ECHA
EUROPEAN CHEMICALS AGENCY

Guidance on the Biocidal Products Regulation

Volume III: Human health
Parts B+C: Assessment & evaluation
Version 5.0, August 2025

LEGAL NOTICE

Guidance on the BPR: Volume III Human health, Assessment & evaluation (Parts B+C)

Reference: ECHA-25-G-05-EN
ISBN: 978-92-9468-508-7
Cat. Number: ED-01-25-021-EN-N
DOI: 10.2823/5976476
Publ.date: August 2025
Language: EN

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DOCUMENT HISTORY

Version	Comment	Date
Version 1.0	First edition	December 2013
Version 1.1	Corrigendum covering the following: (i) Added Annex A, a Commission document on Substances of Concern (ii) Reformatting into ECHA corporate style (iii) Editorial revisions such as punctuation, spelling, etc. (iv) Correcting broken hyperlinks Adding hyperlinks to list of abbreviations and section cross references	April 2015
Version 2.0	Update to section 3 Exposure Assessment The section has been fully revised as follows: • updated text on Exposure Assessment • alignment of the guidance with REACH principles/guidance on exposure • editorial revisions such as punctuation, spelling, etc. • removal of the "technical aspects" into a separate document on Biocides Human Health Exposure Estimation Methodology (available on Biocides webpages). improvement of workflow diagrams	October 2015
Version 2.1	Corrigendum to update the guidance to address Part C Evaluation and to add text and links on "Applicability of Guidance" The text has been revised as follows: • Preface: updated to be in line with the general information in the Part A. • General Introduction: a new paragraph to explain the association of the evaluation and assessment processes. Preface: to add text and links on "Applicability of Guidance"	February 2017
Version 3.0	Update to add a new Section for guidance from ARTFood Project 2 The text has been revised as follows: • To add a new section 5 To revise section 3.4.2 to cross refer to this new section.	November 2017
Version 4.0	Update to add a new Section for guidance from ARTFood Project 1 The text has been revised as follows: • To add a new section 6 To revise section 3.4.2 to cross refer to this new section.	December 2017
Version 5.0	Full revision The guidance was fully reviewed to ensure it is up to date. Sections 5 and 6 of the guidance v. 4.0 were removed from this guidance and moved into a separate guidance on dietary risk assessment, ECHA Guidance Vol III Part D.	August 2025

PREFACE

The Guidance on the Biocidal Products Regulation, Parts B+C (Assessment & evaluation) describes how to assess the information and perform exposure and risk assessment under the Biocidal Products Regulation. For an overview of all the guidance for biocides, please see the ECHA Biocides Guidance website[1].

Guidance on the applicability of new guidance and guidance related documents **for active substance approval** is provided in the document "Applicability time of new guidance and guidance-related documents in active substance approval" available on the BPC Webpage[2].

Guidance on the applicability of new guidance and guidance related documents **for product authorisation** is provided in the CA-document CA-july2012-doc6.2d (final)[3] available on the ECHA Biocides Guidance website[1].

Note that where endpoints refer to classification, this guidance should be read in conjunction with the relevant guidance on CLP.

The guidance documents are generally referred to using short names in *italics*. The full names, references and links to these are provided in Guidance references.

[1] https://echa.europa.eu/guidance-documents/guidance-on-biocides-legislation
[2] Link available under Working Procedures at https://echa.europa.eu/about-us/who-we-are/biocidal-products-committee
[3] Direct link to the document: https://circabc.europa.eu/ui/group/e947a950-8032-4df9-a3f0-f61eefd3d81b/library/a6704d11-5de2-4e17-906f-4bc76fa856aa/details

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GUIDANCE REFERENCES

In this guidance, the various guidance documents are referred to by their short names given in Table 1. For ECHA Guidance, two links are provided: a generic link to the webpage where each guidance can be found, and a direct link that is valid until the guidance is replaced e.g. by a new version.

Table 1: Main guidance referred to

	Short name	Full reference and links
ECHA Guidance on BPR	ECHA Guidance Vol III Part A	Guidance on the Biocidal Products Regulation, Volume III Human health Part A: Information requirements. Available at https://echa.europa.eu/guidance-documents/guidance-on-biocides-legislation Direct link*: https://echa.europa.eu/documents/10162/2324906/bpr_guidance_vol_iii_part_a_en.pdf/05e4944d-106e-9305-21ba-f9a3a9845f93?t=1648525287369
	ECHA Guidance Vol III Part D	Guidance on the Biocidal Products Regulation, Volume III Human health Part D: Dietary risk assessment and livestock exposure[4]. Available at https://echa.europa.eu/guidance-documents/guidance-on-biocides-legislation
	Introduction to ECHA Guidance Part A of Vol I-IV	Introduction to guidance on the Biocidal Products Regulation, Part A: Information requirements, Volumes I – IV. Available at https://echa.europa.eu/guidance-documents/guidance-on-biocides-legislation Direct link*: https://echa.europa.eu/documents/10162/2324906/introduction_part_a_en.pdf/2c188c12-a366-5e45-f843-10abf1e37b6a?t=1648524330674
	ECHA/EFSA ED Guidance	ECHA/EFSA (2018) Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 Available at https://echa.europa.eu/guidance-documents/guidance-on-biocides-legislation Direct link*: https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2018.5311
	ECHA Guidance on Technical Equivalence	Guidance on the Biocidal Products Regulation, Volume V Guidance on applications for technical equivalence Available at https://echa.europa.eu/guidance-documents/guidance-on-biocides-legislation Direct link*: https://echa.europa.eu/documents/10162/2324906/guidance_applications_technical_equivalence_en.pdf/18f72d37-98b6-47c8-98bb-941afeff6968?t=1531380179243

[4] A direct link is not provided because ECHA Guidance Vol III Part D is under revision at the time of publication of the current guidance. Until publication of Part D: where Part D is referred to, please see Sections 5 and 6 of ECHA Guidance Vol III Parts B+C, version 4.1.

	BHHEM	Biocides Human Health Exposure Methodology Available at http://echa.europa.eu/about-us/who-we-are/biocidal-products-committee/working-groups/human-exposure Direct link*: https://echa.europa.eu/documents/10162/992289/bpr_exposuremethodbiochh_en.docx/17e40d4c-5f48-4e12-952b-5372bfe2403c?t=1717067309626
Other ECHA Guidance	CLP Guidance	Guidance on the Application of the CLP Criteria, Part 3: Health Hazards. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Available at https://echa.europa.eu/guidance-documents/guidance-on-clp Direct link*: https://echa.europa.eu/documents/10162/2324906/clp_part3_en.pdf/42e0397a-73f2-0583-958f-3830928e1604?t=1730718832043
	Introductory Guidance on the CLP Regulation	Introductory Guidance on the CLP Regulation Available at https://echa.europa.eu/guidance-documents/guidance-on-clp Direct link*: https://echa.europa.eu/documents/10162/2324906/clp_introductory_en.pdf/b65a97b4-8ef7-4599-b122-7575f6956027?t=1547546145023
	RAAF	Read-Across Assessment Framework (RAAF) Available at https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across
	REACH Guidance Part E	Guidance on Information Requirements and Chemical Safety Assessment, Part E: Risk Characterisation Available at https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment Direct link*: https://echa.europa.eu/documents/10162/17224/information_requirements_part_e_en.pdf/1da6cadd-895a-46f0-884b-00307c0438fd
	REACH Guidance R.3	Guidance on information requirements and chemical safety assessment Chapter R.3: Information gathering Available at https://echa.europa.eu/guidance-documents/guidance-on-reach Direct link*: https://echa.europa.eu/documents/10162/17235/information_requirements_r3_en.pdf/41895234-1125-4977-b058-50a98e36fa48
	REACH Guidance R.4	Guidance on information requirements and chemical safety assessment Chapter R.4: Evaluation of available information Available at https://echa.europa.eu/guidance-documents/guidance-on-reach Direct link*: https://echa.europa.eu/documents/10162/17235/information_requirements_r4_en.pdf/d6395ad2-1596-4708-ba86-0136686d205e
	REACH Guidance R.6	Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of chemicals Available at https://echa.europa.eu/guidance-documents/guidance-on-reach Direct link*: https://echa.europa.eu/documents/10162/17224/information_requirements_r6_en.pdf/77f49f81-b76d-40ab-8513-4f3a533b6ac9

