarily based on its intrinsic properties. However, the degree of bioconcentration also depends on factors such as the degree of bioavailability, the physiology of test organism, maintenance of constant exposure concentration, exposure duration, metabolism inside the body of the target organism and excretion from the body. The interpretation of the bioconcentration potential in a chemical classification context therefore requires an evaluation of the intrinsic properties of the substance, as well as of the experimental conditions under which bioconcentration factor (BCF) has been determined. IR&CSA Chapter R.7c, section 7.10.5.1 discusses the suitability of bioconcentration data, log K_{ow} data and other information (e.g. evidence for limited bioaccumulation potential) for classification purposes. Use of measured biomagnification data is discussed in relation to the screening approach in IR&CSA Chapter R.7c, section 7.10.4.5. Bioaccumulation of metals is discussed in Annex IV.

Information on the bioaccumulation potential of a substance may be available from standardised tests or may be estimated from the structure of the molecule. The interpretation of such bioconcentration data for classification purposes often requires detailed evaluation of test data. Guidance has been developed in IR&CSA in order to facilitate this evaluation. IR&CSA Chapter R.7a, section 7.1.8 gives guidance on n-octanol/water partition coefficient and IR&CSA Chapter R.7c, section 7.10.4 gives guidance on how to evaluate laboratory data on aquatic bioaccumulation. The use of bioaccumulation data for classification purposes is only applicable to substances. Bioaccumulation data on mixtures cannot be used as it does not provide a reliable indication of environmental fate (CLP Annex I, section 4.1.3.3.1).

III.2 Interpretation of bioconcentration data

Aquatic hazard classification of a substance is normally based on existing data on its environmental properties. Test data will only seldom be produced with the main purpose of facilitating a classification. Often a diverse range of test data is available which does not necessarily match the classification criteria. Further guidance on how to use this data is given in IR&CSA Chapter R.7c, section.

Bioconcentration of an organic substance can be experimentally determined in bioconcentration experiments, during which BCF is measured as the concentration in the organism relative to the concentration in water under steady-state conditions and/or estimated from the uptake rate constant and the elimination rate constant. In general, the potential of an organic substance to bioconcentrate is primarily related to the lipophilicity of the substance. A measure of lipophilicity is the n-octanol/water partition coefficient (K_{ow}) which, for lipophilic non-ionised organic substances, undergoing minimal metabolism or biotransformation within the organism, is

correlated with the bioconcentration factor. Therefore, K_{ow} is often used for estimating the bioconcentration of non-ionised organic substances, based on the empirical relationship between log BCF and log K_{ow}. For those organic substances, estimation methods are available for calculating the K_{ow}. Data on the bioconcentration properties of non-ionised organic substances may thus be (i) experimentally determined, (ii) estimated from experimentally determined K_{ow}, or (iii) estimated from K_{ow} values derived by use of Quantitative Structure Activity Relationships ((Q)SARs). Guidance for interpretation of such data is given in IR&CSA Chapter R.7c, sections 7.10.4 and 7.10.5. Guidance is also given on ionised chemicals and other classes that need special attention (see Annex III.3.1).

III.2.1 Bioconcentration factor (BCF)

The bioconcentration factor is defined as the ratio on a weight basis between the concentration of the chemical in biota and the concentration in the surrounding medium, here water, at steady state. BCF can thus be experimentally derived under steady-state conditions, on the basis of measured concentrations. In addition BCF can also be calculated as the ratio between the first-order uptake and elimination rate constants; a method which does not require steady state (equilibrium conditions).

Experimentally derived BCF values of high quality studies are ultimately preferred for classification purposes as such data override surrogate data, e.g. K_{ow}. High quality data are defined as data where the validity criteria for the test method applied are fulfilled and described. Further guidance is provided in IR&CSA Chapter R.7c, section 7.10.4. BCF results of poor or questionable quality may give an erroneous BCF value. Therefore, such data should be carefully evaluated before use and consideration should be given to using K_{ow} instead.

Different test guidelines for the experimental determination of bioconcentration in fish have been documented and adopted, the most generally applied being OECD TG 305 (OECD, 2012; C.13 in Test Methods Regulation 440/2008 is a corresponding test). If there is no BCF value for fish species, high-quality data on the BCF value for invertebrate species may be used. An invertebrate (mussel, oyster or scallop) BCF can be used as a worst case (conservative) value for fish. BCF for algae should not be used.

Experimental BCF data on highly lipophilic substances (e.g. with log K_{ow} above 6) will have a higher level of uncertainty than BCF values determined for less lipophilic substances. For highly lipophilic substances, e.g. with log K_{ow} above 6, experimentally derived BCF values tend to decrease with increasing log K_{ow}. Conceptual explanations of this non-linearity mainly refer to either reduced membrane permeation kinetics or reduced biotic lipid solubility for large molecules. A low bioavailability and uptake of these substances in the organism will thus occur. Other factors relate to experimental considerations, such as equilibrium not being reached, reduced bioavailability due to sorption to organic matter in the aqueous phase, and analytical errors. Special care should thus be taken when evaluating experimental data on BCF for highly lipophilic substances as these data will have a much higher level of uncertainty than BCF values determined for less lipophilic substances.

III.2.1.1 BCF in different test species

BCF values used for classification are based on whole body measurements. As stated previously, the optimal data for classification are BCF values derived using OECD TG 305 or corresponding EU test guideline C.13 or internationally equivalent methods, which uses small fish. Due to the higher gill surface-to-weight ratio in smaller organisms than in larger ones, steady-state conditions will be reached sooner in smaller organisms than in larger ones. The size of the organisms (fish) used in bioconcentration studies is thus of considerable importance in relation to the time used in the uptake phase, when the reported BCF value is based solely on measured concentrations in fish and water at steady-state. Thus, if large fish, e.g. adult salmon, have been used in bioconcentration studies, it should be evaluated whether the uptake period was sufficiently long for steady state to be reached or to allow for a kinetic uptake rate constant to be determined

precisely. Also possible growth dilution should be taken into account when calculating the BCF values for smaller fish that grow during the bioconcentration studies.

Furthermore, when using existing data for classification, it is possible that the BCF values could be derived from several different fish or other aquatic species (e.g. clams) and for different organs in the fish. Thus, to compare diverse measured BCF data from different species to each other and to the criteria, normalisation to common basis lipid content will be required to reduce variability. Detailed guidance can be found in IR&CSA Chapter R.7c, section 7.10.4.1 for 'correction factors'.

Generally, the highest valid BCF value expressed on this common lipid basis is used to determine the wet weight based BCF-value in relation to the cut off value for BCF of 500 of the classification criteria.

III.2.1.2 Use of radio-labelled substances

The use of radio-labelled test substances can facilitate the analytical measurements in water and fish samples. The BCF from radio-labelled studies should, preferentially, be based on the parent compound. If these are unavailable, for classification purposes, the BCF based on total radio-labelled residues can be used. If the BCF, in terms of radio-labelled residues, is ≥ 1000, the identification and quantification of degradation products documented to be ≥ 10 % of total residues in fish tissues at steady state, are strongly recommended. This is because when using radio-labelled substances, the labelling is most often placed in the stable part of the molecule, for which reason the measured BCF value represents both the parent substance and takes account of the radio labelled part of the metabolites (*i.e.*, the BCF represents the whole undegraded molecule). Thus, unless combined with a specific analytical method, the total radioactivity measurements potentially reflect the presence of the parent substance as well as possible metabolite(s) and possible metabolised carbon, which have been incorporated in the fish tissue in organic molecules. BCF values determined by use of radio-labelled test substances are therefore normally overestimated. Also, for some substances it is the metabolite which is the most toxic or which has the highest bioconcentration potential. Therefore, selective measurements of the parent substance as well as the metabolites may thus be important for the interpretation of the aquatic hazard (including the bioconcentration potential) of such substances. In experiments where radio-labelled substances have been used, high radio-label concentrations are often found in the gall bladder of fish. This is interpreted to be caused by biotransformation in the liver and subsequently by excretion of metabolites in the gall bladder (Comotto *et al.*, 1979; Wakabayashi *et al.*, 1987; Goodrich *et al.*, 1991; Toshima *et al.*, 1992).

When fish do not eat, the content of the gall bladder is not emptied into the gut, and high concentrations of metabolites may build up in the gall bladder. The feeding regime may thus have a pronounced effect on the measured BCF. In the literature many studies are found where radio-labelled compounds are used, and where the fish are not fed. In these studies the bioconcentration may in most cases have been overestimated. As these concepts are of limited applicability to metals and metal compounds (relevant for assessment for assessment under Annex IV according to Annex IV.1), please see Annex IV.4 for more information.

III.2.2 Octanol-water-partitioning coefficient (K_{ow})

For organic substances experimentally derived high-quality K_{ow} values are preferred over other determinations of K_{ow}. When no experimental data of high quality are available, validated Quantitative Structure Activity Relationship ((Q)SAR) results for log K_{ow} may be used in the classification process. Such (Q)SAR results may be used without modification to the agreed criteria when they meet the validity criteria indicated in IR&CSA Chapter R.6. These requirements include, among others, the use of a scientifically valid model and that the substance falls within its applicability domain. For substances like strong acids and bases, substances which react with the eluent, or surface-active substances, a (Q)SAR estimated value of $K_{ow,}$ or an estimate based on individual *n*-octanol and water solubilities should be provided instead of an analytical determination of K_{ow}. For ionisable substances, measurements should be taken on their non-ionised form (free acid or free base) only by using an appropriate buffer with pH below pK for free

acid or above the pK for free base. Similarly, (Q)SAR estimates should be based on the non-ionised structure. If multiple log K_{ow} data are available for the same substance, the reasons for any differences should be assessed before selecting a value. Generally, the highest valid value should take precedence.

III.2.2.1 Experimental determination of K_{ow}

For experimental determination of K_{ow} values, several different methods are described in standard guidelines. IR&CSA Chapter R.7a, section 7.1.8.3 gives guidance on direct measurement methods (Shake Flask Method, Generator Column Method, and Slow Stirring Method), and on one indirect measurement method (Reverse Phase HPLC Method).

III.2.2.2 Use of (Q)SARs for determination of log K_{ow} and BCF

Numerous (Q)SARs have been and continue to be developed for the estimation of K_{ow} and BCF. When an estimated K_{ow} value is found, the estimation method has to be taken into account. Further guidance is also provided in IR&CSA Chapter R.7a, section 7.1.8.4. (Q)SARs can also be used to derive BCF values. However, as CLP (I.4.1.2.8) clearly prefers experimental BCF data and experimental log K_{ow} data where available, (Q)SAR BCF data can only be considered as part of a broader WoE approach. As for other endpoints derived using (Q)SARs, careful attention should be paid to the validity of the result as further detailed in IR&CSA Chapter R.6. It should be noted that due to the wording of CLP (I.4.1.2.8), (Q)SAR BCF data cannot be used as a one for one substitute for experimental BCF or log K_{ow} data. Furthermore, following the weighting of data described above (Q)SAR BCFs derived using as input experimental data (*e.g.* log K_{ow} and intrinsic clearance data from OECD TG 319A and B) should generally be given greater weight than those where the log K_{ow} and other source data is calculated. Examples of freely available (Q)SAR software programs that include models for the prediction of log K_{ow} and BCF are EPISuite (https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface), OECD (Q)SAR Toolbox (https://qsartoolbox.org/) and VEGA (https://www.vegahub.eu/).

III.3 Chemical classes that need special attention with respect to BCF and K_{ow} values

There are certain physico-chemical properties of substances, which can make the determination of BCF or its measurement difficult. These may be substances, which do not bioconcentrate in a manner consistent with their other physico-chemical properties, e.g. steric hindrance or substances which make the use of descriptors inappropriate, e.g. surface activity, which makes both the measurement and use of log K_{ow} inappropriate.

III.3.1 Substances difficult to test

The methods presented above are generally designed for non-ionised organic substances. They are therefore of limited usefulness for a large number of other substances, collectively termed difficult substances, which include complex mixtures and chemicals that are charged at environmental pH (such as inorganic compounds). Substances difficult to test may be poorly soluble substances, complex mixtures, high molecular weight substances, surface active substances, inorganic substances, ionisable substances, or organic substances that do not partition to lipid. Some guidance is given in this Chapter. More detailed guidance is provided in IR&CSA Chapter R.7c, mainly in Appendix R.7.10-3.

In order to bioconcentrate in aquatic organisms, an organic substance needs to be present in the water, available for transfer across the fish gills and soluble in lipids. Factors that may alter this availability will thus change the actual bioconcentration of a substance, when compared with the prediction. For example, readily biodegradable substances may only be present in the aquatic compartment for short periods of time. Similarly, volatility, and hydrolysis will reduce the concentration and the time during which a substance is available for uptake. A further important

parameter, which may reduce the actual exposure concentration of a substance, is adsorption, either to particulate matter or to surfaces in general.

There are a number of substances, which have shown to be rapidly transformed in the organism, thus leading to a lower BCF value than expected. Substances that form micelles or aggregates may bioconcentrate to a lower extent than would be predicted from simple physico-chemical properties, *e.g.* water solubility. This is also the case for hydrophobic substances that are contained in micelles formed as a consequence of the use of dispersants. Therefore, the use of dispersants in bioaccumulation tests is discouraged. Further guidance is given in IR&CSA Chapter R.7c, section 7.10.3.4 on how to consider the factors that affect the bioaccumulation potential of many substances and that are important especially in the absence of a fully valid BCF test result.

In general, for substances difficult to test, measured BCF and K_{ow} values – based on the parent substance – are a prerequisite for the determination of the bioconcentration potential. Furthermore, proper documentation of the test concentration is a prerequisite for the validation of the given BCF value.

III.3.2 Poorly soluble and complex substances

Special attention should be paid to poorly soluble substances. Frequently the solubility of these substances is recorded as less than the detection limit, which creates problems in interpreting the bioconcentration potential. Where the test data indicate that the concentrations in the study are below the limit of detection, then the test is invalid and cannot be used. For such substances the bioconcentration potential should be based on experimental determination of log K_{ow} or (Q)SAR estimations of log K_{ow} (see Annex III.2.2). Complex substances contain a range of individual substances which can have a great variation in their physico-chemical and toxicological properties. It is generally not recommended to estimate an average or weighted BCF value. It is preferable to identify one or more representative constituents for further consideration. Further guidance is given in IR&CSA Chapter R.7c, Appendix R.7.10-3.

III.3.3 High molecular weight substances

A number of regulatory systems use molecular weight as an indicator for reduced or minimal bioconcentration. It is, however, concluded in IR&CSA Chapter R.7c, section 7.10.3.4 that molecular mass and size should not be used in isolation as confirmatory evidence of lack of bioaccumulation (ECETOC 2005). However, supported by other data and by employing expert judgement, it may be concluded by a weight of evidence argument that such substances are unlikely to have a high bioconcentration factor (regardless of the log K_{ow} value). More details can be found in PBT assessment guidance (IR&CSA Chapter R.11).

III.3.4 Surface-active substances (surfactants)

Surfactants consist of a non-polar, lipophilic part (most often an alkyl chain) (the hydrophobic tail) and a polar part (the hydrophilic headgroup). According to the charge of the headgroup, surfactants are subdivided into classes of anionic, cationic, non-ionic, or amphoteric surfactants. Due to the variety of different headgroups, surfactants are a structurally diverse class of compounds, which is defined by surface activity rather than by chemical structure. The bioaccumulation potential of surfactants should thus be considered in relation to the different subclasses (anionic, cationic, non-ionic, or amphoteric) instead of to the group as a whole. Surface-active substances may form emulsions, in which the bioavailability is difficult to ascertain. Micelle formation can result in a change of the bioavailable fraction even when the solutions are apparently formed, thus giving problems in interpretation of the bioaccumulation potential. See IR&CSA Chapter R.7c, Appendix R.7.10-3 for further guidance.

Measured (experimentally derived) BCF values on surfactants show that BCF tends to increase with increasing alkyl chain length and be dependent on the site of attachment of the head group, other structural features and whether the alkyl part is subject to biotransformation.