	REACH Guidance R.7a	Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance Available at https://echa.europa.eu/guidance-documents/guidance-on-reach Direct link*: https://echa.europa.eu/documents/10162/17224/information_requirements_r7a_en.pdf/e4a2a18f-a2bd-4a04-ac6d-0ea425b2567f
	REACH Guidance R.7c	Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7c: Endpoint specific guidance Available at https://echa.europa.eu/guidance-documents/guidance-on-reach Direct link*: https://echa.europa.eu/documents/10162/17224/information_requirements_r7c_en.pdf/e2e23a98-adb2-4573-b450-cc0dfa7988e5
	REACH Guidance R.8	Guidance on information requirements and chemical safety assessment Chapter R.8: Characterisation of dose [concentration]-response for human health Available at https://echa.europa.eu/guidance-documents/guidance-on-reach Direct link*: https://echa.europa.eu/documents/10162/17224/information_requirements_r8_en.pdf/e153243a-03f0-44c5-8808-88af66223258
	REACH Guidance R.13	Guidance on information requirements and chemical safety assessment Chapter R.13: Risk management measures and operational conditions Available at https://echa.europa.eu/guidance-documents/guidance-on-reach Direct link*: https://echa.europa.eu/documents/10162/17224/information_requirements_r13_en.pdf/1f6d95d0-a9cb-479d-889e-f7f528e69fbd
	REACH Guidance R.15	Guidance on Information Requirements and Chemical Safety Assessment Chapter R.15: Consumer exposure assessment Available at https://echa.europa.eu/guidance-documents/guidance-on-reach Direct link*: https://echa.europa.eu/documents/10162/17224/information_requirements_r15_en.pdf/35e6f804-c84d-4962-acc5-6546dc5d9a55
	REACH Guidance R.19	Guidance on information requirements and chemical safety assessment Chapter R.19: Uncertainty analysis, available at https://echa.europa.eu/guidance-documents/guidance-on-reach Direct link*: https://echa.europa.eu/documents/10162/17224/information_requirements_r19_en.pdf/d5bd6c3f-3383-49df-894e-dea410ba4335
EFSA Guidance	EFSA Opinion on genotoxicity testing strategies	EFSA, European Food Safety Authority (2011), Scientific Opinion of the Scientific Committee on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 9(9), www.efsa.europa.eu/efsajournal
	EFSA Guidance on dermal absorption	European Food Safety Authority (EFSA) (2017), Guidance on dermal absorption, EFSA Journal 15(6), https://doi.org/10.2903/j.efsa.2017.4873
	EFSA Clarification on genotoxicity	EFSA Scientific Committee (2017), Clarification of some aspects related to genotoxicity assessment, EFSA Journal 15(12), https://doi.org/10.2903/j.efsa.2017.5113

	EFSA WoE Guidance	European Food Safety Authority (EFSA) (2017), Guidance on the use of the weight of evidence approach in scientific assessments, EFSA Journal 15(8), https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2017.4971
	EFSA Guidance on uncertainty analysis	European Food Safety Authority (EFSA) (2018), Guidance on Uncertainty Analysis in Scientific Assessments, EFSA Journal 16(1), https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5123
	EFSA TTC Guidance	European Food Safety Authority (EFSA) (2019), Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment, https://www.efsa.europa.eu/en/efsajournal/pub/5708
	EFSA Opinion on comparative in vitro metabolism studies	European Food Safety Authority (EFSA) (2021), Scientific Opinion of the Scientific Panel on Plant Protection Products and their Residues (PPR Panel) on testing and interpretation of comparative in vitro metabolism studies, EFSA Journal 19(12), https://doi.org/10.2903/j.efsa.2021.6970
	EFSA aneugenicity Guidance	European Food Safety Authority (EFSA) (2021), Guidance on aneugenicity assessment, EFSA Journal 19(8), https://doi.org/10.2903/j.efsa.2021.6770
	EFSA BMD Guidance	European Food Safety Authority (EFSA) (2022), Guidance on the use of the benchmark dose approach in risk assessment, EFSA Journal 20(10), https://doi.org/10.2903/j.efsa.2022.7584
	EFSA Guidance on epidemiological studies	European Food Safety Authority (EFSA) (2024), Scientific Committee guidance on appraising and integrating evidence from epidemiological studies for use in EFSA's scientific assessments, EFSA Journal 22(7), https://doi.org/10.2903/j.efsa.2024.8866
OECD Guidance	OECD GD 20	OECD (2004), Guidance Document for Neurotoxicity Testing, OECD Series on Testing and Assessment, No. 20, OECD Publishing, Paris, https://doi.org/10.1787/16d23a96-en
	OECD GD 28	OECD (2004), Guidance Document for the Conduct of Skin Absorption Studies, OECD Series on Testing and Assessment, No. 28, OECD Publishing, Paris, https://doi.org/10.1787/9789264078796-en
	OECD GD 71	OECD (2017), Guidance Document on the Uterothrophic Bioassay - Procedure to Test for Antioestrogenicity, OECD Series on Testing and Assessment, No. 71, OECD Publishing, Paris, https://doi.org/10.1787/76fa8730-en
	OECD GD 116	OECD (2014), Guidance Document 116 on the Conduct and Design of Chronic Toxicity and Carcinogenicity Studies, Supporting Test Guidelines 451, 452 and 453: Second edition, OECD Series on Testing and Assessment, No. 116, OECD Publishing, Paris, https://doi.org/10.1787/9789264221475-en
	OECD GD 150	OECD (2018), Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption, OECD Series on Testing and Assessment, No. 150, OECD Publishing, Paris, https://doi.org/10.1787/9789264304741-en
	OECD GD 151	OECD (2013), Guidance document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test, OECD Series on Testing and Assessment, No. 151, https://one.oecd.org/document/ENV/JM/MONO(2013)10/en/pdf
	OECD 184	OECD (2017), Revised Guidance Document on Developing and Assessing Adverse Outcome Pathways, Series on Testing & Assessment No. 184. ENV/JM/MONO(2013)6