III.3.4.1 Octanol-water-partition coefficient (K_{ow})

The octanol-water partition coefficient for surfactants cannot be determined using the shake-flask or slow stirring method because of the formation of emulsions. In addition, the surfactant molecules will exist in the water phase almost exclusively as ions, whereas they will have to pair with a counter-ion in order to be dissolved in octanol. Therefore, experimental determination of K_{ow} does not characterise the partition of ionic surfactants (Tolls, 1998). On the other hand, it has been shown that the bioconcentration of anionic and non-ionic surfactants increases with increasing lipophilicity (Tolls, 1998). Tolls (1998) showed that for some surfactants, an estimated log K_{ow} value using LOGKOW could represent the bioaccumulation potential; however, for other surfactants some 'correction' to the estimated log K_{ow} value using the method of Roberts (1989) was required. These results illustrate that the quality of the relationship between log K_{ow} estimates and bioconcentration depends on the class and specific type of surfactants involved. Therefore, the classification of the bioconcentration potential based on log K_{ow} values should be used with caution. Further guidance is provided in IR&CSA Chapter R.7c, Appendix R.7.10-3.

III.4 Conflicting data and lack of data

III.4.1 Conflicting BCF data

When multiple BCF data are available for the same substance, the possibility of conflicting results may arise. In general, conflicting results for a substance, which has been tested several times with an appropriate bioconcentration test, should be interpreted by a 'weight of evidence approach'. This implies that if experimentally determined BCF data, both ≥ and < 500, have been obtained for a substance, the data of the highest quality and with the best documentation should be used for determining the bioconcentration potential of the substance. If differences still remain, for example high-quality BCF values for different fish species are available, generally the highest valid value should be used as the basis for classification. When larger data sets (4 or more values) are available for the same species and life stage, the geometric mean of the BCF values may be used as the representative BCF value for that species (IR&CSA Chapter R.7c, section R.7.10.4.6).

III.4.2 Conflicting log K_{ow} data

When multiple log K_{ow} data are available for the same substance, the possibility of conflicting results might arise. If log K_{ow} data both ≥ and < 4 have been obtained for a substance, then the data of the highest quality and the best documentation should be used for determining the bioconcentration potential of the substance. If differences still exist, generally the highest valid value should take precedence. In such situation, (Q)SAR estimated log K_{ow} could be used as guidance.

III.4.3 Expert judgement

If no experimental BCF or log K_{ow} data or no predicted log K_{ow} data are available, the potential for bioconcentration in the aquatic environment may be assessed by expert judgement. This may be based on a comparison of the structure of the molecule with the structure of other substances for which experimental bioconcentration or log K_{ow} data or predicted K_{ow} are available. IR&CSA Chapter R.7c, gives guidance on read-across and categories in section R.7.10.3.2.

III.5 Decision scheme

Based on the above discussions and conclusions, a decision scheme has been elaborated which may facilitate decisions as to whether or not a substance has the potential for bioconcentration in aquatic species. A conclusion on (the potential for) bioaccumulation is required under CLP and a conclusion should always be presented. In the event that available data is genuinely equivocal,

a conclusion of 'inconclusive' may be acceptable but a positive/negative conclusion is clearly preferred. For metals and metal compounds (relevant for assessment under Annex IV according to Annex IV.1), please see Annex IV.4.

Experimentally derived BCF values of high quality are ultimately preferred for classification purposes. BCF results from poor or questionable quality studies should preferably not be used for classification purposes. If no BCF is available for fish species, high quality data on the BCF for some invertebrates (e.g. blue mussel, oyster and/or scallop) may be used as a worst case surrogate. As discussed in III.2.2.2, BCF (Q)SARs cannot be placed in the decision scheme due to the considerations expressed in CLP (I.4.1.2.8) but can be used as part of a WoE assessment.

For non-ionised organic substances, experimentally derived high quality K_{ow} values, or values which are evaluated in reviews and assigned as the "recommended values", are preferred. If no experimental data of high quality are available, validated Quantitative Structure Activity Relationships ((Q)SARs) for log K_{ow} may be used in the classification process. Such validated (Q)SARs may be used without modification in relation to the classification criteria, if restricted to chemicals for which their applicability is well characterised. For difficult substances like strong acids and bases, metal complexes, and surface-active substances a (Q)SAR estimated value of $K_{ow,}$ or an estimate based on individual *n*-octanol and water solubilities could be provided instead of an analytical determination of K_{ow}.

If data are available but not validated, expert judgement should be used. Attempts to reach a conclusion should be made with all available data under a weight of evidence approach, where possible.

As mentioned in the first paragraph above, whether or not a substance has a potential for bioaccumulation in aquatic organisms could thus be decided in accordance with the following scheme:

Valid/high quality experimentally determined BCF value → YES:

→ BCF ≥ 500: *The substance has a potential for bioaccumulation in the aquatic environment*

→ BCF < 500: *The substance does not have a potential for bioaccumulation in the aquatic environment*

Valid/high quality experimentally determined BCF value → NO:

→ Valid/high quality experimentally determined log K_{ow} value → YES:

→ log K_{ow} ≥ 4: *The substance has a potential for bioaccumulation in the aquatic environment*

→log K_{ow} < 4: *The substance does not have a potential for bioaccumulation in the aquatic environment*

Valid/high quality experimentally determined BCF value → NO:

Valid/high quality experimentally determined log K_{ow} value → NO:

Use of validated (Q)SAR for estimating a log K_{ow} value → YES:

→ log K_{ow} ≥ 4: *The substance has a potential for bioaccumulation in the aquatic environment*

→ log K_{ow} < 4: *The substance does not have a potential for bioaccumulation in the aquatic environment*

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IV ANNEX IV: METALS AND INORGANIC METAL COMPOUNDS

IV.1 Introduction

The harmonised system for classifying chemical substances is a hazard-based system, and the basis of the identification of hazard is the aquatic toxicity of the substances, and information on the degradation and bioaccumulation behaviour (OECD 2001). Since this document deals only with the hazards associated with a given substance when the substance is dissolved in the water column, exposure from this source is limited by the solubility of the substance in water and bioavailability of the substance to organisms in the aquatic environment. Thus, the hazard classification schemes for metals and metal compounds are limited to the acute and long-term hazards posed by metals and metal compounds when they are available (*i.e.* exist as dissolved metal ions, for example, as M+ when present as M-NO_3), and do not take into account exposures to metals and metal compounds that are not dissolved in the water column but may still be bioavailable, such as metals in foods. This section does not take into account the non-metallic ion (e.g. CN^-) of metal compounds which may be toxic. For such metal compounds the hazards of the non-metallic ions must also be considered.

Also organometal compounds may be of concern given they may pose bioaccumulation or persistence hazards. Organometals do not dissociate or dissolve in water as the metal ion, as metals and inorganic metal compounds do. Organometals (e.g. methyl mercury or tributyltin) that do not release metal ions are thereby excluded from the guidance of this section and should be classified according to the general guidance provided in part 4. Metal compounds that contain an organic component but that dissociate easily in water or dissolve as the metal ion should be treated in the same way as metal compounds and classified according to this annex (e.g. zinc acetate).

The level of the metal ion which may be present in solution following the addition of the metal and/or its compounds, will largely be determined by two processes: the extent to which it can be dissolved, *i.e.* its water solubility, and the extent to which it can react with the media to transform to water soluble forms. The rate and extent at which this latter process, known as 'transformation' for the purposes of this guidance, takes place can vary extensively between different compounds and the metal itself, and is an important factor in determining the appropriate hazard class. Where data on transformation are available, they should be taken into account in determining the classification. The Protocol for determining this rate is available as Annex 10 to UN GHS.

Generally speaking, the rate at which a substance dissolves is not considered relevant to the determination of its intrinsic toxicity. However, for metals and many poorly soluble inorganic metal compounds, the difficulties in achieving dissolution through normal solubilisation techniques are so severe that the two processes of solubilisation and transformation become indistinguishable. Thus, where the compound is sufficiently poorly soluble that the levels dissolved following normal attempts at solubilisation do not exceed the available L(E)C_{50}, it is the rate and extent of transformation, which must be considered. The transformation will be affected by a number of factors, not least of which will be the properties of the media with respect to pH, water hardness, alkalinity, temperature etc. In addition to these properties, other factors such as the size and, in particular, the specific surface area of the particles which have been tested, the length of time over which exposure to the media takes place and, of course the mass or surface area loading of the substance in the media will all play a part in determining the level of dissolved metal ions in the water. Transformation data can generally, therefore, only be considered as reliable for the purposes of classification if conducted according to the standard protocol in Annex 10 to UN GHS. This protocol aims at standardising the principal variables such that the level of dissolved ion can be directly related to the loading of the substance added. It is this loading level which yields the level of metal ion equivalent to the available L(E)C_{50} or NOEC/EC_{10} that can then be used to determine the acute or long-term hazard category appropriate for classification. The testing methodology is detailed in Annex 10 to UN GHS. The strategy to be adopted in using the

data from the testing protocol, and the data requirements needed to make that strategy work, are described in Annex IV.2, IV.3 and in more detail in Annex IV.5 of this document.

In considering the classification of metals and metal compounds, both readily and poorly soluble, recognition has to be paid to a number of factors. As defined in Annex II.1, the term 'degradation' refers to the decomposition of organic molecules. For inorganic compounds and metals, clearly the concept of degradability, as it has been considered and used for organic substances, has limited or no meaning. Rather, the substance may be transformed by normal environmental processes to either increase or decrease the bioavailability of the toxic species. Equally, the log K_{ow} cannot be considered as a measure of the potential to accumulate. Nevertheless, the concept that a substance, or a toxic metabolite/reaction product may not be rapidly lost from the environment and/or may bioaccumulate, are as applicable to metals and metal compounds as they are to organic substances.

Speciation of the soluble form can be affected by pH, water hardness and other variables, and may yield particular forms of the metal ion which are more or less toxic. In addition, metal ions could be made non-available from the water column by a number of processes (e.g. mineralisation and partitioning). Sometimes these processes can be sufficiently rapid to be analogous to degradation in assessing chronic (long-term) aquatic hazard. However, partitioning of the metal ion from the water column to other environmental media does not necessarily mean that it is no longer bioavailable, nor does it necessarily mean that the metal has been made permanently unavailable.

Information pertaining to the extent of the partitioning of a metal ion from the water column, or the extent to which a metal has been or can be converted to a form that is less toxic or non-toxic is frequently not available over a sufficiently wide range of environmentally relevant conditions, and thus, a number of assumptions will need to be made as an aid in classification. These assumptions may be modified if available data show otherwise. In the first instance it should be assumed that the metal ions, once in the water, are 'not rapidly partitioned' from the water column. Underlying this is the assumption that, although speciation can occur, the species will remain available under environmentally relevant conditions. This may not always be the case, as described above, and any evidence available that would suggest changes to the bioavailability over the course of 28 days, should be carefully examined.

The bioaccumulation of metals and inorganic metal compounds is a complex process and bioaccumulation data should be used with care. The application of bioaccumulation criteria will need to be considered on a case-by-case basis taking due account of all the available data.

A further assumption that can be made, which represents a cautious approach, is that, in the absence of any solubility data for a particular metal compound, either measured or calculated, the metal compound will be assumed to be sufficiently soluble to cause toxicity at the level of the ecotoxicity reference value (ERV), being the acute ERV (expressed as $L(E)C_{50}$), and/or the chronic ERV (expressed as the NOEC/EC_x or an HC5 for extensive data sets) and thus may be classified in the same way as other soluble salts of the metal. Again, this is clearly not always the case, and it may be wise to generate appropriate solubility data. Absence of solubility data on the metallic form for a metal for which the soluble salts are classified for the environment, will therefore lead to a default classification due to potential hazard concerns.

This Annex IV deals with metals and inorganic metal compounds. Within the context of this guidance document, metals and metal compounds are characterised as follows:

a. metals (M^0) in their elemental state are not soluble in water but may transform to yield the available form (e.g. Fe^0 will not dissolve as such, but the Fe^0 molecules present at the surface of a massive/powder will be first transformed into Fe^{2+} or Fe^{3+} compounds prior to their solubilisation). This means that a metal in the elemental state may react with water or a dilute aqueous electrolyte to form soluble cationic or anionic products, and in the process the metal will oxidise, or transform, from the neutral or zero oxidation state to a higher one;

b. in a simple metal compound, such as an oxide or sulphide, the metal already exists in the oxidised state, so that further metal oxidation is unlikely to occur when the compound is introduced into an aqueous medium.

Organo-metals are outside the scope of this section.

While oxidisation may not change, interaction with the media may yield more soluble forms. A sparingly soluble metal compound can be considered as one for which a solubility product can be calculated, and which will yield a small amount of the available form by dissolution. However, it should be recognised that the final solution concentration may be influenced by a number of factors, including the solubility product of some metal compounds precipitated during the transformation/dissolution test, e.g. aluminium hydroxide.

IV.2 Application of aquatic toxicity data and solubility data for classification

IV.2.1 Interpretation of aquatic toxicity data

Ecotoxicity data of soluble inorganic compounds are used and combined to define the toxicity of the metal ion under consideration. The ecotoxicity of soluble inorganic metal compounds is dependent on the physico-chemistry of the medium, irrespective of the original metal species released in the environment. Reading across metal compounds can therefore be conducted by comparing the soluble metal ion concentration (µg Me/L) causing the ecotoxicity effect and translating this towards the compound under investigation. A molecular weight correction of the ecotoxicity reference value may be required to classify soluble metal compounds (MW soluble substance/MW metal ion[137]). Poorly soluble metal compounds and metals do not require Molecular weight correction given the amount used for Transformation Dissolution already recognises this into the loading calculation. The comparison is therefore directly done by comparing the soluble fraction measured after Transformation Dissolution with the ecotoxicity reference values of the soluble metal ion (based on the UN GHS, 2009).

When evaluating ecotoxicity data, the general guidance on the weight of evidence (see section 4.1.3.2.4 of this document) is also applicable to metals.

The term adequacy covers here both the **reliability** *(*inherent quality of a test relating to test methodology and the way that the performance and results of a test are described) and the **relevance** *(*extent to which a test is appropriate to be used for the derivation of an ecotoxicity reference value) of the available ecotoxicity data.

Under the reliability criteria, metal specific considerations include the description of some abiotic parameters in the test conditions for enabling the consideration of the bioavailable metal concentration and free metal ion concentration:

- **Description of the physical test conditions**: further to the general parameters (O_2, T°, pH, …) abiotic parameters such as dissolved organic carbon (DOC), hardness, alkalinity of the water that govern the speciation and hence the metal bioavailability is required. A proper description of culture conditions related to the level of essential metals is required to avoid artefacts due to acclimatisation/adaptation (see also below);
- **Description of test materials and methods**: to calculate the free metal ion concentration with speciation models the concentrations of dissolved major ions and cations like Al, Fe, Mg, Ca… are required;
- **Concentration-effect relationship; hormesis**: sometimes an increased performance in growth or reproduction is seen at low metal doses that exceed the control values, referred to as hormesis. Such effects can be important especially for major trace nutrients such as

[137] Note that this calculation needs to be adjusted to reflect the stoichiometry of the compound, for example for $Zn_3(PO_4)_2$ the MW metal would be multiplied by three.

Fe, Zn and Cu but can also occur with a wide variety of non-essential substances. In such cases, positive effects should not be considered in the derivation of acute ERV's and especially chronic ERV's, likely other models than the conventional log-logistic dose-response model should be used to fit the dose-response curve and consideration should be given to the adequacy of the control diet/exposure. Due to the essential nutritional needs, caution is needed with regards to extrapolation of the dose-response curve (e.g. to derive an acute ERV) below the lowest tested concentration.

Under the relevancy criteria, certain considerations need to be made, related to the relevancy of the test substance and to acclimatisation/adaptation:

- **Relevance of the test substance**: soluble metal salts should be used for the purpose of classification of inorganic metals/metal compounds. The ecotoxicity adapted from organic metal compounds exposure should not be used.
- **Acclimatisation/adaptation**: for essential metals, the culture medium should contain a minimal concentration not causing deficiency for the test species used. This is especially relevant for organisms used for long-term toxicity tests where the margin between essentiality and toxicity may become small. As an example, for algae, depletion of the strong complexing agent EDTA from the medium may result in iron deficiency.

Aquatic toxicity studies carried out according to a recognised protocol should normally be acceptable as valid for the purposes of classification. Annex I should also be consulted for generic issues that are common to assessing any aquatic toxicity data point for the purposes of classification.

IV.2.1.1 Metal complexation and speciation

The toxicity of a particular metal in solution, appears to depend primarily on (but is not strictly limited to) the level of dissolved free metal ions and the physico-chemistry of the environment. Abiotic factors including alkalinity, ionic strength and pH can influence the toxicity of metals in two ways: (i) by influencing the chemical speciation of the metal in water (and hence affecting the availability) and (ii) by influencing the uptake and binding of available metal by biological tissues. For the classification of metals, Transformation/Dissolution is carried out over a pH range. Ideally both T/D and ecotoxicity data are compared at a similar pH since both parameters will vary with pH. However, the majority of ecotoxicity tests are performed at the higher pH range (*i.e.* > pH 7.5) and ecotoxicity data obtained at lower pH are often scarce. Bioavailability and speciation models (e.g. respectively Biotic Ligand Models and WHAM (Tipping, 1994), as discussed below) may allow to normalise ecotoxicity data obtained at a given pH to other pH values, relevant to the T/D data. The applicability of the bioavailability models to the biological species for which data are available must be evaluated. Guidance on the Bioavailability correction for metals can be found in IR&CSA Chapter R.7c, section R.7.13.1).

Where chemical speciation is important, it may be possible to model the concentrations of the different chemical forms of the metal, including those that are likely to cause toxicity. Analysis methods for quantifying exposure concentrations, which are capable of distinguishing between the complexed and uncomplexed fractions of a test substance, may not always be available or economic.

Complexation of metals to organic and inorganic ligands in test media and natural environments can be estimated from metal speciation models. Speciation models for metals, including pH, hardness, DOC, and inorganic substances such as MINTEQ (Brown and Allison, 1987), WHAM (Tipping, 1994) and CHESS (Santore and Driscoll, 1995) can be used to calculate the uncomplexed and complexed fractions of the metal ions.

Alternatively, and when available for the metal, the Biotic Ligand Model (BLM), allows, for the calculation of the acute and/or chronic ERV's of the metal ion, for different pH values, through integration of metal speciation and its interaction with the organism. The BLM model has at present been validated for a number of metals, organisms, and endpoints (Santore and Di Toro, 1999). The models and formula used for the characterisation of metal complexation in the media should always be clearly reported, allowing for their translation back to natural environments (OECD, 2000). In case a metal-specific BLM is available covering an appropriate pH range, a normalised comparison of aquatic toxicity data can be made using the entire effects database for different reference pH values.

IV.2.2 Interpretation of solubility data

When considering the available data on solubility, their validity and applicability to the identification of the hazard of metal compounds should be assessed. In particular, the pH and the medium in which the data were generated should be known.

IV.2.2.1 Assessment of existing data

Existing data will be in one of the three forms: **for soluble, insoluble metal compounds and the metallic form**. For some well-studied metals, there will be solubility products and/or solubility data for the various inorganic metal compounds. It is also possible that the pH relationship of the solubility will be known. However, for many metals or metal compounds, it is probable that the available information will be descriptive only, e.g. poorly soluble or resulting from the water solubility test from OECD TG 105 physico-chemical water dissolution test. Unfortunately there appears to be very little (consistent) guidance about the solubility ranges for such descriptive terms. Where these are the only information available it is most probable that solubility data will need to be generated using the Transformation/Dissolution Protocol (Annex 10 to UN GHS).

IV.2.2.2 Screening T/D test for assessing solubility of metal compounds

In the absence of solubility data, a simple 'Screening Test' for assessing solubility, based on the high rate of loading (100 mg/L) for 24 h and rigid stirring conditions, should be used for metal compounds as described in the Transformation/Dissolution Protocol (Annex 10 to UN GHS). The function of the screening test is to identify those metal compounds which undergo either dissolution or rapid transformation such that they are indistinguishable from soluble forms and hence may be classified based on the dissolved ion concentration and those who dissolves slowly and can be assessed in the same way as the metallic form. Where data are available from the screening test detailed in the Transformation/Dissolution Protocol, the maximum solubility obtained over the tested pH range should be used. Where data are not available over the full pH range, a check should be made that this maximum solubility has been achieved by reference to suitable thermodynamic speciation models or other suitable methods (see Annex IV.2.1 of this document). It should be noted that this test is only intended to be used for inorganic metal compounds. Metals should immediately be assessed at the level of the full T/D test.

IV.2.2.3 Full T/D test for assessing solubility of metals and metal compounds

The Full Transformation Dissolution test should be carried out at the pH[138] that maximises the concentration of dissolved metal ions in solution and that expresses the highest toxicity.

Based on the data from the Full Test, it is possible to generate a concentration of the metal ions in solution after 7 days (short-term test) for each of the three loadings (*i.e.* 1 mg/L as 'low', 10 mg/L as 'medium' and 100 mg/L as 'high loading') used in the test. If the purpose of the test is to assess the long-term hazard of the substance, then the loadings[139] should be 0.01 mg/L, 0.1 mg/L or 1 mg/L depending on the transformation rate and the duration of the test being extended to 28 days (long-term test).

[138] The UN GHS transformation/dissolution protocol specifies a pH range of 6-8.5 for the 7days test and 5.5 to 8.5 for the 28 days test. Considering the difficulty in carrying out transformation/dissolution tests at pH 5.5, the OECD only validated the test in the pH range of 6-to 8.5.

[139] The standard protocol in Annex 10 to UN GHS presently only foresees a long-term loading rate of 1 mg/l and lower loading rates may not even be practically feasible for each case. While TDp testing at lower loading rates is in principle the best way forward it is technically often not feasible for the lower chronic loading rates. Extensive experience with the T/D protocol demonstrated that reliable predictions can be made for other loading rates. In order to make maximal use of existing Transformation Dissolution data, the 28 days results for the lower chronic loading rates (0,1 and 0,01 mg/l) can therefore be derived by extrapolation from TDp evidence from other loading rates. Such read-across should be justified on a case by case basis and supported by reliable information on the T/D at different loading rates, *e.g*. over 7 and/or 28 days. It should be noted that the relationship between loading rate and dissolved metal concentration may well not be linear. Therefore, extrapolation of T/D data to lower loadings should preferably be made by using the equations of section A10.6.1 of the UN-Annex 10 transformation dissolution protocol or alternatively by extrapolating in a precautionary way.

The UN announced to change/update Annex 10 in the near future to bring it better in line with the chronic classification strategy an aim that is already anticipated in this guidance note for the CLP.

IV.2.3 Comparison of aquatic toxicity data and solubility data

A decision on whether or not the substance is classified will be made by comparing aquatic toxicity data and solubility data. Depending on the available data two approaches can be followed.

1. When only a **limited dataset** is available existing data should be taken together irrespective of whether the toxicity and dissolution data are at the same pH and the lowest data point should give the basis for classification (this should be used as the default approach). This default approach may lead to the lowest toxicity data point compared with the highest Transformation Dissolution result each derived at different pH levels used for the purpose of classification.
2. When a more **extensive toxicity/dissolution dataset** is available, a split of the acute and chronic ecotoxicity reference values can be performed according to their pH used during T/D test. The worst case classification entry across pHs should be used based on comparing TDp data with relevant ecotox data across the pH range. Meaning that toxicity data and transformation data are in this case always compared at the same pH.

This split of the effects data into pH classes would apply in an equal way to the acute and the long-term effects data sets.

IV.3 Assessment of environmental transformation

Environmental transformation of one species of a metal to another species of the same metal does not constitute 'degradation' as applied to organic compounds and may increase or decrease the availability and bioavailability of the toxic species. In addition naturally occurring geochemical processes can partition metal ions from the water column while also other processes may remove metal ions from the water column (e.g. by precipitation and speciation). Data on water column residence time, transformation in the aquatic environment, and the processes involved at the water – sediment interface (*i.e.* deposition and re-mobilisation) are fairly extensive for some metals. Using the principles and assumptions discussed above in Annex IV.1 of this document, it may therefore be possible to incorporate this approach into the classification.

Such assessments are difficult to give guidance for and will normally be addressed on a case-by-case approach. However, the following multiple lines of evidence that could be considered may be taken into account:

a. Changes in speciation if they are to non-available forms, however, the potential for the reverse change to occur must also be considered;
b. Changes in speciation to species which are considerably less soluble than that of the metal compound being considered.

Some caution is recommended; see Annex IV.1 of this document, the 5th and 6th paragraph.

⚠ Comment by ECHA: Two workshops were held on the issue, in 2012 and 2019. The 2012 workshop defined " types of metals in respect to Rapid Removal (RR) mechanisms: 1) metals that methylate and are by default not considered as rapidly transformed (*e.g.* Hg), 2) metals that quickly hydrolyse and form different products that quickly precipitate in the water column (Al and Fe) for which the new species can be considered as rapidly transforming and 3) metals for which the key question is 'irreversibility' (*i.e.*, binding to a non-bioavailable form under a range of environmental conditions). A second workshop (WS) was held in June 2019 to discuss the issue of Rapid Removal (RR) for type 3 metals. The conclusion of the WS was that at this stage neither RR nor the extended T/D protocol (T/Dp-E) were suitable for use under CLP (CA_68_2019) and that metals shall only be assessed on a case-by-case basis evaluating the available evidence. This conclusion was brought to CARACAL 32, which agreed with the conclusion (CA_101_2019). Consequently, neither RR nor the T/Dp-E are considered

suitable for use under CLP without further technical advancements of the science and case by case data sets. Instead, environmental transformation as described in IV.3 shall be used for classification and labelling. Annex IV example D provides a practical example, taking both chemical transformation and potential removal mechanisms into account.

IV.4 Bioaccumulation

While log K_{ow} is a good predictor of BCF for certain types of organic compounds e.g. non-polar organic substances, it is irrelevant for inorganic substances such as inorganic metal compounds because metals, in contrast to organic substances, are not lipophilic and are not passively transported through cellular membranes. Uptake of metal ions occurs through active processes.

The mechanisms for uptake and depuration rates of metals are very complex and variable and there is at present no general model to describe this. Instead the bioaccumulation of metals according to the classification criteria should be evaluated on a case-by-case basis using expert judgement.

While BCFs are indicative of the potential for bioaccumulation there may be a number of complications in interpreting measured BCF values for metals and inorganic metal compounds. For most metals and inorganic metal compounds the relationship between water concentration and BCF in aquatic organisms is inverse, and bioconcentration data should therefore be used with care. This is particularly relevant for metals that are biologically essential. Metals that are biologically essential are actively regulated in organisms in which the metal is essential (homeostasis). Removal and sequestration processes that minimise toxicity are complemented by an ability to up-regulate concentrations for essentiality. Since nutritional requirement of the organisms can be higher than the environmental concentration, this active regulation can result in high BCFs and an inverse relationship between BCFs and the concentration of the metal in water. When environmental concentrations are low, high BCFs may be expected as a natural consequence of metal uptake to meet nutritional requirements and can in these instances be viewed as a normal phenomenon. Also, while a metal may be essential in a particular organism, it may not be essential in other organisms. Therefore, where the metal is not essential or when the bioconcentration of an essential metal is above nutritional levels, special consideration should be given to the potential for bioconcentration and environmental concern.

Non-essential metals are also actively regulated to some extent and therefore also for non-essential metals, an inverse relationship between the metal concentration and the external concentration may be observed (McGeer *et al*., 2003).

Consequently for both essential and non-essential elements, measured BCFs decline as external concentration increases. When external concentrations are so high that they exceed a threshold level, or overwhelm the regulatory mechanism, this can cause harm to the organism.

BCF and BAF may be used to estimate metal accumulation by:

a. Considering information on essentiality and homeostasis of metals/ metal compounds. As a result, of such regulation, the 'bioaccumulative' criterion is not applicable to these metals.

b. Assessing bioconcentration factors for non-essential metals, should preferably be done from BCF studies using environmentally relevant concentrations in the test media.

IV.5 Classification strategies for metals and metal compounds

IV.5.1 Introduction

⚠ Notice! *Acute and long-term hazards* are assessed individually.

For determination of long-term hazards preference should be given in applying the approach based on chronic toxicity data. Such evidence is often frequently available for the bioavailable forms of metals.

The schemes for the determination of acute and long-term aquatic hazards of metals and metal compounds are described below and summarised diagrammatically in the figures:

IV.5.2.1 (acute hazard classification of metals);

IV.5.2.2 (a and b) (long-term hazard of metals);

IV.5.3.1 (acute hazard classification of metal compounds);

IV.5.3.2 (a and b) (long-term hazard of metal compounds).

There are several stages in these schemes where data are used for decision purposes. It is not the intention of the classification schemes to generate new ecotoxicity data. In the absence of valid data, it will be necessary to use all available data and expert judgement.

In the following sections, the reference to the acute and chronic ERV's refer to the data point(s) that will be used to select the hazard category(ies) for the metal or metal compound.

When considering acute and chronic ERV's data for metal compounds, it is important to ensure that the data point to be used as the justification for the classification is expressed in the weight of the molecule of the metal compound to be classified. This is known as correcting for molecular weight. Thus while most metal data is expressed in, for example, mg/L of the metal (ion), this value will need to be adjusted to the corresponding weight of the metal compound. Thus:

Acute $ERV_{\mathbf{compound}}$ = acute ERV of the metal compound = acute ERV of metal ion x (Molecular weight of metal compound /atomic weight of the metal).

Chronic $ERV_{\mathbf{compound}}$ = chronic ERV of the metal compound = chronic ERV of metal ion x (Molecular weight of metal compound /atomic weight of the metal).

IV.5.2 Classification strategies for metals

⚠ Notice! *Acute and long-term hazards* are assessed individually.

IV.5.2.1 Classification strategy for determining acute aquatic hazard for metals

The scheme for the determination of *acute* aquatic hazard for metals are described in this section and summarised diagrammatically in Figure IV.1.

Where *the acute ERV* for the metal ions of concern is greater than 1 mg/L the metals need not be considered further in the classification scheme for acute hazard.

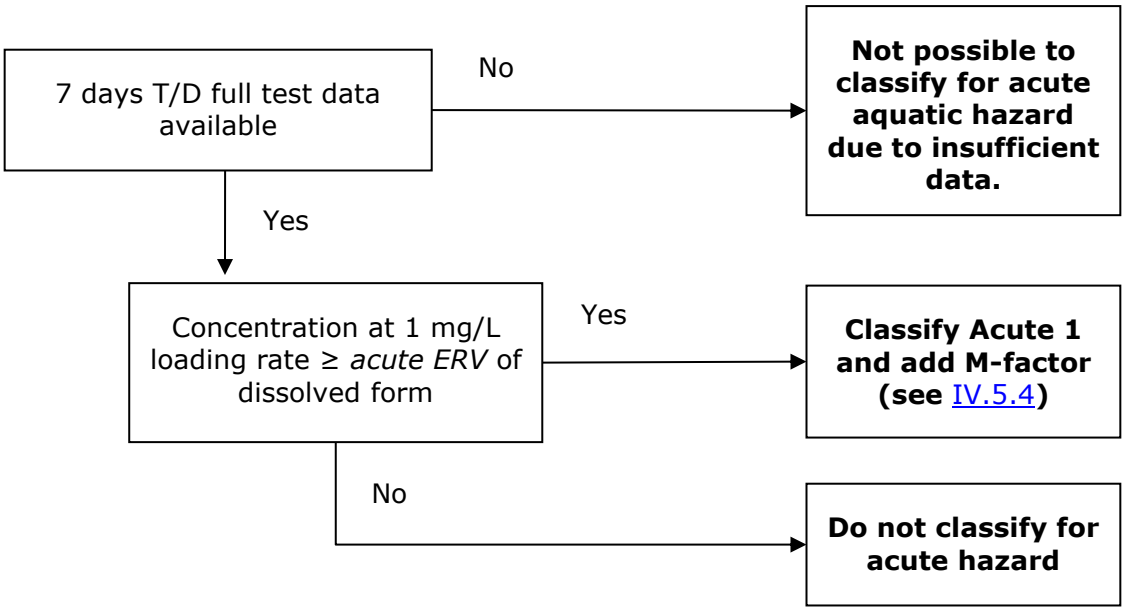
Where the acute ERV for the metal ions of concern is less than or equal to 1 mg/L consideration must be given to the data available on the rate and extent to which these ions can be generated from the metal. Such rate and extend data, to be valid and useable should have been generated using the Transformation/Dissolution Protocol (Annex 10 to UN GHS) for a 7d period.