	OECD GD 203	OECD (2017), Guidance Document on an Integrated Approach on Testing and Assessment (IATA) for Skin Corrosion and Irritation, OECD Series on Testing and Assessment, No. 203, OECD Publishing, Paris, https://doi.org/10.1787/9789264274693-en
	OECD 207	OECD (2014), New Scoping Document on in vitro and ex vivo Assays for the Identification of Modulators of Thyroid Hormone Signalling, OECD Series on Testing and Assessment, No. 207, OECD Publishing, Paris, https://doi.org/10.1787/9789264274716-en
	OECD GD 256	OECD (2016). Guidance Document on the reporting of Defined Approaches and Individual Information Sources to be used within Integrated Approaches to Testing and Assessment for Skin Sensitisation, OECD Series on Testing and Assessment No. 256, ENV/JM/MONO(2016)29, ENV/JM/MONO(2016)29/ANN1 and ENV/JM/MONO(2016)29/ANN2
	OECD GD 260	OECD (2017), Guidance Document for the Use of Adverse Outcome Pathways in Developing Integrated Approaches to Testing and Assessment (IATA), OECD Series on Testing and Assessment, No. 260, OECD Publishing, Paris, https://doi.org/10.1787/44bb06c1-en
	OECD GD 263	OECD (2024), Guidance Document on Integrated Approaches to Testing and Assessment (IATA) for Serious Eye Damage and Eye Irritation, Third Edition, OECD Series on Testing and Assessment, OECD Publishing, Paris, https://doi.org/10.1787/cdb440be-en
	OECD GD 286	OECD (2018), Guidance Document on Good In Vitro Method Practices (GIVIMP), OECD Series on Testing and Assessment, No. 286, OECD Publishing, Paris, https://doi.org/10.1787/9789264304796-en
	OECD GD 331	OECD (2021), Guidance document on the characterisation, validation and reporting of Physiologically Based Kinetic (PBK) models for regulatory purposes, OECD Series on Testing and Assessment, No. 331, OECD Publishing, Paris, https://doi.org/10.1787/d0de241f-en
	OECD GD 397	OECD (2024), Guidance Document on Integrated Approaches to Testing and Assessment (IATA) for Phototoxicity Testing, OECD Series on Testing and Assessment, No. 397, OECD Publishing, Paris, https://doi.org/10.1787/8a979653-en
	OECD 20	OECD (2019), Guidance for Receiving Authorities on the Review of the GLP Status of Non-Clinical Safety Studies, OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, No. 20, OECD Publishing, Paris, https://doi.org/10.1787/cd5bf97e-en
	OECD 156	OECD (2022), Guidance notes on dermal absorption studies, OECD Series on Testing and Assessment, No. 156, OECD Publishing, Paris, https://one.oecd.org/document/ENV/JM/MONO(2011)36/REV1/en/pdf
	OECD 238	OECD (2017), Overview on genetic toxicology TGs, OECD Series on Testing and Assessment, No. 238, OECD Publishing, Paris, https://doi.org/10.1787/9789264274761-en
	OECD 403	OECD (2024), Thyroid in vitro methods: assessment reports by the thyroid disruption methods expert group: Reports assessing the validation status of assays from the EU-NETVAL activities, OECD Series on Testing and Assessment, No. 403, OECD Publishing, Paris, https://doi.org/10.1787/3786c75f-en
	OECD 405	OECD (2024), (Q)SAR Assessment Framework: Guidance for the regulatory assessment of (Quantitative) Structure Activity Relationship models and predictions, Second Edition, OECD Series on Testing and Assessment, No. 405, OECD Publishing, Paris, https://doi.org/10.1787/bbdac345-en

	OECD 497	OECD (2023), Guideline No. 497: Defined Approaches on Skin Sensitisation, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/b92879a4-en
	OECD TG	OECD Test Guidelines for testing of chemicals; available at https://www.oecd.org/env/ehs/testing/seriesontestingandassessmentadoptedguidanceandreviewdocuments.htm
Other Guidance	ICH Photosafety Guidance	ICH Guidance S10 on Photosafety Evaluation of Pharmaceuticals (2015) EMA/CHMP/ICH/752211/2012 Committee for Human Medicinal Products: https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/ich-guideline-s10-photosafety-evaluation-pharmaceuticals-step-5_en.pdf
	JECFA ARfD guidance	World Health Organization, Food and Agriculture Organization of the United Nations & Joint FAO/WHO Expert Committee on Food Additives. (2017). Guidance document for the establishment of Acute Reference Dose (ARfD) for veterinary drug residues in food: Joint FAO/WHO Expert Committee on Food Additives (JECFA). World Health Organization. https://iris.who.int/handle/10665/340568
	NAFTA DNT Guidance	North American Free Trade Agreement (NAFTA) Technical Working Group on Pesticides (TWG), Developmental Neurotoxicity Study Guidance Document, 2016, https://www.epa.gov/sites/default/files/2017-02/documents/developmental_neurotoxicity_study_internal_guidance_document_final_0.pdf
	US EPA Guidance for thyroid assays	US EPA (2015), Guidance for Thyroid Assays in Pregnant Animals, Fetuses and Postnatal Animals, and Adult Animals. https://www.epa.gov/pesticide-registration/guidance-thyroid-assays-pregnant-animals-fetuses-and-postnatal-animals-and
	WHO/IPCS Immunotoxicity guidance	World Health Organization & International Programme on Chemical Safety. (2012). Guidance for immunotoxicity risk assessment for chemicals. World Health Organization. https://iris.who.int/handle/10665/330098
	WHO/IPCS Guidance on uncertainty and data quality in exposure assessment	International Programme on Chemical Safety. (2008). Uncertainty and data quality in exposure assessment. World Health Organization. https://iris.who.int/handle/10665/44017
	WHO/IPCS Guidance on principles for human exposure models	International Programme on Chemical Safety. (2005). Principles of characterizing and applying human exposure models. World Health Organization. https://iris.who.int/handle/10665/43370

ABBREVIATIONS

Standard term / Abbreviation	Explanation
ACH	Air changes per hour
ADI	Acceptable daily intake
ADME	Absorption, distribution, metabolism, and excretion
AEC	Acceptable Exposure Concentration
AEL	Acceptable exposure level
AF	Assessment factor
AOEL	Acceptable Operator Exposure Level
AOPs	Adverse Outcome Pathways
APF	Assigned Protection Factors
ARfD	Acute Reference Dose
ATP	Adenosine triphosphate
AUC	Area under the curve
BMD	Benchmark dose
BPC	Biocidal Products Committee (ECHA body)
BPR	Biocidal Products Regulation. Regulation (EU) No 528/2012 of the European Parliament and of the Council concerning the making available on the market and use of biocidal products
bw	Body weight
CA	Competent Authority Evaluating CA (eCA) is the Competent Authority that evaluates the application for an active substance approval or an application for a Union authorisation. Receiving CA is the Competent Authority that receives an application for a national authorisation.
Cat	Category
CLP (Regulation)	Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging of substances and mixtures
C&L	Classification and labelling
ConsExpo	Software enabling estimation of consumer exposure
Cmax	Peak plasma concentration
CNS	Central nervous system

DMEL	Derived Minimal Effect Level For non-threshold effects, the underlying assumption is that a no-effect-level cannot be established and a DMEL therefore expresses an exposure level corresponding to a low, possibly theoretical, risk, which should be seen as tolerable risk.
DNA	Deoxyribonucleic acid
DNEL	Derived No Effect Level
DNT	Developmental neurotoxicity
EATS	Estrogen, Androgen, Thyroid, Steroidogenesis
EC_{50}	Median effective concentration
ECVAM	(see "EURL ECVAM")
EFSA	European Food Safety Agency
EN	European norm
EOGRTS	Extended one generation reproductive toxicity study
EPA (USA)	Environmental Protection Agency of the United States of America
EURL ECVAM	EU Reference Laboratory for alternatives to animal testing
FAO	Food and Agriculture Organization
FDA	U.S. Food and Drug Administration
GI	Gastrointestinal
GLP	Good laboratory practice
HBM	Human biomonitoring
HI	Hazard index
HQ	Hazard quotient
IC_{50}	Median immobilisation concentration or median inhibitory concentration
IOEL	Indicative occupational exposure level
IPCS	International Programme on Chemical Safety of the World Health Organisation
ISO (TC, SC, WG)	International Organisation for Standardisation (Technical Committee, Scientific Committee, Working Group)
JECFA	Joint FAO/WHO Expert Committee on Food Additives and Contaminants
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
KE	Key event
KER	Key event relationship
Km	Michaelis constant, describes the concentration at which half the enzyme's active sites are occupied by substrate

K_{ow}	Octanol-water partition coefficient
K_P	Solid-water partitioning coefficient of suspended matter
LEV	Local exhaust ventilation
LLNA	Local lymph node assay
LOAEC	Lowest Observed Adverse Effect Concentration
LOAEL	Lowest Observed Adverse Effect Level
log P	Octanol/water partition coefficient
M	Molarity
MMAD	Mass median aerodynamic diameter
mmHg	Millimetre(s) of mercury, a unit of pressure equal to 0.001316 atmosphere
mN/m	Millinewton(s) per metre, a unit of torque
MoA	Mode of action
MOA	Mechanism of action
mol	Mole(s)
MRL	Maximum residue level
MTD	Maximum tolerated dose
NAMs	New approach methodologies
NOAEC	No observed adverse effect concentration
NOAEL	No observed adverse effect level
NOEL	No observed effect level
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational exposure limit OELs are regulatory values which indicate levels of exposure that are considered to be safe (health-based) for a chemical substance in the air of a workplace. Such limits are set by regulatory authorities at EU and national levels.
OSHA	Occupational Safety and Health Administration (European Agency for Safety and Health at Work)
Pa	Pascal(s)
PBK	Physiologically based kinetic (modelling)
pKa	Negative decadic logarithm of the acid dissociation constant (describes how acidic (or not) a given hydrogen atom in a molecule is)
PoD	Point of Departure
PPE	Personal Protective Equipment