Where 7d data from the Transformation/Dissolution protocol are available, then the results should be used to classify, according to the following rule:

Classify the metal as ***Category Acute 1*** if the dissolved metal ion concentration after a period of 7 days (or earlier for a significant time period) at a loading rate of 1 mg/L exceeds

that of the acute ERV, an M-factor must also be established as part of this classification (see IV.5.4).

Figure IV. 1 Classification strategy for determining acute aquatic hazard for metals



IV.5.2.2 Classification strategy for determining long-term aquatic hazard for metals

The scheme for the determination of *long-term* aquatic hazard for metals are described in this section and summarised diagrammatically in Figure IV.2 and IV.3.

Metals can be classified for long-term aquatic hazards:

1. using chronic reference data when available; or
2. using the surrogate approach in absence of appropriate chronic toxicity reference data.

In case relevant chronic ecotoxicity data (chronic ERV) are available the approach comparing chronic ERV with 28 days transformation/dissolution reference should be applied as described under IV.5.2.2.1 while otherwise the surrogate approach (see IV.5.2.2.2) should be followed.

IV.5.2.2.1 Approach based on available chronic toxicity reference data

Where *the chronic ERV* for the metal ions of concern is greater than 1 mg/L, the metals need not be considered further in the classification scheme.

Where the chronic ERV for the metal ions of concern is less than or equal to 1 mg/L consideration must be given to the data available on the rate and extent to which these ions can be generated from the metal. Such rate and extend data, to be valid and useable should have been generated using the Transformation/Dissolution Protocol (Annex 10 to UN GHS) for a 28 d period.

Where such T/Dp data are unavailable the surrogate approach should be applied (see Annex IV.5.2.2.2). Where 28d data from the Transformation/Dissolution protocol are available, then, the results should be used to aid classification according to the following rules:

a. **Classify** the metal as ***Category Chronic 1*** if the dissolved metal ion concentration obtained at a loading rate of 0.1 mg/L is greater than or equal to the chronic ERV, an M-factor must also be established as part of this classification (see IV.5.4); or

b. **Classify** the metal as ***Category Chronic 2*** if the dissolved metal ion concentration obtained at a loading rate of 1 mg/L is greater than or equal to the chronic ERV.

If there is evidence of rapid environmental transformation:

a. **Classify** the metal as ***Category Chronic 1*** if the dissolved metal ion concentration obtained at a loading rate of 0.01 mg/L is greater than or equal to the chronic ERV, an M-factor must also be established as part of this classification (see IV.5.4); or

b. **Classify** the metal as ***Category Chronic 2*** if the dissolved metal ion concentration obtained at a loading rate of 0.1 mg/L is greater than or equal to the chronic ERV; or

c. **Classify** the metal as ***Category Chronic 3*** if the dissolved metal ion concentration obtained at a loading rate of 1 mg/L is greater than or equal to the chronic ERV.

Do not classify for long-term hazard if the dissolved metal ion concentration obtained from the 28 day Transformation/Dissolution test at ***a loading rate of 1 mg/L*** is less than the chronic ERV of the metal ion.

IV.5.2.2.2 The surrogate approach

Where the acute ERV for the metal ions of concern is less than or equal to 100 mg/L consideration must be given to the data available on the rate and extent to which these ions can be generated from the metal. Such rate and extend data, to be valid and useable should have been generated using the Transformation/Dissolution Protocol (Annex 10 to UN GHS) for a 7d period.

Where such T/Dp data are unavailable, *i.e.* there is no clear data of sufficient validity to show that the transformation to metal ions will not occur; the safety net classification (Category Chronic 4) should be applied since the known classifiable toxicity of these soluble forms is considered to give rise to sufficient concern.

Where T/Dp data are available classification should be according to the following rules:

a. **Classify** the metal as ***Category Chronic 1*** if the dissolved metal ion concentration obtained from the 7 day transformation test at the low loading rate (1 mg/L) is greater than or equal to the acute ERV, an M-factor must also be established as part of this classification (see IV.5.4);

b. **Classify** the metal as ***Category Chronic 2*** if the dissolved metal ion concentration obtained from the 7 day transformation test at the medium loading rate (10 mg/L) is greater than or equal to the acute ERV;

c. **Classify** the metal as ***Category Chronic 3*** if the dissolved metal ion concentration obtained from the 7 day transformation test at the high loading rate (100 mg/L) is greater than or equal to the acute ERV.

d. **Classify** the metal as ***Category Chronic 4*** if the dissolved metal ion concentration obtained from the 7 day transformation test at the high loading rate (100 mg/L) is lower than the acute ERV.

Figure IV. 2 Classification strategy for determining long-term aquatic hazard for metals

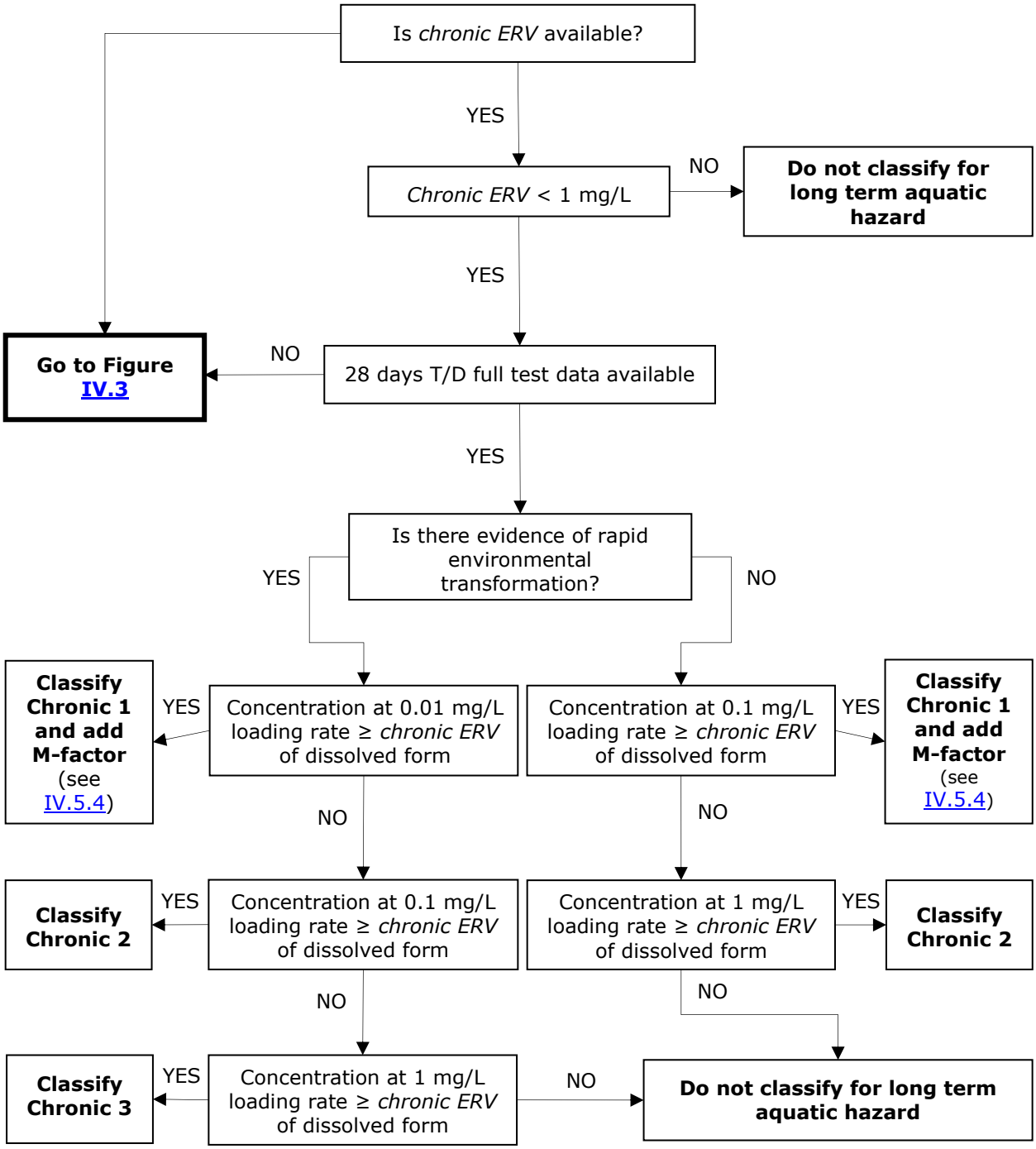
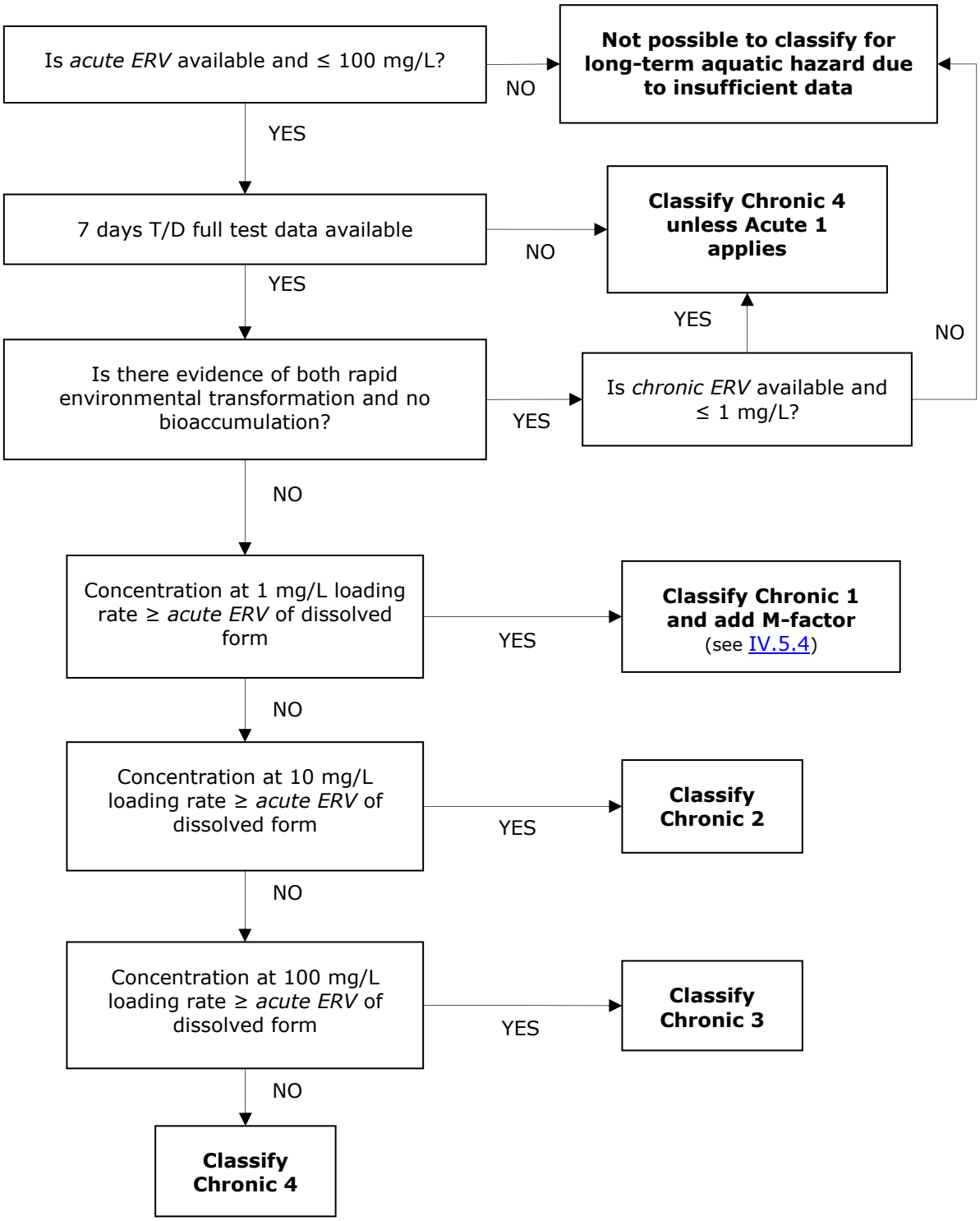


Figure IV. 3 Classification strategy for determining long-term aquatic hazard for metals in absence of appropriate chronic toxicity reference and/or T/Dp data



IV.5.3 Classification strategies for metal compounds

⚠ Notice! Acute and long-term hazards are assessed individually

A metal compound will be considered as ***readily soluble*** if:

- the water solubility (measured through a 24-hour Dissolution Screening test or estimated e.g. from the solubility product) is greater or equal to the acute ERV of the dissolved metal ion concentration; or
- if such data are unavailable, *i.e.* there are no clear data of sufficient validity to show that the transformation to metal ions will not occur.

Care should be exercised for metal compounds whose solubility is close to the acute toxicity reference value as the conditions under which solubility is measured could differ significantly from those of the acute toxicity test. In these cases the results of the Dissolution Screening Test are preferred.

Metal compounds that have lower water solubility than the acute ERV through a 24-hour Dissolution Screening test or estimated from the solubility product, are considered as ***poorly soluble metal compound.***

IV.5.3.1 Classification strategies for determining acute aquatic hazard for metal compounds

The scheme for the determination of *acute* aquatic hazard for metal compounds are described in this section and summarised diagrammatically in Figure IV.4.

Where the acute ERV for the metal ions of concern corrected for the molecular weight of the compound (further called as *acute* $ERV_{compound}$) is greater than 1 mg/L, the metal compounds need not to be considered further in the classification scheme for acute hazard.

Where the acute $ERV_{compound}$ is less than or equal to 1 mg/L, consideration must be given to the data available on the rate and extent to which these ions can be generated from the metal compound. Such data, to be valid and useable should have been generated using the T/D (Annex 10 to UN GHS).

Readily soluble metal compounds

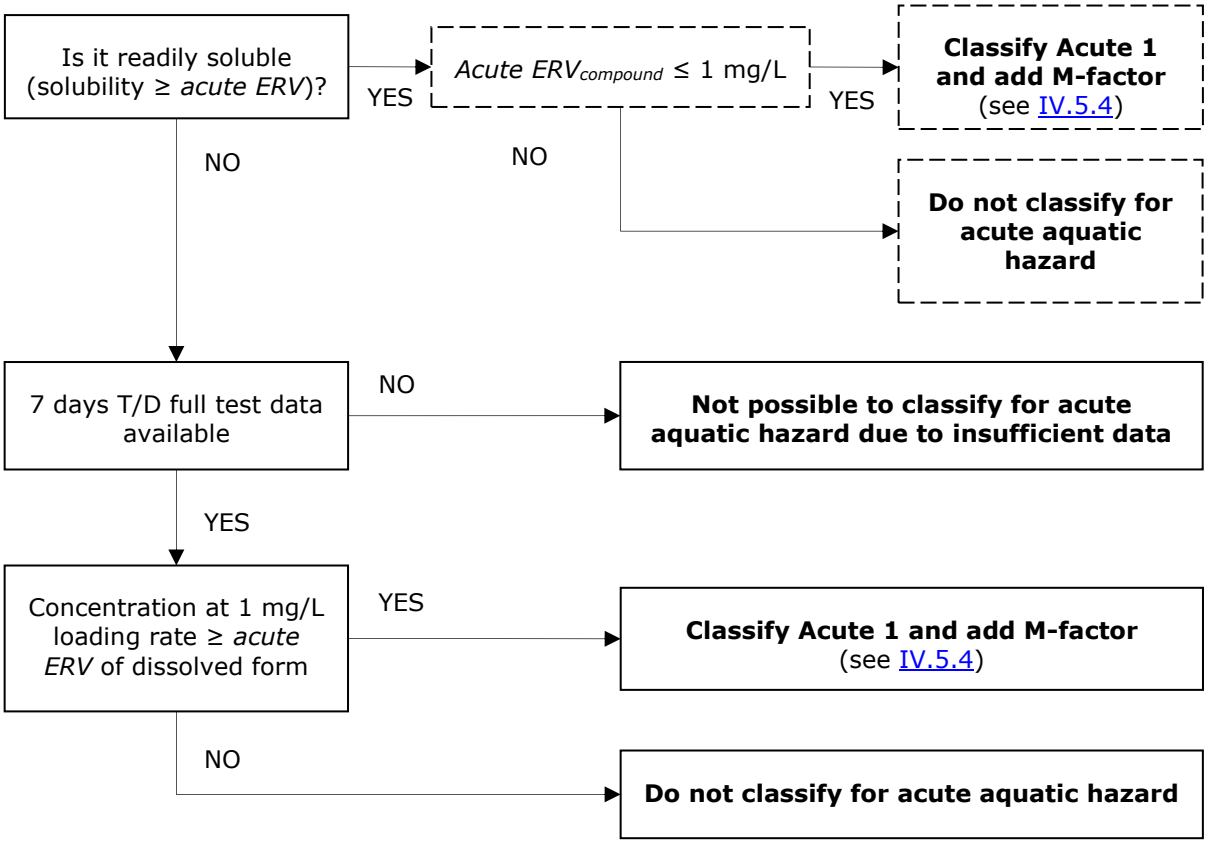
Classify the metal compound as ***Category Acute 1*** if the acute $ERV_{compound} \leq 1$ mg/L, an M-factor must also be established as part of this classification (see IV.5.4).

Poorly soluble metal compounds

Where 7d data from the Transformation/Dissolution protocol are available, then the results should be used to classify sparingly soluble metal compounds, according to the following rule:

> Classify the metal compound as *Category Acute 1* if the dissolved metal ion concentration after a period of 7 days (or earlier for a significant time period) at a loading rate of 1 mg/L exceeds that of the acute ERV, an M-factor must also be established as part of this classification (see IV.5.4).

Figure IV. 4 Classification strategy for determining acute aquatic hazard for metal compounds



IV.5.3.2 Classification strategy for determining long-term aquatic hazard for metal compounds

The scheme for the determination of *long-term* aquatic hazard for metal compounds are described in this section and summarised diagrammatically in Figure IV.5 and IV.6.

Metal compounds can be classified for long-term aquatic hazards:

1. using chronic reference data when available; or
2. using the surrogate approach in absence of appropriate chronic toxicity reference data.

In case relevant chronic ecotoxicity data (chronic ERV) are available the approach comparing chronic ERV of the dissolved metal ion with release data of 28 days transformation/dissolution, should be applied as described under IV.5.3.2.1 while otherwise the surrogate approach (see IV.5.3.2.2) should be followed.

IV.5.3.2.1 Approach based on available chronic toxicity reference data

Where the chronic ERV for the metal ions of concern corrected for the molecular weight of the compound (further called as *chronic ERV$_{compound}$*) is greater than 1 mg/L, the metal compounds need not to be considered further in the classification scheme for long-term hazard.

Readily soluble metal compounds

Readily soluble metal compounds are classified on the basis of chronic ERV of the dissolved metal ion, corrected for the molecular weight of the compound (further called as chronic ERV$_{compound}$) .

If there is *no evidence* of rapid environmental transformation:

a. **Classify** the metal compound as ***Category Chronic 1*** if the chronic ERV$_{compound}$ ≤ 0.1 mg/L, an M-factor must also be established as part of this classification (see IV.5.4); or

b. **Classify** the metal compound as ***Category Chronic 2*** if the chronic ERV$_{compound}$ > 0.1mg/L and ≤ 1 mg/L.

If there is *evidence* of rapid environmental transformation:

a. **Classify** the metal compound as ***Category Chronic 1*** if the chronic ERV$_{compound}$ ≤ 0.01 mg/L, an M-factor must also be established as part of this classification (see IV.5.4); or

b. **Classify** the metal compound as ***Category Chronic 2*** if the chronic ERV$_{compound}$ > 0.01mg/L and ≤ 0.1 mg/L; or

c. **Classify** the metal compound as ***Category Chronic 3*** if the chronic ERV$_{compound}$ > 0.1mg/L and ≤ 1 mg/L.

Poorly soluble metal compounds

Where *the chronic ERV* for the metal ions of concern is greater than 1 mg/L, the metals need not be considered further in the classification scheme.

Where the chronic ERV$_{\mathbf{compound}}$ is less than or equal to 1 mg/L consideration must be given to the data available on the rate and extent to which these ions can be generated from the metal compound. Such rate and extend data, to be valid and useable should have been generated using the Transformation/Dissolution Protocol (Annex 10 to UN GHS) for a 28d period.

Where 28d T/Dp data are unavailable, the surrogate approach should be applied (see Annex IV.5.3.2.2).

Where 28d data from the Transformation/Dissolution protocol are available, then classify according to the following rules:

a. **Classify** the metal compound as ***Category Chronic 1*** if the dissolved metal ion concentration obtained from the 28 day transformation test at a loading rate of 0.1 mg/L is greater than or equal to the chronic ERV, an M-factor must also be established as part of this classification (see IV.5.4); or

b. **Classify** the metal compound as ***Category Chronic 2*** if the dissolved metal ion concentration obtained from the 28 day transformation test at a loading rate of 1 mg/L is greater than or equal to the chronic ERV.

If there is evidence of rapid environmental transformation:

a. **Classify** the metal compound as ***Category Chronic 1*** if the dissolved metal ion concentration obtained from the 28 day transformation test at a loading rate of 0.01 mg/L is greater than or equal to the chronic ERV, an M-factor must also be established as part of this classification (see IV.5.4); or

b. **Classify** the metal compound as ***Category Chronic 2*** if the dissolved metal ion concentration obtained from the 28 day transformation test at a loading rate of 0.1 mg/L is greater than or equal to the chronic ERV; or

c. **Classify** the metal compound as ***Category Chronic 3*** if the dissolved metal ion concentration obtained from the 28 day transformation test at a loading rate of 1 mg/L is greater than or equal to the chronic ERV.

Do not classify for long-term hazard if the dissolved metal ion concentration obtained from the 28 day Transformation/Dissolution test at a loading rate of 1 mg/L is less than the chronic ERV of the dissolved metal ion.

IV.5.3.2.2 The surrogate approach

Readily soluble metal compounds

In absence of relevant chronic toxicity data, and unless there is evidence of both rapid environmental transformation and evidence of no bioaccumulation (see Annex IV.3 and IV.4), *readily soluble metal compounds* are classified as:

a. **Category Chronic 1** if the acute $ERV_{compound} \leq 1$ mg/L, an M-factor must also be established as part of this classification (see IV.5.4); or

b. **Category Chronic 2** if the acute $ERV_{compound} > 1$mg/L and ≤ 10 mg/L; or

c. **Category Chronic 3** if the acute $ERV_{compound} > 10$mg/L and ≤ 100 mg/L.

Poorly soluble metal compounds

Where the acute $ERV_{compound}$ is less than or equal to 100 mg/L consideration must be given to the data available on the rate and extent to which these ions can be generated from the metal. Such rate and extend data, to be valid and useable should have been generated using the Transformation/Dissolution Protocol (Annex 10 to UN GHS) for a 7d period.

Where such 7d T/Dp data are unavailable, *i.e.* there is no clear data of sufficient validity to show that the transformation to metal ions will not occur; the safety net classification (Category Chronic 4) has to be applied.

Where T/Dp data are available but relevant chronic ERVs are absent, the results should be used to aid classification according to the following rules:

a. **Classify** the metal compound as ***Category Chronic 1*** if the dissolved metal ion concentration obtained from the 7 day transformation test at the low loading rate (1 mg/L) is greater than or equal to the acute ERV and there is no evidence of rapid environmental transformation and no bioaccumulation, an M-factor must also be established as part of this classification (see IV.5.4);

b. **Classify** the metal compound as ***Category Chronic 2*** if the dissolved metal ion concentration obtained from the 7 day transformation test at the medium loading rate (10 mg/L) is greater than or equal to the acute ERV and there is no evidence of rapid environmental transformation and no bioaccumulation;

c. **Classify** the metal compound as ***Category Chronic 3*** if the dissolved metal ion concentration obtained from the 7 day transformation test at the high loading rate (100 mg/L) is greater than or equal to the acute ERV and there is no evidence of rapid environmental transformation and no bioaccumulation;

d. **Classify** the metal compound as ***Category Chronic 4*** if the dissolved metal ion concentration obtained from the 7 day transformation test at the high loading rate (100 mg/L) is lower than the acute ERV and there is no evidence of rapid environmental transformation and no bioaccumulation.

Figure IV. 5 Classification strategy for determining long-term aquatic hazard for metal compounds

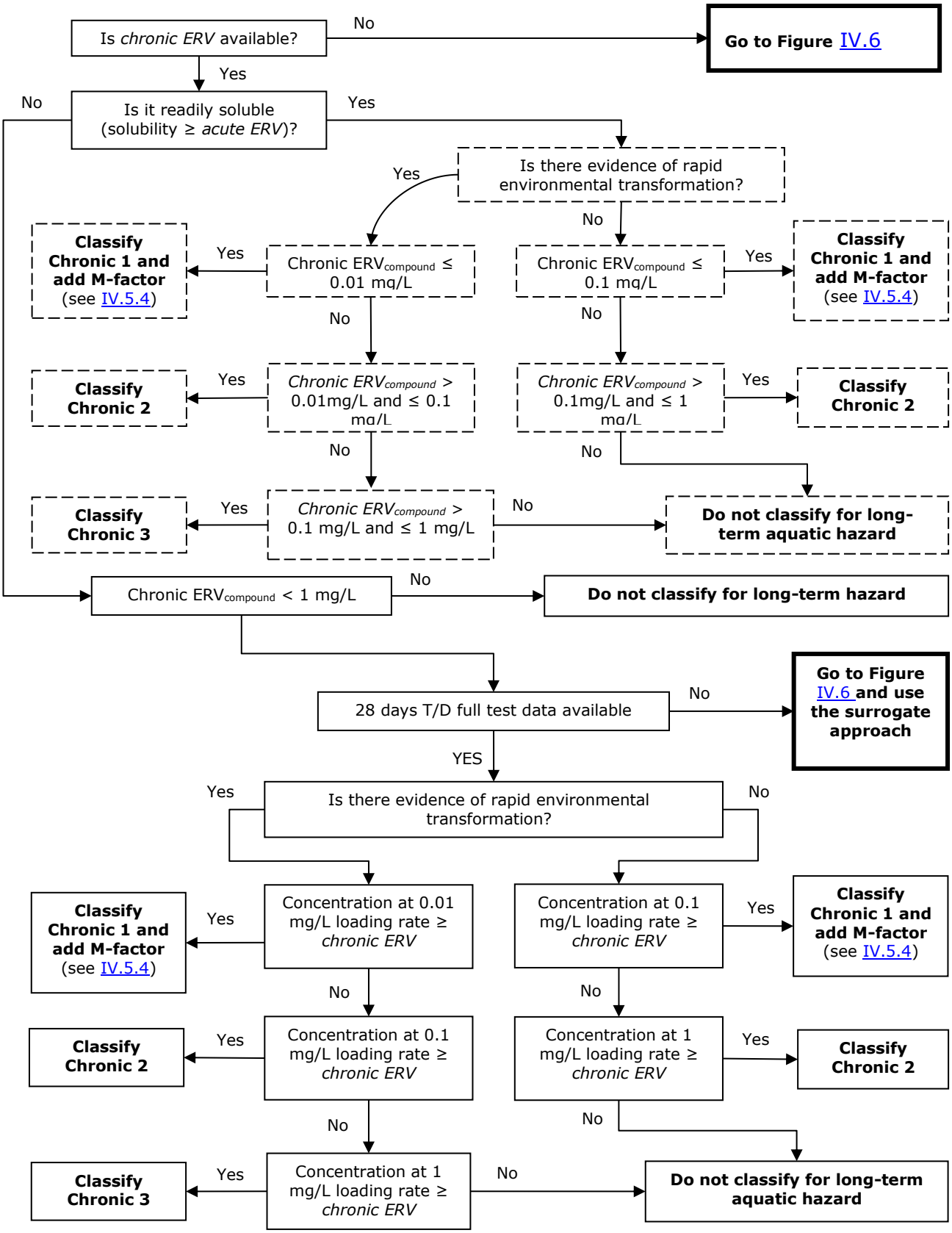
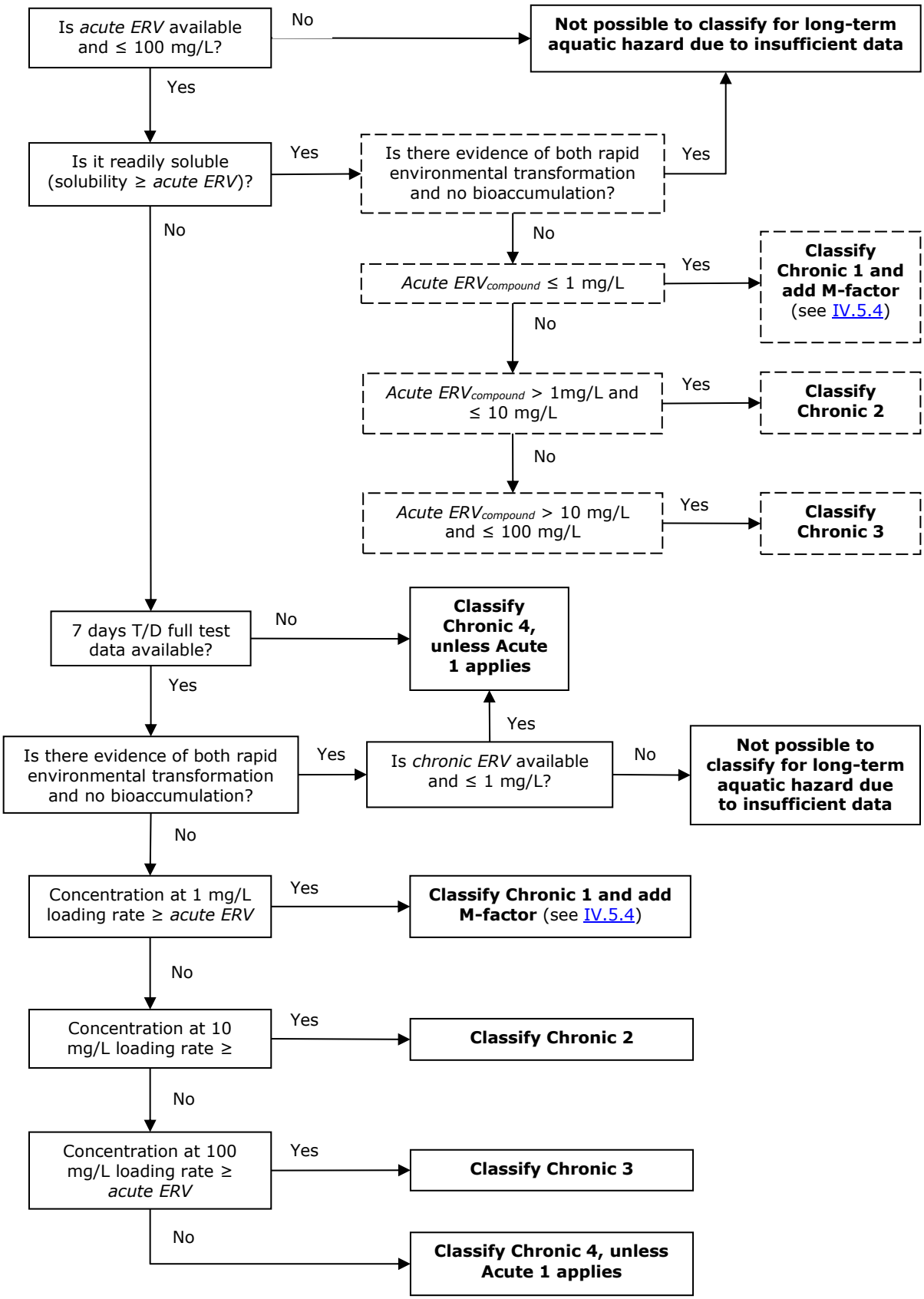


Figure IV. 6 Classification strategy for determining long-term aquatic hazard for metal compounds in absence of appropriate chronic toxicity reference and/or T/Dp data



IV.5.4 Setting M-factors for metals and inorganic metal compounds

For the hazard class "Hazardous to the Aquatic Environment", SCLs are not applicable. Instead the M-factors concept is used.