PT	Product type
(Q)SAR	(Quantitative) structure activity relationship
RA	Risk Assessment
RAC	Committee for Risk Assessment (ECHA body)
RC	Risk Characterisation
REACH	Regulation (EC) No 1907/2006 on Registration, Evaluation, Authorisation and Restriction of Chemicals
RMM	Risk Management Measures
RPE	Respiratory Protective Equipment
SDS	Safety data sheet
SoC	Substance of concern
STOP	Substitution, Technical measures, Organisational measures, Personal protection. This STOP principle gives a hierarchy for the selection of risk management measures at the workplace in the order of priority.
TD	Toxicodynamics
TDAR	T-cell dependent antibody response assay
TK	Toxicokinetics
TG	Test guideline
TGR	Transgenic rodent (assay)
TTC	Threshold of toxicological concern
UDS	Unscheduled DNA synthesis
Vmax	Maximum velocity, reflects how fast the enzyme can catalyse the reaction
w/w	Weight per weight ratio
WHO	World Health Organisation
WoE	Weight of evidence

GLOSSARY OF TERMS

Standard term / Abbreviation	Explanation
Abuse	is intentional misuse, for example inhaling aerosol propellant - as such, it is not included in exposure estimation.
Actual dermal exposure	is the amount of active substance or in-use biocide formulation (biocidal product) that reaches the skin through e.g. (work) clothing or gloves and is available for uptake through the skin.
Assessment factor (AF)	Assessment factors reflect the degree of uncertainty in extrapolation from experimental test data (e.g. obtained in a limited number of subjects from a limited number of species) to the situation in the human (sub-) population for which the risk characterisation is performed. Sources of uncertainty typically considered by using AFs include inter- and intraspecies variability in terms of toxicodynamics and/or TK, differences in route, frequency or duration of exposure between the experimental data and the scenario considered for risk characterisation, particular severity of effect, or a poor Database. Synonyms of AF under other legislative frameworks and historically include uncertainty factor, extrapolation factor, modifying factor, safety factor.
Biological monitoring	is the sampling of blood, urine, saliva or exhaled air at suitable times before, during and after the task, and analysing for the substance or a metabolite to determine the body dose. The sampling regime needs expert advice and ethical clearance.
Bystanders	are those who could be located within or directly adjacent to the area where a biocidal product has been applied; their presence is quite incidental and unrelated to work involving biocides, but whose position might lead them to be exposed for a short period of time (acute exposure); and who take no action to avoid or control exposure.
Central tendency	in a distribution is a value that describes best the central value. The central tendency may be used in exposure estimates where well trained operators show practically continuous use.
Consumer	A member of the general public who may be exposed to biocides by using a consumer product.
Dislodgeable residues	are post-application residues that are available for uptake through human contact with substances on surfaces.
exposure via the environment	is an element of secondary exposure. It includes bystanders and consumers, including children, who are inadvertently exposed to biocides by inhalation and/or ingesting contaminated food or water.
Industrial users	are professional users involved in manufacturing, handling and/or packaging of active substances or products in industry as well as those using biocidal products in their own processes at industrial setting, for example, manufacturers of timber cladding using wood preservatives or food companies using disinfectants.
Ingestion	arises from the swallowing of biocides. Ingestion can also occur through poor hygiene practice (e.g. through dislodging from contaminated skin to food or cigarettes, by hand-mouth contact, or through applying cosmetics).
Inhalation exposure	reflects the airborne concentration that is available in the breathing zone. The substance is then available for uptake via the lungs or following mucociliary elevator action from the gastrointestinal tract.

Intended use	of a biocidal product means what is supposed to be used according to the manufacturer's specifications, instructions, and other information.
Mechanism of action	Molecular sequence of events that produce a specific biological outcome.
Mixing & loading	handling biocide concentrates, diluting them and where necessary, putting the in-use formulation into the application apparatus.
Mode of action	Key events by which a chemical exerts its biological effects.
Non-professional user	Non-professional users belong to the general population and are exposed to the biocidal products they are applying, mainly consumer products intended for domestic use. Non-professional users include also employed persons at workplaces, where the use of a biocidal product is not directly related to the main objective of the business (e.g. use of a domestic fly spray in an office environment). A clear definition of the use and user is required to distinguish between professionals and non-professionals. It is assumed that non-professionals will generally comply with instructions for use of a product, but have no access to controls or PPE (with rare exceptions).
Overall assessment factor	The combined AFs covering all uncertainties in deriving a reference value, calculated by multiplication of all individual assessment factors. See also definition of AF.
Penetration of PPE	that proportion of biocide that by-passes PPE, e.g. by soaking through seams and zips, being drawn in at the neck, cuffs and ankles by the "bellows effect", that gets inside protective gloves by them being donned with contaminated hands.
Permeation of PPE	the migration of biocide through the PPE barrier, e.g. solvent-based product through latex-based gloves.
Personal protective equipment (PPE)	includes head, eye, respiratory (RPE), body, hand and foot protection that is designed to protect the wearer.
Post-application	covers the scenarios of sampling, maintaining and cleaning and may give rise to secondary exposure.
Potential dermal exposure	is the deposition of active substance or biocidal product on the outer surface of clothing and on any bare skin.
Preparation or formulation	is the biocidal product as placed on the market; the active substance with its co-formulants, diluents, carrier materials and stabilisers.
Primary exposure	is that which occurs to the user (i.e. the person who applies the biocide).
Probabilistic (stochastic) modeling	is used to combine data in order to derive fair 'central tendency' and 'realistic worst case' values. It is based on distributions of parameters. See deterministic estimates.
Professional users (e.g. employees and the self-employed)	The professional or industrial user comes into contact with the biocidal product as a consequence of their professional life. Professional users are trained and skilled in the main objectives of their occupation and may have some experience and skill in the use of the PPE if that is necessary for their normal work. In general, the professional user is subject to worker protection legislation (e.g. EU Chemical Agents Directive) and has residual risk controlled through control measures, which may include the use of PPE. Some workers will have limited knowledge and skills to handle hazardous biocidal products, particularly if not routinely required in their workplace (e.g. incidental use of slimicides, insecticides, irregular disinfection and use of products containing preservatives). The exposure conditions of these users might be similar to those of non-professional users. See also 'trained professional'.