The M-factors are used in application of summation method for classification of mixtures containing substances that are classified as very toxic. The concept of M-factors has been established to give an increased weight to very toxic substances when classifying mixtures. M-factors are only applicable to the concentration of a substance classified as hazardous to the aquatic environment (categories Acute 1 and Chronic 1) and are used to derive by the summation method the classification of a mixture in which the substance is present. They are, however, substance-specific and it is important that they are being established already when classifying substances.

M-factors should have been established in accordance with Article 10 of CLP and be available in the C&L Inventory.

For the harmonised classifications in Annex VI to CLP, M-factors shall be set by the manufacturer, importer or downstream user in case there is no M-factor provided, in accordance with Article 10(4) of CLP.

For *soluble metal compounds* M-factors are applied as for organic substances (see Table IV.1).

For poorly soluble metal compounds and metals, M-factors can be estimated from the ratio of the soluble metal ions concentrations obtained from Transformation Dissolution (7 d at a loading of 1 mg/L or 28 d at the loading used to determine classification as Chronic 1, *i.e.*, 0.1 or 0.01 mg/L, depending on rapid/no-rapid transformation) and the ERV of the dissolved metal ion taking the considerations mentioned in IV.2.3 into account. If this ratio is:

- below 10 then an M-factor of 1 should be applied;
- 10 and < 100 then the M-factor would be 10;
- 100 and < 1000 then the M-factor would be 100.

Continue in factor 10 intervals.

Table IV. 1 M-factors for inorganic substances

Acute ERV (mg/L)	Multiplying factors (M)
0,1 < Acute ERV ≤ 1	1
0,01 < Acute ERV ≤ 0,1	10
0,001 < Acute ERV ≤ 0,01	100
0,0001 < Acute ERV ≤ 0,001	1000
Continue in factor 10 intervals	10000

Chronic ERV (mg/L)	Multiplying factors (M)	
	No rapid environmental transformation	Rapid environmental transformation
0,01 < Chronic ERV ≤ 0,1	1	1
0,001 < Chronic ERV ≤ 0,01	10	1
0,0001 < Chronic ERV ≤ 0,001	100	10
0,00001 < Chronic ERV ≤ 0,0001	1000	100
Continue in factor 10 intervals		

IV.5.5 Particle size and surface area

Surface area is a crucial parameter in that any variation in surface area tested may cause a significant change in the levels of metals ions released in a given time window. Thus, particle size or surface area is fixed for the purposes of the transformation test, allowing the comparative classifications to be based solely on the loading level. Normally, the classification data generated would have used the smallest particle size marketed to determine the extent of transformation. There may be cases where data generated for a particular metal powder are not considered as suitable for classification of the massive forms. For example, where it can be shown that the tested powder is structurally a different material (e.g. different crystallographic structure) and/or it has been produced by a special process and is not generally generated from the massive metal, classification of the massive can be based on testing of a more representative particle size or surface area, if such data are available. The powder may be classified separately based on the data generated on the powder. However, in normal circumstances it is not anticipated that more than two classification proposals would be made for the same metal.

Metals with a particle size smaller than the default diameter value of 1 mm can be tested on a case-by-case basis. One example of this is where metal powders are produced by a different production technique or where the powders give rise to a higher dissolution (or reaction) rate than the massive form leading to a more stringent classification.

The particle sizes tested and/or used for classification and labelling depend on the substance being assessed and are shown in the table below:

Type	Particle size	Comments
Metal compounds	Smallest representative size sold	Never larger than 1 mm
Metals – powders	Smallest representative size sold	May need to consider different sources if yielding different crystallographic/ morphologic properties
Metals – massive	1 mm	Default value may be altered if sufficient justification

Massives will usually be tested as 1 mm particles. Alternatively, the T/D testing of materials with different surface area's may result in highly reliable dissolution kinetic equations that allows to define the 'Critical Particle Diameter' (CPD) for appropriate loadings for the acute and long-term hazard assessment.

For most metals and some metal compounds, it is possible, using the Transformation/ Dissolution Protocol (Annex 10 to UN GHS), to obtain a correlation between the concentration of the metal ion after a specified time interval as a function of the surface area loadings of the forms tested. Such correlations should be established for the relevant pH ranges as specified in the protocol. In such cases, it could then be possible to estimate the level of dissolved metal ion concentration at a given pH of the metal with different particles, using the critical surface area approach (Skeaff *et al.,* 2000). From this correlation and a linkage to the appropriate toxicity data at corresponding pH level, it is possible to determine a "Critical Surface Area" (CSA) of the substance that delivers the L(E)C_{50} to the dissolution medium and then to convert the CSA to a Critical Particle Diameter (CPD) (see example). This CPD at appropriate mass loadings for acute and long-term hazard assessment can then be used to:

- determine the classification category of powders based on the finest representative powder on the market; and
- determine an accurate classification of the massive metal by applying a 1 mm (default) diameter.

Within the CSA Approach an equation is developed to predict metal ion release (based on previously measured metal ion release from different loadings of the metal), which is correlated to measured surface area, and a corresponding calculated equivalent particle diameter. The basis of the CSA Approach is that ***the release of metal ions is dependent on the surface area of the substance***, with this release being predictable once the relationship has been established. The CSA is the surface area loading (mm^2/l) to a medium that delivers a selected ecotoxicity reference value to that medium. The term *SA* is the measured specific surface area (m^2/g) of the metal sample. The measured specific critical surface area (SA_{crit}) (m^2/g) is the measured specific surface areas for the corresponding low, medium and high loadings which are associated with the respective acute and long-term aquatic toxicity classification categories in the classification scheme for metals and metal compounds. A typical equation for this relationship for a given substance, aquatic medium, pH and retention time is:

$$\log (C_{Me(aq)}, \text{mg/L}) = a + b \log(A_{meas})$$

$C_{Me(aq)}$ = total dissolved concentration of metal ion (mg/L) at a particular length of test time (*i.e.* 168 hours for acute toxicity transformation testing) under certain conditions (*i.e.* pH, specified medium, etc.), as determined by transformation/dissolution testing of different surface area loadings

a, *b* = regression coefficients

A_{meas} = initial surface area loading (mm^2/l) [equals (measured specific surface area, *SA*, in m^2/g) X (substance mass loading in g/l) X 10^6], where *SA* was measured with the BET nitrogen adsorption-desorption technique.

IV.5.6 Classification of mixtures of metals and metal compounds

Simple composed metal or metal compound mixtures should be handled as mixtures and classified according to the mixtures rules described in section 4.1.4 given they normally express toxicity as a function of their composing ingredients. Ores and concentrates and UVCB inorganics are considered as substances in respect to CLP but follow in general the mixture ruling to determine their classification unless specific ecotoxicity data are available for the mineral(s) under consideration.

Ores and concentrates and inorganic UVCBs are considered substances under CLP. In the absence of substance specific ecotoxicity data, their classification can be assessed by applying the mixtures rule. The metals industry has developed classification tools that allow for the hazard ID and environmental classification of these complex materials, by integrating all aspects of this guidance with a knowledge of their mineralogical and other typical metal properties.

Metal alloys are considered as mixtures for the purpose of classification and labelling (Article 2(27) of CLP), although due to their (eco)toxicity profile differing from that of their constituents, they may require special consideration. Further information on how to assess the environmental hazard classification of alloys and other complex metal containing materials is provided hereunder.

IV.5.6.1 Classification of alloys and complex metal containing materials

Metal alloys, or alloy manufacturing products are not simple mixtures of metals or metal compounds, since the alloy has clearly distinctive properties compared to a classical mixture of its metal components. Justified by their intrinsic properties, the solubility properties can differ substantially from what is observed for each individual constituent in that alloy (e.g. the rate and extend of metals release from pure metals are different from the ones from alloys). The rate and extend to which the ingredients of the alloy react with the media to transform to water soluble forms can be measured in the same way as with metals (by using the OECD Transformation/Dissolution test (Annex 10 to UN GHS)). However, alloys often react slowly and to a very limited extent, making the application of the T/D protocol more complex. Special care should be taken in this respect to the detection limit and the accurate determination of the measured surface. Initial testing of alloys, using the T/D protocol, shows that this can be useful but **further additional guidance on this aspect is recommended**.

More complex metals or metal compounds containing inorganic substances like e.g. ores and concentrates are not simple mixtures of metals or metal compounds. Justified by their intrinsic properties, the solubility properties can differ substantially from what is observed for each individual constituent of that complex substance (e.g. the rate and extent of metals release from e.g. ores/concentrates are different from the ones from simple metals). All these materials are typically not readily soluble in any aqueous medium. In addition, these materials are often heterogeneous in size and composition on a microscopic/macroscopic scale. Therefore, adequate amounts of the material could be used to evaluate the extent to which the substances can be dissolved, *i.e.* its water solubility and/or the extent to which the metals can react with the media to transform to water soluble forms e.g. through Transformation/Dissolution tests. Additional guidance on this aspect is needed for complex metal mixtures.

An **ecotoxicity validation step** may be important for alloys and complex metal containing materials (e.g. ores, concentrates, slags), where binding of the metal to abiotic and biological binding sites will in many cases be competitive. Therefore the 'additivity mode' is not necessarily valid and additional information may be relevant.

Therefore, information from ecotoxicity validation steps could be useful in cases where a significant uncertainty is associated with the existing toxicity data. This ecotoxicity validation should have been derived from tests using most sensitive species at dissolved ion concentrations equivalent to those measured in the T/D medium. However, information from ecotoxicity testing directly in the T/D medium is not recommended because the composition of this medium is unlikely to meet the requirements for standard test media to ensure proper survival and/or

reproduction. Therefore, ecotoxicity tests should have been conducted in standard media dosed at metal concentration equivalent to the concentration level actually measured in the T/D medium.

IV.6 References

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IV.7 Decision on classification: examples for metals and metal compounds

List of examples:

- **Example A**: Soluble metal compound with acute and chronic toxicity data and no evidence of rapid environmental transformation (Me_2 $(SO4)_2$).
- **Example B**: Poorly soluble metal compound with acute and chronic toxicity data, Transformation/Dissolution data at 7 days (low loading rate) and at 28 days (only low and medium loading rates) and no evidence of rapid environmental transformation.
- **Example C**: Metal in powder and massive form with acute and chronic toxicity data and Transformation/Dissolution data at 7 days (low, medium and high loading rates) and at 28 days (only the high loading rate) and no evidence of rapid environmental transformation.
    - *Explanatory note to Example D* - Critical Surface Area (CSA) Approach.
- **Example D**: Hazard classification of a soluble metal salt: the case of rapid environmental transformation through speciation in the water column.

IV.7.1 Example A: Soluble metal compound with acute and chronic toxicity data and no evidence of rapid environmental transformation ($Me_2(SO4)_2$).

DATA ELEMENTS	Value	Test method ((EC) No. 440/2008) or OECD guideline / remarks
Transformation dissolution protocol evidence		
Screening test (24 h) at 100 mg/L loading	pH 6 : 6240 µg/L pH 8 : 840 µg/L	Metals TDp, non-GLP
7 d TDp test	Not applicable	
28 d TDp test	Not applicable	
MWT of the metal ion versus compound		
	60 / 312	
Acute aquatic toxicity of metal ion[140]		
Fish: *Oncorhynchus mykiss*	120 µg/L (96 h LC_{50}) at pH 7,8 106 µg/L (96 h LC_{50}) at pH 7,8 104 µg/L (96 h LC_{50}) at pH 7,8 78 µg/L (96 h LC_{50}) at pH7,8 (species mean: 102 µg/L at pH 7,8)	C.1. / static, GLP C.1. / static, non-GLP C.1. / static, GLP C.1. / static, non-GLP
Crustacea: *Daphnia magna*	180 µg/L (48 h EC_{50}) at pH 8	C.2. / static, non-GLP
Algae/aquatic plants: *Scenedesmus subspicatus* *Lemna gibba*	 154 µg/L (72 h ErC_{50}) at pH 8 670 µg/L (7 d ErC_{50}) at pH 8	 C.3. / static, GLP C.26. / semi-static, GLP
Chronic aquatic toxicity[141]		
Fish: *Danio rerio*	24 µg/L (28 d NOEC) at pH 6	OECD 210 / 28 d flow-through, non-GLP
Marine Fish	87 µg/L (28 d NOEC) at pH 8	OECD 210 /28 d flow through, GLP)
	1414 µg/L (28 d EC_{10})	OECD 210 /28 d flow through, GLP)

[140] Tests performed with readily soluble salts such as metal sulphates and metal chlorides.

[141] Tests performed with readily soluble salts such as metal sulphates and metal chlorides.

Crustacea: *Daphnia magna*	37 µg/L (21 d EC_{10}) at pH 7.8 8.6 µg/L (21 d NOEC) at pH 6.4	C.20. / semi-static, GLP C.20./semi-static non-GLP
Marine decapoda	1612 µg/L (21 d NOEC)	Non-standard test
Algae/aquatic plants: *Scenedesmus subspicatus*	21.6 µg/L (72 h NOEC) at pH 8 8.7 µg/L (72 h NOEC) at pH 6.2	C.3. / static, GLP C.3. / static, non-GLP
Degradation (evidence of rapid degradation)		
Rapid environmental transformation	No evidence.	
Bioaccumulation		
Bioconcentration factor in fish	+/- 200 at NOEC level	

Aquatic hazard assessment, conclusions and comments:

Transformation Dissolution:

- The substance passes the 24 h screening TDp test at pH 6 given the dissolution at a loading of 100 mg/L is 6240 µg/L > acute ERV of the soluble ion being 102 µg/L at pH 7.8.

Acute aquatic toxicity:

- The acute ecotoxicity reference value is driven by the Fish data. No data are available for the low pH end.
- The acute ERV for the metal compound is 102 * (312/(2*60)) = **265 µg/L**.

Evidence of rapid environmental transformation:

- No information available, so substance considered as not rapidly transformed by normal environmental processes.

Chronic aquatic toxicity:

- The chronic aquatic ecotoxicity reference toxicity value based on the lowest of the available toxicity values is slightly below 10 µg/L for Daphnia magna at pH 6,4 for the metal ion.
- The chronic ERV for the metal compound is 8.6 * (312/(2*60)) = **22.4 µg/L**.

Aquatic hazard classification and, where applicable, established M-factor(s):

- Acute (short-term) aquatic hazard: category Acute 1, M-factor: 1
- Long-term aquatic hazard: category Chronic 1, M-factor: 1

Reasoning:

Acute aquatic hazard

- The acute ecotoxicity reference value is driven by the Fish data. A species mean of 102 µg/L for the metal ion, is calculated for *Oncorhynchus mykiss* given 4 or more toxicity data for the same species under comparable conditions are available.
- Acute aquatic hazard expressed as the ERV for the metal compound after molecular weight correction ≤ 1 mg/L. M-factor is 1 given the acute ERV is between 1 and 0.1 mg/L.
- The molecular weight correction recognises that 2 metal ions are included.

- The substance passes the 24 h screening dissolution test by comparing acute toxicity data at pH 7.8 with TDp data at pH6 given an acute toxicity data set at pH 6 is lacking and the chronic toxicity data indicate more toxic behaviour of the metal at the lower pH end.

Long-term aquatic hazard:

- Adequate information on chronic toxicity (all 3 trophic levels) is available allowing long-term hazard classification (no use of the surrogate approach).[142]
- Marine toxicity data are not included in the chronic ERV assessment given far less sensitive as freshwater toxicity references and data for 3 trophic levels for the freshwater are available.
- The Daphnia magna reference at pH6 is the lowest and determines the chronic ERV.
- A molecular weight correction is applied to the substance recognising that 2 metal ions are included.
- Rapid environmental transformation cannot be demonstrated given the lack of sufficient information.
- The M-factor of 1 is based on the chronic ERV of 22 µg/L (so between 0.01 and 0.1 mg/L.) without rapid environmental transformation.

Labelling elements based on the classification:

Element	Code
GHS Pictogram	GHS09
Signal Word	Wng
Hazard Statement	H400, H410 → H410[143]
Precautionary statement(s)	P273, P391, P501

[142] In absence of adequate chronic toxicity data for all trophic levels, the subsequent step is to combine two types of information, *i.e.,* chronic info for the trophic level with such data and acute aquatic toxicity data and environmental fate information for lacking info on trophic levels. For details see section 4.1.3.3 and Table 4.1.0.

[143] In accordance with Article 27 of CLP, the hazard statement H400 may be considered redundant on the label and therefore not included on the label because hazard statement H410 also applies, see section 4.1.6 of this document.

IV.7.2 Example B: Poorly soluble metal compound with acute and chronic toxicity data, transformation/dissolution data at 7 days (low loading rate) and at 28 days (only low and medium loading rates) and no evidence of rapid environmental transformation

DATA ELEMENTS	Value	Test method ((EC) No. 440/2008) or OECD guideline / remarks
Transformation dissolution protocol evidence		
Screening test (24 h) at 100 mg/L loading	pH 6: 74 µg/L pH 8: 34 µg/L	Metals TDp, non-GLP
7 d TDp test at 1 mg/L loading	pH 6: 50 µg/L pH 8: 16 µg/L	Metals TDp, non-GLP Metals TDp, non-GLP
28 d TDp test at 0.1 mg/L loading at 0.01 mg/L loading	pH 6: no data available pH 8: no data available pH 6: 9 µg/L pH 8: <1 (DL)	Metals TDp, non-GLP Metals TDp, non-GLP Metals TDp, non-GLP Metals TDp, non-GLP
MWT of the metal ion versus compound		
MWT of the metal ion versus compound	60 / 91	
Acute aquatic toxicity of metal ion[144]		
Fish: *Oncorhynchus mykiss*	186 µg/L (48 h LC_{50}) at pH 7 120 µg/L (96 h LC_{50}) at pH 7.8 106 µg/L (96 h LC_{50}) at pH 7.8 104 µg/L (96 h LC_{50}) at pH 7.8 78 µg/L (96 h LC_{50}) at pH 7.8 (species mean for four values : 102 µg/L at pH 7.8) *78 µg/L* (96 h LC_{50}) at pH 6.4	C.1. / static, non-GLP C.1. / static, GLP C.1. / static, non-GLP C.1. / static, GLP C.1. / static, non-GLP
Crustacea: *Daphnia magna*	180 µg/L (48 h EC_{50}) at pH 8 106 µg/L (48 h EC_{50}) at pH 8	C.2. / static, non-GLP
Algae/aquatic plants *Scenedesmus subspicatus*	154 µg/L (72 h ErC_{50}) at pH 8 78 µg/L (72 h ErC_{50}) at pH 6	C.3. / static, GLP
Lemna gibba	670 µg/L (7 d ErC_{50}) at pH 8	C.26. / semi-static, GLP

[144] Tests performed with readily soluble salts such as metal sulphates and metal chlorides.

Chronic aquatic toxicity[145]		
Fish: *Danio rerio*	24 µg/L (28 d NOEC) at pH 6 87 µg/L (28 d NOEC) at pH 8	OECD 210 / 28 d flow-through, non-GLP OECD 210 /28 d flow through, GLP)
Crustacea: *Daphnia magna*	37 µg/L (21 d EC_{10}) at pH 7.8 8.6 µg/L (21 d NOEC) at pH 6.4	C.20. / semi-static, GLP C.20. / semi-static, non-GLP
Algae/aquatic plants: *Scenedesmus subspicatus*:	21.6 µg/L (96 h NOEC) at pH 8 8.7 µg/L (72 h EC_{10}) at pH 6.2	C.3. / static, GLP C.3. / static, non-GLP
Degradation (evidence of rapid degradation)		
Rapid environmental transformation	No data available therefore considered as not rapidly transformed.	
Bioaccumulation		
Bioconcentration factor in fish	+/- 200 at NOEC level	

Aquatic hazard assessment, conclusions and comments:

Transformation Dissolution screening outcome:

- The substance fails the 24 h screening Transformation Dissolution test given the dissolution at a loading of 100 mg/L :
  - at pH 6 is 74 µg/L < acute ERV of the soluble ion being 78 µg/L (borderline case)
  - at pH 8 is 34 µg/L < acute ERV of the soluble ion being 102 µg/L

Acute aquatic toxicity:

- Adequate data on pH 6 and 8 are available allowing to derive an acute ERV for the (soluble) metal ion :
  - at the lower pH end (around pH 6) : 78 µg/L
  - at the higher pH end (around pH 8) : 102 µg/L

7 days Transformation/Dissolution outcome :

- The acute release after 7 d is the highest at pH 6 (50 µg/L) being lower than the acute toxicity level (78 µg/L) at this corresponding pH
- The acute release is lower at or around pH 8 (16 µg/L), which is significantly lower than the acute toxicity level (102 µg/L) at this corresponding pH

Evidence of rapid environmental transformation:

- No information available and therefore substance considered as not rapidly transformed by normal environmental processes.

Chronic aquatic toxicity for a substance not rapidly transformed:

[145] Tests performed with readily soluble salts such as metal sulphates and metal chlorides.

- The chronic ERV for the (soluble) metal ion is ***8.6 µg/L*** around pH 6 and ***21.6 µg/L*** around pH 8.

28 days Transformation dissolution outcome for a substance not rapidly transformed:

- The release after 28 d at pH 6 at a loading of 0.1 mg/L is not available and needs to be extrapolated from the 0.01 loading rate assuming a 10 times higher dissolution level (10x9=90 µg/L), which is significantly larger than the chronic ERV at pH 6 (8.6 µg/L).
- The release for the 0.1 mg/L loading is also extrapolated in the same way and is much lower at pH 8. The calculated release rate of < 10 µg/L is still lower than the chronic toxicity level 21.6 µg/L at this pH level. The calculated release rates at 1 mg/L loading would be < 100 µg/L which is significantly larger than the chronic ERV at pH 8.

Aquatic hazard classification and, where applicable, established M-factor(s):

- Acute (short-term) aquatic hazard: no acute classification
- Long-term aquatic hazard: category Chronic 1, M-factor 10

Reasoning:

The metal compound is considered as poorly soluble since it fails the OECD transformation dissolution screening test at a 100 mg/L loading. The test confirmed pH 6 as the pH of the highest release rate.

Acute aquatic hazards:

- The acute ecotoxicity reference value is driven by the Fish data for the high pH and by algae data for the low pH level. For the high pH end (around pH 8) a species mean of 102 µg/L for the metal ion is calculated for *Oncorhynchus mykiss* and a single reference of 78 µg/L for *Scenedesmus subspicatus* at around pH 6.
- A poorly soluble substance is evaluated for classification by comparing the dissolved metal ion level resulting from the TDp at 7d, at a loading rate of 1 mg/L with the acute ERV as determined for the (soluble) metal ion. A molecular weight correction for the poorly soluble metal compound is consequently not required given this factor has already been included for the loading rate of the TDp test.
- The dissolution level of the poorly soluble metal compound from the 7d TDp at 1 mg loading is lower than the acute ERVs of the soluble metal ion for both pH levels, thereby not resulting in an acute classification.

Long-term aquatic hazard:

- Adequate information on chronic toxicity (all 3 trophic levels) for the higher and lower pH levels are available allowing direct long-term hazard classification (no use of the surrogate approach).
- No valid info is available on rapid transformation by normal environmental processes, so the poorly soluble metal compound is considered to be not rapidly transformed.
- No Molecular Weight Correction is applied for the poorly soluble metal compound given the classification scheme is based on the comparison of the dissolved fraction of the poorly metal compound with the chronic ERV of the soluble metal ion at both pH 6 and pH 8.
- No TDp data are available for the 0.1 mg/L and 1 mg/L loading. The calculated dissolution level from the 28d TDp at pH 6 at 0.1mg/L loading (+/- 90 µg/L) for the poorly soluble metal compound is much higher than the chronic ERV's of the soluble metal ion for pH 6 (8.6 µg/L) warranting a chronic 1 classification. The classification is much less sensitive at pH 8 given a less toxic and a lower dissolution rate.
- The M-factor associated with the long-term hazard classification is derived by using the solubility level derived from the 28d TDp test at the 0,1 mg/L loading (90 µg/L at pH 6)

divided by the ERV of the dissolved metal ion (8.6 µg/L at pH 6): 90/8.6=10.45. According to Annex IV.5.4 the substance will get an M-factor 10, given this factor was between 10 and 100.

Labelling elements based on the classification:

Element	Code
GHS Pictogram	GHS09
Signal Word	Wng
Hazard Statement	H410
Precautionary statement(s)	P273, P391, P501

IV.7.3 Example C: Metal in powder and massive form with acute and chronic toxicity data and Transformation/Dissolution data at 7 days (low, medium and high loading rates) and at 28 days (only the high loading rate) and no evidence of rapid environmental transformation

DATA ELEMENTS	Value	Test method ((EC) No. 440/2008) or OECD guideline / remarks
Transformation dissolution protocol evidence **For metal in POWDER form**		
Screening test (24 h) at 100 mg/L loading	Not applicable for metals	Metals TDp, non-GLP
7 d TDp test at 1 mg/L loading at 10 mg/L loading at 100 mg/L loading	pH 6 : 1.7 µg/L (.) pH 8 : 3 µg/L pH 6 : 24 µg/L pH 8 : 29 µg/L pH 6 : 340 µg/L pH 8 : 280 µg/L	Metals TDp, non-GLP
28 d TDp test at 1 mg/L loading at 0.1 mg/L loading at 0.01 mg/L loading	pH 6: 2.3 µg/L pH 8: 3.5 µg/L no measured data available no measured data available	Metals TDp, non-GLP
MWT of the metal		
MWT of the metal	59	
Acute aquatic toxicity of metal ion[146]		
Fish:	Large data sets available for the 2 pH ends but less sensitive than crustacean at high pH end and Algae at low pH end	C.1. / static, non-GLP C.1. / static, GLP
Crustacea: *Ceriodaphnia dubia*	Most sensitive species at high pH end (pH 8.3-8.7) : Geometric mean for 6 values under comparable test conditions (EC_{50} 48h): 68 µg metal ion/l	C.2. / static, non-GLP

[146] Tests performed with readily soluble salts such as metal sulphates and metal chlorides.

Algae/aquatic plants: *Pseudokirchneriella subcapitata*	Data sets available for the 2 pH ends but less sensitive than crustacean at high pH end and most sensitive endpoint at low end. Most sensitive value (96 h EC_{10}) at the low pH range: 120 µg metal ion/l	C.3. / static, GLP And non-GLP C.26. / static, non-GLP
Chronic aquatic toxicity[147]		
Fish	Large data sets available for different pHs but less sensitive than crustacean at high and low pH	
Crustacea: *Ceriodaphnia dubia*	Most sensitive species at high and low pH end: - At low pH (NOEC 21d): 20 µg/L - At high pH: (EC_{10} 21d): 2.4 µg /l	C.20. / semi-static, non-GLP
Algae/aquatic plants:	Large data sets available for different pH's but less sensitive than crustacean at high and low pH	C.3. / static, GLP C.3. / static, non-GLP
Degradation (evidence of rapid degradation)		
Rapid environmental transformation	No information.	.
Bioaccumulation		
Bioconcentration factor in fish	<< 500 at NOEC or EC_{50} level	

Transformation Dissolution screening outcome: not applicable for metals

Acute aquatic toxicity:

- Adequate data at high and low pH are available allowing deriving an acute ERV for the (soluble) metal ion:
  - at the lower pH end (around pH 6) : ***120 µg/L***
  - at the higher pH end (above pH 8) : ***68 µg/L***

[147] Tests performed with readily soluble salts such as metal sulphates and metal chlorides.

7 days Transformation/Dissolution outcome for the powder form:

- The release after 7 d's is the highest at pH 8 while lower at pH 6. The table below compares the TDp results with the acute ERV values at the corresponding pH ranges

Loading (mg metal ion/l)	pH*	Highest dissolution (mg metal/L)	Reference toxicity value (mg metal/L)	Dissolution > toxicity reference value?
1	low	0.0017	0.12	No
10	low	0.024	0.12	No
100	low	0.35	0.12	Yes
1	high	0.003	0.068	No
10	high	0.029	0.068	No
100	high	0.28	0.068	Yes

* pH value at which dissolution testing was conducted and similar to the pH for the acute toxicity reference value

- The release from the metal powder[148] at a loading of 100 mg/L is for both pH ranges higher than the acute ERV.

7 days Transformation/Dissolution outcome for the massive form :

- The CSA Approach can be used to calculate a Critical Particle Diameter (CPD) for the dissolution rates from the metal powder. The metal in massive form will be classified as hazardous to the aquatic environment if the CPD is above or equal to 1 mm. The measured critical surface area (SA_{crit}) that releases sufficient ions to reach the acute ERV for the most critical pH (6) is ***SA_{crit}*** **0.101 m^2/g** corresponding to an equivalent critical spherical particle diameter (CD_{spec}) of 6.67 μm at a 100 mg/L loading rate. This is far less than 1 mm.

Evidence of rapid environmental transformation:

- No information available and therefore substance considered as not rapidly transformed by normal environmental processes.

Chronic aquatic toxicity:

- The chronic ERV for the (soluble) metal ion is ***2.4 μg/L*** at around pH 8 and ***20 μg/L*** around pH 6 which is an inverse relationship with pH as for the acute level.

28 days Transformation/Dissolution outcome for a substance not rapidly transformed:

- The release after 28 d at a loading of 1 mg/L is slightly higher at ***pH 8*** (3.5 μg/L) than at pH 6 (2.3 μg/L).
- TDp data for lower loadings are not available and were calculated given that the rate of metal ion release from the metal in the OECD 203 medium at high pH at the 28 days can be predicted by the equation: log (CMe(aq)) = -5.144 + 1.0229log(Ameas), whereby

$C_{me(aq)}$ = total dissolved concentration of metal (mg/L)

[148] The finest representative metal powder should be used for TDp testing.

A_{meas} = initial surface area loading (mm^2/l) [equals (measured specific surface area, *SA*, in m^2/g) × (substance mass loading in g/l) X 10], where *SA* was measured with the BET nitrogen adsorption-desorption technique.

An equal approach can be followed for the lower pH level.

- Measured and estimated transformation dissolution data for the *metal powder* are listed in the table below

Loading (mg metal ion/l)	Measured or *calculated*	pH*	Highest dissolution (mg metal/L)	Reference toxicity value (mg metal/L)	Dissolution > toxicity reference value?
1	Measured	low	0.0023	0.020	No
1	Measured	high	0.0035	0.0024	Yes
0.1	Estimated	Low	0.00023	0.020	No
0.1	Estimated	High	0.00035	0.0024	No

* pH value at which dissolution testing was conducted and similar to the pH for the acute toxicity reference value

- The release after 28 days at the 1 mg/L loading for the higher pH level slightly exceeds the chronic ERV, while no such effect is noted at pH 6 mainly due to the lower sensitivity of the species.

Aquatic hazard classification and, where applicable, established M-factor(s):

Acute (short-term) aquatic hazard:

- for the powder form: no acute hazard classification
- for the massive form: no acute hazard classification

Long-term aquatic hazard:

- for the powder form: category Chronic 2
- for the massive form: no long-term hazard classification

Reasoning:

The single environmental classification for all ***metal powders*** (spherical diameter ≤ 1 mm) of the considered metal can be derived by comparing the transformation/dissolution data for the smallest commercially representative metal powder with the acute and chronic toxicity reference values (for the soluble metal compounds).