Realistic worst case	is the situation where the exposure is estimated using from a range of factors (i.e. duration, amount, exposure controls), where applicable, the ones that would be expected to lead to maximum amount of exposure. The realistic worst case does not include deliberate misuse.
Reference value	This term is used for dose levels which serve as reference for assessing whether a particular exposure scenario can be considered to be without appreciable risk to human health. In general, reference values are established by dividing the point of departure (NOAEL/LOAEL) for a critical effect observed in an experimental study by an appropriate overall assessment factor. External reference values are given as concentrations (e.g. in ambient air or solution) and refer to both a specific time frame (short-, medium- or long-term) and route of exposure. In contrast, systemic/internal reference values are given as dose levels on a mg/kg bw basis. They reflect the share of externally applied dose which is systemically available and are thus independent of the route of application, but are also derived for a specific time frame.
Residents	are those who live or work adjacent to an area that has been treated with a biocidal product; whose presence is quite incidental and unrelated to work involving biocides but whose position might lead them to be exposed; who take no action to avoid or control exposure and who might be in the location for 24 hours per day (longer term exposure).
Scenario	is one or a number of well defined tasks for which exposure can be characterised.
Secondary exposure	is that which is not primary. It is characterised through the exposed person having little or no control over their exposure, which may be acute or prolonged. It includes re-entry to treated zones (contact with treated surfaces, inhalation of residual vapours, ingestion of residues).
Synergism	A situation where expected effects are higher than those expected with concentration (dose) addition approach.
Task	covers the phases of use of a biocide. It is a unit of operation within one or several scenarios.
Test Methods Regulation	Regulation (EC) No 440/2008 laying down test methods pursuant to the REACH Regulation
Toxicokinetics	Toxicokinetics describes how the body handles a chemical, as a function of dose and time, in terms of the concept of ADME (absorption, distribution, metabolism and excretion)
Toxicodynamics	Toxicodynamics refers to the molecular, biochemical, and physiological effects of chemicals or their metabolites in biological systems.
Trained professional (trained worker)	A trained professional (synonym: trained worker) has received specialised training in handling hazardous chemicals. They will have received appropriate training 1) to perform their tasks safely, including regarding the process, maintenance and cleaning activities, 2) on the use of RMM including selection and wearing and maintenance of PPE to minimise exposure to the hazardous substance, and 3) to consider the risks to themselves and other persons via secondary exposure, as well as to non-target species where relevant. Trained professionals may use biocidal products more frequently, for longer duration and/or in greater quantities than other types of users.

GENERAL INTRODUCTION

Evaluation

The process of evaluation of active substance applications is given in BPR Article 8 and the common principles for the evaluation of dossiers for biocidal products (including the representative biocidal product in the context of active substance approval) are given in BPR Annex VI.

The evaluating CA (eCA) uses the data submitted in support of an application for active substance approval or authorisation of a biocidal product to make a risk assessment based on the proposed use of the (representative) biocidal product. The evaluating body will base its conclusions on the outcome of the evaluation and decide whether the (representative) biocidal product complies with the criteria for authorisation or whether the active substance may be approved.

This guidance explains how to perform the risk assessment for human health.

Assessment

The risk assessment in relation to human health entails a sequence of actions outlined below.

(1) **Assessment of effects**, comprising:

   (a) **hazard identification:** identification of the adverse effects which a substance has an inherent capacity to cause; and

   (b) **hazard characterisation:** dose (concentration) - response (effects) assessment: estimation of the relationship between dose or concentration to which exposure takes place, and the incidence and severity of an effect.

(2) **Exposure assessment:** estimation of the concentrations/doses to which human populations (workers, consumers) may be exposed either directly or via the environment.

(3) **Risk characterisation:** an assessment on the likelihood that the hazard identified for a substance will occur in a human population due to actual or predicted exposure to that substance. This may include "risk estimation", i.e. the quantification of that likelihood. Combined exposure to multiple chemicals and dietary risk assessment should be considered where relevant.

Risk assessment containing all the above steps must be carried out for all biocidal active substances. Biocidal products may in addition contain substances of concern that also need to be assessed (see BPR Art. 3(1)(f) and the relevant documents agreed at the CA meeting[5]).

Possible results of the risk assessment for biocidal active substances:

- Recommendation for (renewal of) approval of an active substance for use in biocidal products, where necessary subject to certain conditions.
- Recommendation not to approve an active substance for use in biocidal products.

[5] Documents agreed at CA meetings: https://circabc.europa.eu/ui/group/e947a950-8032-4df9-a3f0-f61eefd3d81b/library/386abfea-55ce-4764-8a31-f9d4f6ceaf0a?p=1&n=10&sort=modified_DESC; direct link to CA-Nov14-Doc.5.11: https://circabc.europa.eu/ui/group/e947a950-8032-4df9-a3f0-f61eefd3d81b/library/7bfe2156-3dbc-4793-a2a6-1a8b08a29a23/details

Possible results of the risk assessment for biocidal products:

- Recommendation to authorise a biocidal product (family), where necessary subject to certain restrictions or requirements.
- Recommendation not to authorise a biocidal product (family).

The risk assessment for human health shall address all potential toxic effects and human (sub)populations, considering each population's exposure by the inhalation, oral and dermal routes. This includes but is not limited to acute toxicity, irritation, corrosivity, sensitisation, repeated dose toxicity, mutagenicity, carcinogenicity, toxicity for reproduction, (developmental) neurotoxicity and immunotoxicity and endocrine disruption. The human populations to consider are:

- professional users and industrial workers;
- non-professional users including the general public;
- humans exposed via secondary pathways.

The human exposure assessment is normally based on exposure scenarios (i.e. model calculations) and, if available, on representative monitoring data. If appropriate, available information on substances with analogous use and exposure patterns or analogous properties is taken into account. Expert judgment is needed to assess the availability of representative and reliable monitoring data and/or the amount and the necessary detail of the information to derive realistic exposure levels by modelling. Information may be limited in particular for later stages in the life cycle of a substance (e.g. during and after use in preparations and articles).

The risk assessment should be carried out on the basis of all data available, applying the principles described in the following sections of the document.

It may often be useful to conduct initially a risk assessment using exposure estimates based on worst-case assumptions. If the outcome of such an assessment is "no concern", further risk assessment for that human population will not be necessary, while an outcome "of concern" indicates the need to refine the assessment if possible.

General principles

In brief, human health risk assessment consists of comparing the exposure levels to which the populations are (likely to be) exposed with the exposure levels at which no adverse effects are expected to occur. Where possible, this takes place by comparing the exposure level (the outcome of the exposure assessment), with the relevant AEL or AEC that are derived on the basis of experimental threshold levels such as NOAEL, LOAEL, NOAEC, BMD, etc. with the use of assessment factors (AF; the outcome of the hazard characterisation).

The exposure levels are derived based on available monitoring data and/or model calculations. The experimental threshold values are determined on the basis of results from animal testing or available historical human data (BPR Annex IV, Section 1.1.3). Studies on humans should not be performed for the purposes of BPR.

For some substances, it is not possible to derive an AEL value that would be protective for all systemic effects. As an example, for genotoxic substances it is considered prudent to assume that a threshold exposure level cannot be identified (see section 1.8.5 for exceptions and further guidance).

The derivation and use of dose-response relationships for each of the effects to be considered are discussed in detail in section 2.

To assess effects and exposure, data on physico-chemical properties including chemical reactivity may be needed. Information on physico-chemical properties are required, for example to estimate emissions and human exposure scenarios and to assess the design of toxicity tests. This information may also provide indications regarding absorption of the substance for various routes of exposure. Chemical reactivity may also be relevant in e.g. estimating the exposure of the substance and its breakdown or reaction products, and it has an impact on toxicokinetics and metabolism.

Historical control data helps for example in judging the severity of the effects, reversibility, biological relevance, and the normal biological variation of an effect. In test guidelines a criterium for the evaluation is the comparison with historical control ranges for the negative controls. This helps to clarify the results and to reach a conclusion. In some cases, stored samples from previous positive control animals (i.e. historical control database for the tissues of interest) can replace a concurrent positive control group in an *in vivo* study. Comparison with concurrent study control data should however always take precedence over comparison with historical control data.

The decision whether a substance presents an unacceptable risk to human health is taken on the basis of whether exposure level exceeds the AEL/AEC value. If it is not possible to derive an AEL or AEC, a qualitative evaluation is carried out of the likelihood that an adverse effect may occur.

The comparison of exposure and AEL/AEC is done separately for each human population (likely to be) exposed to the substance. In any particular human population, sub-populations may be identified (e.g. with different exposure scenarios and/or different susceptibility) which may need to be considered individually in risk characterisation. Thus, exposure levels are derived separately for each relevant population/sub-population, and the most critical AELs and/or AECs are identified for the critical endpoints, and ratios of exposure level to AEL/AEC values are established.

The risk assessment relies on expert judgement in interpreting both effects and exposure.

Requirements for further information on effects and on exposure are inter-related, and are to a large extent addressed in the toxicity testing strategies in *ECHA Guidance Vol III Part A*. However, when all effects and expected human exposure patterns are considered, the need of further testing may be considered, possibly using more than one route of exposure. In deciding which tests and routes of exposure should be studied, one should consider toxicokinetic, metabolic and mechanistic information, the intended use and primary and secondary exposure routes. At each stage, integrated requirements for further testing must be developed, using expert judgment to ensure that the necessary information is obtained using the least amount of testing in animals.

1 Effect assessment – hazard identification

1.1 Introduction

The effects assessment comprises the following steps of the risk assessment:

- **hazard identification:** the aim of the hazard identification is to identify the effects of concern and to determine or review classification.
- **hazard characterization: dose (concentration) - response (effect) assessment** is the estimation of the relationship between dose, or level of exposure to a substance, and the incidence and severity of an effect. In this section it is referred to as "dose-response". At this step the NOAEL or NOAEC (or LOAEL, LOAEC) shall be determined for the observed effects, where possible and appropriate. The shape of the dose-response curve should also be considered (see Section 2) where relevant.

At all steps of the effects assessment, the data is evaluated for adequacy and completeness. The evaluation of adequacy shall address the reliability and relevance of the data.

For effects for which it is not possible to determine a NOAEL/LOAEL, it is generally sufficient to evaluate whether the substance has an inherent capacity to cause the effect. Where it is possible to draw a relationship between the dose or concentration of the substance and the severity of an adverse effect, this relationship should be determined.

If well-reported and relevant historical human data are available for any endpoint, it is generally given preference in the risk assessment over animal/non-animal data. Potential differences in sensitivity of human studies and studies in animals should be considered when performing the risk assessment. In hazard identification, the relative lack of sensitivity of human data may cause particular difficulty: negative data from studies in humans will usually not be used to override the classification of substances which have been classified on the basis of data from studies in animals in accordance with the criteria given in the CLP Regulation (Regulation (EC) No 1272/2008) unless the classification is based on an effect which clearly would not be expected to occur in humans.

For hazard identification, *ECHA Guidance Vol III Part A* needs to be considered together with this Guidance as well as with the *CLP Guidance*. As the first steps in hazard assessment, all available information is collected and assessed before deciding if additional testing needs to be performed. Once new test results become available, using *ECHA Guidance Vol III Part A*, these results should be evaluated according to the guidance in this section (i.e. Effects Assessment).

There are various sources for gathering all available information on chemicals:

- The eChemPortal (http://www.echemportal.org), ECHA CHEM (https://chem.echa.europa.eu/), the QSAR Toolbox (http://www.qsartoolbox.org) and US EPA CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard/) are recommended for the collection of existing information on toxicological properties as well as for the determination of potential application of non-test methods in the hazard assessment of biocidal active substances. In addition, toxicological information can be obtained from publicly available study reports (e.g. from NCI) and assessment reports from risk assessment bodies/institutions (e.g. expert panels from EFSA or the European Commission), unpublished studies, databases and publications such as books, scientific journals, criteria documents, monographs and other publications (see *REACH Guidance R.3* for further general guidance).

- Useful databases and resources to obtain data or to aid in the toxicological evaluation of the data are available online. Some examples of freely accessible databases are the DevTox database (https://devtox.org/index_en.php), Fraunhofer ITEM RepDose database (http://fraunhofer-repdose.de/), ToxRefDB by US-EPA (http://www.epa.gov/comptox/toxrefdb/) and ECHA CHEM (http://www.echemportal.org). The last three databases are also freely available within the OECD QSAR Toolbox (www.qsartoolbox.org).

1.2 Evaluation of data

In all stages of effects assessment, it is important to evaluate the adequacy and completeness of the data. This is particularly important for substances where a number of test results are available for each effect, but some or all of them may not have been carried out to current standards. This section puts forward general guidelines on data evaluation. The term adequacy is used here to cover the reliability of the available data and the relevance of that data for human hazard and risk assessment. In addition to this section, the *REACH Guidance R.4* provides further guidance for assessing the relevance, reliability, and adequacy of the information. Further information and tools for this purpose include the Critical Appraisal Tool and the SciRAP (https://scirap.org).

1.2.1 Completeness of data

For biocidal active substances and products, the BPR gives the dispositions on data requirements for authorisation. Annexes II and III of the BPR detail core data requirements to all active substances and biocidal products, respectively, and Annex IV specifies the general rules for the adaptation of the data requirements. For completeness of data, see *ECHA Guidance Vol III Part A*.

1.2.2 Adequacy, reliability and relevance of data

1.2.2.1 Adequacy of data

The adequacy of data can be defined by two basic elements:

- reliability, covering the inherent quality of a test relating to test methodology and the way that the performance and results of the test are described;
- relevance, covering the extent to which a test is appropriate for a particular hazard or risk assessment.

Reliable, relevant data can be considered valid for use in the risk assessment. When there is more than one set of data for an effect, the greatest weight is given to the most reliable and relevant. For a transparent assessment of the reliability and relevance, tools are available such as SciRAP (https://scirap.org), the Critical Appraisal Tool and ToxRTool (https://joint-research-centre.ec.europa.eu/scientific-tools-and-databases/toxrtool-toxicological-data-reliability-assessment-tool_en).

1.2.2.2 Reliability of data

When appropriate tests are available that are conducted according to the EU Test Methods Regulation (Regulation (EC) No 440/2008) and OECD Test Guidelines, in compliance with the

principles of GLP, many of the issues addressed in this section will not be relevant. For guidance on verifying the GLP status of a study, please refer to *OECD 20*.

For some substances, the test data have been generated prior to the requirements of GLP and the standardisation of test methods. That data may still be used for risk assessment, but the data and the methodology used must be evaluated to determine their reliability for assessment purposes. The evaluation requires expert judgement and must be transparent, so that the use made of a particular data set is clearly justified. The requirements of the appropriate standardised test method and GLP principles should be regarded as a reference when evaluating the available test data. Studies carried out according to current methods (e.g. EU Test Methods Regulation, OECD Test Guidelines Programme (http://www.oecd.org/env/ehs/) or U.S. EPA Test Guidelines (https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances) and appropriately reported should be considered most reliable for risk assessment. In accordance with Point 5 of BPR Annex II, the latest version of an adopted test guideline should always be used when generating new data, independently of whether it is published by the EU or OECD. The scoring system developed by (Klimisch et al., 1997) is recommended to assess the reliability of data:

1= reliable without restrictions: "studies or data […] generated according to generally valid and/or internationally accepted testing guidelines (preferably performed according to GLP) or in which the test parameters documented are based on a specific (national) testing guideline […] or in which all parameters described are closely related/comparable to a guideline methods."

2= reliable with restrictions: "studies or data […] (mostly not performed according to GLP), in which the test parameters documented do not totally comply with the specific testing guideline, but are sufficient to accept the data or in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable."

3= not reliable: "studies or data […] in which there were interferences between the measuring system and the test substance or in which organisms/test systems were used which are not relevant in relation to the exposure (e.g. non-physiological pathways of application) or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for assessment and which is not convincing for an expert judgment."

4= not assignable: "studies or data […] which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature (books, reviews, etc.)."

The use of scoring tools allows ranking the information and organising it for further review. This implies focusing on the most relevant ones for the endpoint being measured or estimated. The evaluation of reliability is performed considering certain formal criteria using international standards as references. The scoring of information should not exclude all unreliable data from further consideration because they might still be pertinent to the evaluated endpoints. In general, data that are not reliable or for which reliability cannot be assessed, e.g. due to insufficient documentation, may only be used as supporting data.

In a test report, the assessor should consider whether:

- the purity, impurities and the origin of the test substance are reported;
- a complete test report is available or the test has been described in sufficient detail and the test procedure is in accordance with generally accepted scientific standards;

- the reliability of the data cannot be fully established or the test procedure differs in some respects from the test guidelines and/or generally accepted scientific standards.