Acute hazard classification:

- The ***dissolution rate for the finest powder*** on the market does not reach the concentration corresponding with the ERV, within 7 days at a loading of 1 mg/L. This is only reached at a loading of 100 mg/L. Therefore, **no acute hazard classification is required.**
- The ***dissolution rate for the massive forms*** (spherical diameter > 1 mm) is lower than those for powders given the lower available surface area. The Critical surface area approach confirms that above a diameter of 6.7 µm the acute ERV cannot be reached within 7 days at a loading of 1 mg/L. (Not even at a 100 mg/L loading.) Thereby confirming no need for an acute hazard classification. More explanation on the CSA assessment of the powder form for this metal is included in the explanatory note to example D (see below).

Long-term hazard classification:

- The metal does not fulfil the criterion for rapid environmental transformation.
- T/D data are only available for 1 mg/L loading rate. The medium loading rate of 0,1 mg/L required for the long-term hazard assessment could be safely extrapolated from existing evidence given clear relationships between concentration and dissolution were established for both pH levels.
- The comparison of chronic ERV's with the 28 days TDp results concludes that the chronic ERV for the metal ion is only reached at a loading rate of 1 mg/L at pH 8. Therefore, ***chronic 2 hazard classification for the metal in the powder form is warranted.***
- Given the surface of the particle reference ***for massive metal*** is > 100 larger than for the smallest commercially representative form this corresponds to a Critical Particle Diameter > 1 mm at the high loading rate. Therefore there is no need to classify the massive form for long-term hazard.

Labelling elements based on the classification for the powder form:

Element	Code
GHS Pictogram	GHS09
Signal Word	none
Hazard Statement	H411
Precautionary statement(s)	P273, P391, P501

Labelling elements based on the classification for the massive form: none

Element	Code
GHS Pictogram	none
Signal Word	none
Hazard Statement	none
Precautionary statement(s)	none

IV.7.3.1 Explanatory note to Example C - Critical Surface Area (CSA) approach

Acute hazard:

For the metal powder in this example, the data showed that the concentration of metal released in the OECD 203 medium at pH 8 at the 168 hr can be predicted by the equation:

$\log (C_{Me(aq)}) = -5.122 + 0.9875 \log (A_{meas})$

$C_{Mel(aq)}$ = total dissolved concentration of Metal ion (mg/L) at 168 hr and pH 8;

A_{meas} = initial surface area loading (mm^2/l) [equals (measured specific surface area, *SA*, in m^2/g) × (substance mass loading in g/l) × 10^6], where *SA* was measured with the BET nitrogen adsorption-desorption technique.

The CSA approach can subsequently determine what surface areas and particle diameters would result in different levels of aquatic toxicity classification using the regression coefficients from the above equation, *a* (-5.122) and *b* (0.9875), and the proposed acute toxicity reference value (0.068 mg Me/l) as the $C_{Me(aq)}$. The critical surface area (*CSA*) would be the A_{meas} at which the metal ion is released at the concentration of the acute toxicity reference value. The following equations can be used to derive these values for this case:

$\log L(E)C_{50} = -5.122 + 0.9875 \log CSA$

$L(E)C_{50}$ = acute ecotoxicity reference value for classification (mg/L)

CSA = critical surface area (mm^2/l) that releases metal ion in the concentration of the acute ecotoxicity reference value to the aquatic medium

The *CSA* can be derived as follows:

$$\log CSA = \left(\frac{\log L(E)C_{50} + 5.122}{0.9875} \right)$$

For an acute toxicity reference value of 0.068 mg Me/l, the *CSA* is thus 10,100 mm^2/l. This is the surface area loading of metal that will deliver the reference value amount of metal ion to the OECD 203 medium at pH 8 and at a time of 168 hr.

The critical specific surface areas, SA_{crit}s for a loading of 1 mg/L will deliver the acute toxicity reference value to the OECD 203 medium at pH 8 and a time of 168 hr can be calculated by:

SA_{crit} = critical specific surface area (m^2/g) corresponding to the acute ecotoxicity reference value

CP = classification cut-off loading of 1 mg/L that yield a classification as acute 1)

Thus, for the metal powder under consideration a *CSA* of 10.100 mm^2/l and the *CP* of 1 mg/L, the SA_{crit} is 10,1 m^2/g.

The equivalent critical spherical particle diameter (CD_{spec}) associated with the acute ecotoxicity reference value is determined by:

$$CDspec = \left(\frac{6}{SA_{crit} \times \rho Me} \right)$$

ρ_{Me} = density of the metal (g/cm^3)

CD_{spec} = critical diameter of the sphere (μm) corresponding to the acute ecotoxicity reference value

For the above SA_{crit} of 10,1 m^2/g, corresponding to the 1 mg/L loading, the critical diameter would be 0.067 μm. The EU-CLP system defines that the finest representative metal powder should be used for TDp testing and classification of the metal powder form.

An acute hazard classification can therefore be assigned to all metal powders (diameter ≤ 1 mm) by **measuring the real surface area** using the BET nitrogen adsorption-desorption technique and comparing it to SA_{crit}. If the surface area of the reference material is greater than the SA_{crit} for the associated acute hazard classification then the representative metal sample would classify for that acute hazard category ***and classify all powder types of that metal in the same way***. If the measured surface area is less than the SA_{crit}s of all of the classification categories then all powders of this metal would not classify for aquatic toxicity.

The CSA Approach can consequently be used to assign an acute hazard classification to the metal powders based on measured surface area using the **measured surface area of0.43 m^2/g** for the smallest representative size powder on the EU market. Since this surface area is greater than 0.1 m^2/g but less than 1 m^2/g, there is according to this approach no need for an ***acute hazard classification of the metal powders in this example***.

The CSA Approach can also be used to calculate a Critical Particle Diameter (CPD) to be used to determine an accurate classification of the **metal massive** (diameter > 1 mm), where the measured surface area of the tested granules is 0.086 m^2/g. This surface area is far less than all of the SA_{crit} so there is ***no need for an acute classification for the metal massive***.

Long-term hazard: For this example it has been shown that rate of metal ion release from the metal in OECD TG 203 medium at high pH at the 672 hr can be predicted by the equation:

$$\log (C_{Me(aq)}) = -5.144 + 1.0229 \log(A_{meas})$$

$C_{me(aq)}$ = total dissolved concentration of metal (mg/L)

A_{meas} = initial surface area loading (mm^2/l) [equals (measured specific surface area, *SA*, in m^2/g) × (substance mass loading in g/l) X 10^6], where *SA* was measured with the BET nitrogen adsorption-desorption technique.

The CSA Approach can determine what surface areas and particle diameter would result in chronic (long-term) hazard classification by using the regression coefficients from the above equation, *a* (-5.144) and *b* (1.0229), and the proposed chronic toxicity reference value (0.0024 mg Me/l) as the $C_{Me(aq)}$. The critical surface area (*CSA*) would be the A_{meas} at which metal ion is released at the concentration of the chronic toxicity reference value. The following equations can be used to derive these values.

$$\log \textit{chronic toxicity} = -5.144 + 1.0229 \log \textit{CSA}$$

chronic toxicity = chronic ecotoxicity reference value for classification (mg/L), using calculated EC_{10}s or measured NOECs (if the EC_{10} is less than the NOEC)

CSA = critical surface area (mm^2/l) that releases metal in the concentration of the chronic toxicity reference value to the aquatic medium

The *CSA* can be derived as follows:

$$\log CSA = \left(\frac{\log chronictoxicity + 5.144}{1.0229} \right)$$

For the long-term hazard classification derivation exactly the same approach as for the acute hazard assessment can be followed to define SA_{crit} and CD_{spec}. For this metal powder example this results in a CSA of 3,420 mm^2/l and the CP of 1 mg/L, the SA_{crit} is 0.342 m2/g.

For a SA_{crit} of 0.342 m^2/g, corresponding to the 1 mg/L loading, the critical diameter would be 2 μm.

Equivalent as for the assessment of the acute hazard the CSA Approach can be used to assign a long-term hazard classification to all powders based on measured surface area of the reference powder, using the measured surface area at 100 mg/L loading (0.43 m^2/g) for the smallest representative size powder on the EU market. Since this surface area is greater than 0.342 m^2/g, ***all metal powders would be classified as Chronic 3***.

The CSA Approach can also be used to **classify the massive metal (diameter > 1 mm)**, where the measured surface area of the massive at 100 mg/L loading) is 0.086 m^2/g. This surface area is less than the chronic SA_{crit} so the massive metal form would ***not be classified for long-term environmental hazard***.

IV.7.4 Example D: Hazard classification of a soluble metal salt: the case of rapid environmental transformation through speciation in the water column

General approach

This example was selected to:

i. illustrate the use of information on the metal oxidation and resulting transformation of metal ions in the water column for classification decisions;

ii. provide further information related to testing of sparingly soluble metal salts.

The metal ion selected for this example, Me(II), is unstable when its solutions are exposed to air, and it oxidises to the Me(III), which then forms the familiar insoluble, hydrated, amorphous, gelatinous precipitate, $Me(OH)_3$ (metal hydroxide). The question then arises as to whether the metal hydroxide precipitate forms rapidly enough to decrease the concentration of Me(II) and Me(III) ions to levels below which there is no cause for concern over the aquatic environment. Consideration of the rates at which Me(II) oxidises to Me(III) is relevant to this question to proof rapid environmental transformation.

Additionally, the classification of substances of concern for the aquatic environment requires evaluation of aquatic toxicity. Results for this case were evaluated against standard acceptability criteria for use in this classification assessment.

Results

<u>Assessment of the rapid environmental transformation:</u>

A review of the scientific literature on the oxidation of metal sulphate reveals the following: Metal sulphate reacts with oxygen in water to form metal hydroxide ($MeOH_2$), moderately insoluble, Ksp = 1.6×10^{-14}) this in turn undergoes further oxidation to form metal hydroxide ($MeOH_3$) which is highly insoluble (Ksp = 1×10^{-36}). Formation of metal hydroxide at pH levels above 5.0 limits the presence of metal ions in aqueous systems. In sediments the metal hydroxide is expected to result in enriched concentrations of insoluble metal sulphide.

The rates at which dissolved metal sulphate (Me^{++}) oxidises to (Me^{+++}) and forms the metal hydroxide [$Me(OH)_3$] precipitate:

- Is highly dependent on pH (100 fold from pH 6 to 8);
- decreases with increase in ionic strength of the aqueous medium (pristine waters contain less metal ions);
- dependent to some extent on the anions present in solution such as sulphate and chloride;
- increases 10-fold for a 15 °C increase in temperature;
- exhibits a linear dependence on the partial pressure of oxygen; and
- dependent on the initial concentration of metal sulphate and exhibits linear reaction kinetics at Me(II) loadings less than ~50 micromolar (~3 mg/L). At concentrations greater than 50 micromolar, rates of reaction increase with increasing concentration of metal sulfate (about 4× for each order of magnitude).

Based on literature data and empirical reaction kinetics, it can be calculated that, at low pH (reasonable worst case scenario) in the OECD 203 medium (diluted by 10 as per the Transformation/Dissolution Protocol), the half-times for the oxidation of Me(II) are 11, 9 and 3.6 hr, for 1, 10 and 100 mg/L loadings of $MeSO_4$, respectively. At high pH, the reaction is estimated to be as short as 8 seconds. The rapid precipitation of metal ions from aqueous systems accounts for low 'metal' concentrations found in most natural aquatic systems (all except natural waters at very low pH values (*i.e.* < pH 5.5)). Under the reasonable worst case scenario of low pH and a low initial concentration of 1 mg/L $MeSO_4$, the 70 % removal from solution is calculated to be achieved in 19hr and 90 % removal would be achieved by 36hr. Since the removal of the metal sulphate are due to reaction with oxygen in water to form highly insoluble and non-classifiable metal hydroxide and the half-life for the removal of the soluble species are less than 16 days this can be considered as rapidly transformed in the water column and the substance considered for classification purposes as rapidly degradable.

To support this, evidence of rapid loss of 'Metal ions' (and other metals) from the water column has been reported in mesocosm lake experiments (Perch Lake). The data are presented as half-lives as a function of time, partition coefficient and first stability constant. Half-lives for metal ions in the mesocosms are calculated to be approximately 11 days under the given conditions. The data support that half-lives are short and loss from the water column can be related to both formation of the metal hydroxide but also to sorption to suspended particles that are settling.

Aquatic Toxicity

Acute ERV values lie in the range of 1-37 mg/L (see Table). Two values for *Daphnia magna* were less than 10 mg/L. Four *Daphnia magna* studies were performed and the geometric mean value for this species is 5.77 mg/L. The values for fish were all greater than 10 mg/L. No algal studies were deemed reliable. All these values are expressed as mg/L Me. If the classification relates specifically to metal sulphate of which the most common form is the heptahydrate $MeSO_4.7H_2O$. The numerical ERV values detailed should be adjusted according to the table below and the species under consideration to calculate the toxicity on a metal sulfate basis.

Chemical Species	Molecular Weight	Ratio
$MeSO_47H_2O$	278.0	4.978
$MeSO_4H_2O$	169.91	3.043
$MeSO_4$	151.90	2.720
Me	55.84	1.0

The data cover all the reliable results available for aquatic toxicity of binary 'metal' and any observed toxicity effects could relate to the Me ion which could be in Me(II) or metal Me(III) oxidation states.

Conversion of the acute ERV values for the metal ion to those appropriate for $MeSO_4.7H_2O$ implies an acute toxicity range of 6.4 to 199 mg/L.

Table IV. 2 Acute toxicity data deemed reliable for 'Metal' are presented as mg/L Me

Test substance	Test organism	Duration	Endpoints	L(E)C_{50} (mg Me L^{-1})
$MeCl_3.6H_2O$	*Pimephales promelas* *Lepomis macrochirus*	96h 96h	Survival Survival	21.8 20.3
$MeSO_4.7H_2O$	*Oncorhynchus mykiss*	96h	Survival	16.6
$Me_2(SO_4)_3$	*Oncorhynchus mykiss*	96h	Survival	>27.9
$MeSO_4$	*Daphnia pulex*	24h	Immobility	36.9
$MeSO_4$	*Daphnia magna*	24h	Immobility	17
$MeCl_3.6H_2O$	*Daphnia pulex*	48h	Immobility	12.9
$Me_2(SO_4)_3$	*Daphnia longispina*	48h	Immobility	11.5
$MeCl_3.6H_2O$	*Daphnia magna*	48 h	Immobility	9.6
$MeSO_4$	*Daphnia magna*	24h	Immobility	5.25
$MeSO_4.7H_2O$	*Daphnia magna*	48h	Immobility	1.29

Table IV. 3 Chronic toxicity data deemed reliable for 'Metal' are presented as mg/L Me

Test substance	Test organism	Duration	Endpoints	NOEC/LOEC (mg Me L^{-1})
$Fe(OH)_3$	*Salvelinus fontinalis*	30 days	Hatching Growth Survival	 >10.3
$Fe(OH)_3$	*Oncorhynchus kisuth*	30 days	Hatching Growth Survival	>10.3 2.81/>10.3 >10.3
$FeCl_3.6H_2O$	*Pimephales promelas*	33 days	Survival Length Weight	 1.0/1.6 1.61/2.81
$FeCl_3.6H_2O$	*Daphnia pulex*	21 days	Immobility Total offspring Brood size	2.51/5.01 0.63/1.26 1.26/2.51
$FeCl_3.6H_2O$	*Daphnia magna*	21 days	Immobility Reproduction	5.9 EC50 4.4 EC16

Aquatic hazard classification*:*

Acute hazard: Not classified.

Long-term hazard: Not classified.

Reasoning:

Acute aquatic toxicity > 1 mg/L.

Since all chronic aquatic toxicity values are higher than 1 mg/L and rapid transformation to a metal hydroxide takes place by normal environmental processes, no classification is warranted.

Labelling elements based on the classification:

Element	Code
GHS Pictogram	none
Signal Word	none
Hazard Statement	none
Precautionary statement(s)	none

EUROPEAN CHEMICALS AGENCY
P.O. BOX 400,
FI-00121 HELSINKI, FINLAND
ECHA.EUROPA.EU