The following factors, among others, can support the acceptability of data for use in a risk assessment:

- the data is consistent with other studies or calculations on the substance;
- there are other studies on e.g. isomers with similar structure activity profile, homologues, relevant precursors, breakdown products or other chemical analogues, and the data under consideration are consistent with them;
- an approximate value is sufficient for taking a decision on the result of the risk characterisation;

If critical information is not reported (e.g. species tested, substance identity, dosing procedure) the test data should be considered unreliable for risk assessment.

In principle, the same criteria apply to test data reported in the published literature. The amount of information presented will provide the basis to decide on the reliability of the data reported. In general, publications in peer-reviewed journals are preferable. High-quality reviews may be used as supporting information. Summaries or abstract publications may also supply supporting material. See also specific considerations on the use of public literature in *Introduction to ECHA Guidance Part A of Vol I-IV*.

1.2.2.3 Relevance of data

To evaluate the relevance of the available data, it is necessary to judge, among other things, if an appropriate species has been studied, if the route of exposure is relevant for the population and exposure scenario under consideration, and if the substance tested is representative of the substance as supplied. To assess the latter, it is necessary that the substance is properly identified, and any significant impurities described and relevant impurities identified.

Relevant human data of an adequate quality can sometimes be the best available data but, more frequently, the available human, animal, and other data are considered together to conclude on the relevance to humans of effects observed in animals.

The evaluation of the relevance for humans of data from studies in animals is aided by use of data on the TK, including metabolism of a substance in both humans and the animal species used in the toxicity tests. Well-documented evidence for a species-specific effect/response (e.g. light hydrocarbon-induced nephropathy in the kidney of male rats) can be used as justification for the conclusion that a particular effect is not expected to occur in humans exposed to the substance.

In the absence of such information on the substance itself or by justified read-across, threshold adverse effects observed in studies in animals will normally be assumed likely to occur also in humans exposed to the substance above a certain level of exposure.

In the interpretation of relevance of *in vitro* data, it should be taken into account whether the results seen have been observed or could be expected to occur (e.g. from a knowledge of the TK of the substance) *in vivo*. In general, the relevance of an alternative (non-animal) test, such as an *in vitro* test, is assessed according to the scientific basis of the test system (scientific relevance) and the predictive capacity (predictive relevance) of the prediction model, which is an algorithm for extrapolating from *in vitro* data to an *in vivo* endpoint (see also *OECD GD 286*).

I*n vitro* tests are used as standard test guideline protocols for the assessment of specific endpoints. However, in general, the results of *in vitro* tests provide supplementary information which, for instance, may be used to facilitate the interpretation of the relevance for humans of data from studies in animals, or to gain a better understanding of the mechanism of action of a substance.

1.2.2.4 Types of data

This section covers information apart from specific animal testing performed to assess the hazard properties of the substance.

1.2.2.4.1 Human data

The evaluation of human data usually requires more elaborate and in-depth critical assessment of the reliability of the data than animal data. Epidemiological studies with negative results are not sufficient to show the absence of an intrinsic hazardous property of a substance but well documented "negative" studies of good quality may be useful in risk assessment. Four major types of human data may be submitted: (1) analytical epidemiology studies on exposed populations, (2) descriptive or correlation epidemiology studies, (3) case reports and (4) in very rare, justified cases, controlled studies in human volunteers.

(1) Analytical epidemiology studies

Analytical epidemiology studies are useful for identifying a relationship between human exposure and effects such as biological effect markers, early signs of chronic effects, disease occurrence or mortality. Such studies may provide the best data for risk assessment. Study designs include:

- case-control (case-referent) studies, where a group of individuals with (cases) and without (controls/referents) a particular effect is identified and compared to determine differences in exposure;
- cohort studies, where a group of "exposed" and "non-exposed" individuals are identified and differences in effect occurrence are studied;
- cross-sectional studies, where a population (e.g. a workforce) is studied, so that morbidity at a given point in time can be assessed in relation to concurrent exposure.

The strength of the epidemiological evidence for specific health effects depends, among other things, on the type of analyses and on the magnitude and specificity of the response. Confidence in the findings is increased when comparable results are obtained in several independent studies on populations exposed to the same agent under different conditions and using different study designs.

Criteria for assessing the adequacy of epidemiology studies include:

- proper selection and characterisation of the exposed and control groups;
- adequate characterisation of exposure;
- sufficient length of follow-up for disease/toxicity occurrence;
- valid ascertainment of effect;

- proper consideration of bias and confounding factors; and
- reasonable statistical power to detect an effect.

(2) Descriptive epidemiology studies

Descriptive epidemiology studies examine differences in disease rates among human populations in relation to age, gender, race, and differences in temporal or environmental conditions. These studies are useful for identifying areas for further research but less useful for risk assessment. Typically, these studies can only identify patterns or trends in disease occurrence over time or in different geographical locations but cannot ascertain the causal agent or degree of human exposure.

(3) Case reports

Case reports describe a particular effect in an individual or a group of individuals who were exposed to a substance. They may be particularly relevant when they demonstrate effects which cannot be observed in experimental animal studies.

(4) Human exposure studies in volunteers

Well-conducted, controlled human exposure studies in volunteers, including low exposure TK studies, can be used in risk assessment in some rare cases if such information is already available. However, few human experimental toxicity studies are available due to the practical and ethical considerations involved in deliberate exposure of individuals. Such studies, e.g. studies carried out for the authorisation of medical products, must have been conducted in line with the World Medical Association Declaration of Helsinki, which describes the general ethical principles for medical research involving human subjects (World Medical Association, 2000). However, for studies preceding this declaration, case-by-case consideration is necessary.

Criteria for a well-designed study include the use of a double-blind study design, inclusion of a matched control group, and an adequate number of subjects to detect an effect. The results from human experimental studies are often limited by a relatively small number of subjects, short duration of exposure and/or low dose levels resulting in poor sensitivity in detecting effects.

Experimental human toxicity studies shall not be conducted specifically for the purpose of BPR. Testing with human volunteers is strongly discouraged but when good quality data are already available, they may be used in well justified cases.

For further guidance on assessing epidemiology studies, please refer to *EFSA Guidance on epidemiological studies*.

1.2.2.4.2 In vitro data

It can be expected that some of the available data have been derived from studies conducted *in vitro* or *in chemico*. The usefulness of these studies will be determined by their adequacy in light of some of the general criteria already discussed, e.g. how well the study is reported, how well the test substance is characterised, and to what extent the requirements of the method described in the EU Test Methods Regulation (Regulation (EC) No 440/2008) have been met for the endpoint under consideration.

More detailed information on the use and assessment of *in vitro* studies can be found in *OECD GD 286*. Some criteria require particular attention when assessing the adequacy of *in vitro* studies, e.g.:

- the range of exposure levels used, taking into account the toxicity of the substance towards the bacteria/cells, its solubility and, as appropriate, its effect on the pH and osmolality of the culture medium;
- the maintenance of effective concentrations of volatile substances in the test system;
- use of an appropriate exogenous metabolism mix (e.g. S9 from induced rat liver or from hamster liver) when necessary;
- use of appropriate negative and positive controls as integral parts of the tests;
- use of an adequate number of tests and replicates within the tests;
- use of the appropriate test system (e.g. appropriate cell lines).

1.2.2.4.3 Read-across

Read-across is used in predicting endpoint information for one substance ('target substance') by using data from the same endpoint from other substances ('source substance'). According to the Read-Across Assessment Framework (*RAAF*), substances that have physicochemical, toxicological and ecotoxicological properties that are likely to be similar or follow a regular pattern as a result of structural similarity may be considered as a group, or 'category' of substances.

Guidance on the application of read-across on grouping approaches is provided in *REACH Guidance R.6* and *RAAF*.

RAAF is applicable for biocides and the legal requirements of Annex IV of BPR for the use of read-across are to a large extent similar to the requirements of REACH Annex XI 1.5. It provides the eCAs and applicants a structured framework to ensure that all relevant scientific aspects of the read-across adaptation have been considered and covered.

As highlighted in *RAAF*, it has to be understood that each read-across case is unique and *RAAF* is a living framework for analysis, rather than a series of steps to be followed mechanically. Deviations from *RAAF* are possible depending on the case being assessed. The uncertainty related to the use of read-across for a specific endpoint should be separately addressed. A correction of the point of departure (PoD) should be made for molecular weights of the source substance and the target substance, if applicable.

1.2.2.4.4 (Q)SAR predictions

When experimental data do not exist, or when data are limited, the use of (Quantitative) Structure-Activity Relationships ((Q)SARs) predictions and other *in silico* models can be used together with other information in the Weight of Evidence (WoE) assessment. (Q)SAR models can be particularly useful in assessing properties of e.g. specific (low level) impurities or metabolites, and/or substances for which data are absent and difficult to generate for a given endpoint. In addition, (Q)SAR predictions may also support read-across. (Q)SAR concept and terminology are explained in *REACH Guidance R.6*.

When using (Q)SARs to predict a substance property, it is important to ensure that the model is valid and the predictions are of acceptable quality (*OECD 405*). The general provisions outlined

in *REACH Guidance R.6* and *OECD 405* should be followed. Transparent documentation must be provided for the model and the prediction, compiled according to the reporting formats available in Annexes 1 and 2 of *OECD 405*.

Currently, (Q)SAR models do not sufficiently well predict the outcome of test guideline studies and hence do not provide sufficient confidence for regulatory decision making. They may however provide valuable support for read-across justification and contribute to a WoE assessment. Most models are of qualitative nature, predicting the substance to have or not have a particular property, and are not addressing quantitative aspects such as the N(L)OAEL required for risk assessment.

References to guidance documents with examples of available (Q)SAR models as well as examples for commonly used tools are provided throughout this document. This does not provide a complete overview of all available models and software.

Commonly used software tools with models predicting human health hazard properties are provided in Table 2 below.

Table 2: Commonly used software tools

Tool	Link
ACD/Labs	https://www.acdlabs.com/
ChemTunes ToxGPS	https://mn-am.com/products/chemtunestoxgps/
Danish QSAR database and Danish QSAR models	https://qsar.food.dtu.dk/
Derek NEXUS and Sarah NEXUS	https://www.lhasalimited.org/
iSafeRat	https://www.kreatis.eu/isaferat_page
HazardExpert Pro	https://compudrug.com/hazardexpertpro
Leadscope	https://www.instem.com/solutions/insilico/computational-toxicology.php
MolCode ToolBox	https://compudrug.com/molcode_toolbox
Multicase CASE	https://multicase.com/
Onco-Logic®	https://www.epa.gov/tsca-screening-tools/oncologictm-expert-system-evaluate-carcinogenic-potential-chemicals
ProtoTOX	https://protopred.protoqsar.com/ProtoTOX_info
OPERA	https://ntp.niehs.nih.gov/whatwestudy/niceatm/comptox/ct-opera/opera
TEST	https://www.epa.gov/comptox-tools/toxicity-estimation-software-tool-test
TIMES	https://oasis-lmc.org/products/software.aspx

TOPKAT	https://www.3ds.com/products/biovia/discovery-studio/qsar-admet-predictive-toxicology
ToxTree	https://toxtree.sourceforge.net/
VEGA-QSAR	https://www.vegahub.eu/about-vegahub/

Several (Q)SAR models can be used through the OECD QSAR Toolbox (https://qsartoolbox.org/), a freely available software developed by Laboratory of Mathematical Chemistry, Bourgas University, Bulgaria, in collaboration with ECHA and OECD. The OECD QSAR Toolbox functionalities can also be used to identify analogues and chemical categories, which can serve as sources for read-across and trend analysis predictions. In addition, it has functionalities for retrieving experimental data, simulating metabolism and profiling properties of chemicals which can be used to support the predictions.

1.2.2.4.5 Integrated approaches for testing and assessment

In vitro, *in silico* and omics methods, usually captured under the term "new approach methodologies" (NAMs) can provide useful mechanistic information as well as indications of adverse effects. Due to significant advances in high throughput and high content methods, combined with a wide variety of assays that investigate specific molecular effects, it is possible to gather relatively easily additional information that can help to quantify and characterise molecular and cellular responses to individual substances, especially where OECD TGs are available, e.g. *in vitro* tests for skin/eye corrosion and irritation, skin sensitisation, genotoxicity and endocrine disruption.

To assist in integrating this data in an objective and systematic way, the OECD has provided guidance on integrated approaches to testing and assessment (*OECD GD 260*). IATAs are pragmatic, science-based approaches for chemical hazard or risk characterisation that rely on an integrated analysis of existing information in a WoE assessment coupled with the generation of new information using testing strategies. IATAs necessarily always include some level of expert judgement, though the prescriptiveness of an IATA ranges from flexible, non-formalised judgment-based approaches (e.g. grouping and read-across) to more structured, prescriptive, rule-based approaches (e.g. Integrated Testing Strategy; ITS). In some specific cases, data generated by alternative (non-animal) methods can already be evaluated by means of a fixed rule-based data interpretation procedure (i.e. defined approach), e.g. in the assessment of skin sensitisation (*OECD GD 256*; see also Section 1.6) and skin corrosion and irritation (*OECD GD 203*; see also Section 1.5).

Ideally, IATAs should be mechanistically informed, relying on existing knowledge of the molecular mechanisms through which chemicals exert their toxic effect. This way, the mechanistic understanding provides a frame for the organisation and analysis of information from methods that target different levels of biological organisation, enabling the contribution of these test results in deciding on the likelihood of the adverse outcome of interest (Tollefsen et al., 2014). The sequence from the molecular level up to the organ or organism level can be provided by Adverse Outcome Pathways (AOPs), which can be described as a logical sequence of key events (KEs) triggered by chemical exposure and occurring at the molecular (the molecular initiating event) up to the cellular, organ and whole organism or even population level. These KEs, which should be measurable effects, are causally linked to the adverse outcome under consideration, which are the reported endpoints from the test required under the BPR or observations in other toxicological or epidemiological investigations. The link between an upstream KE and a downstream KE in an AOP is called the key event relationship (KER). The

KERs include the available evidence supporting the causal relationship between a pair of KEs (Villeneuve et al., 2014a, 2014b; Edwards et al., 2016). Published AOPs, as well as AOPs pending publication, are accessible via AOPwiki (https://aopwiki.org/).

1.2.2.5 Weight of evidence

The weight of evidence (WoE) approach is an iterative, structured method used to assess scientific questions by integrating diverse types or lines of evidence. It can be used for hazard identification, hazard characterisation and risk assessment purposes. A WoE assessment may be needed for example if information does not fulfil all the requirements but is nevertheless considered sufficient to cover the information needs, or where pieces of information taken separately might allow differing conclusions. The approach generally includes the following steps/actions:

1. **Problem formulation** – Define the scientific question(s) to address.
2. **Collection and documentation of all information** - Assemble and document all available information and search strategies, building complementary or standalone lines of evidence.
3. **Assessment of quality of individual evidence** – Evaluate the quality of the evidence on the basis of adequacy, reliability and relevance. Ensure consistency across the lines of evidence through a conceptual model (e.g. flowchart or list of logical steps) to enhance transparency.
4. **Integration and weighing of evidence (WoE analysis)** – Integrate the evidence and perform a WoE analysis. Different methodologies can be used, but the elements that should be considered are consistency, specificity, likelihood/biological plausibility and temporality. In general, *in vivo* and *in vitro* data have more weight than computational methods, though these methods can still contribute within a WoE approach (https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/weight-of-evidence).
5. **Applications of levels of confidence –** Assign confidence levels to individual evidence and/or to the overall assessment.
6. **Uncertainty analysis –** Conduct an uncertainty analysis, including a sensitivity analysis if needed, to identify and assess which evidence and uncertainties have most influence on the conclusion. An iterative refinement process covering any of the above steps can help reduce uncertainty (see also *ECHA Guidance R.19*).
7. **Conclusions and reporting** - Conclusions may be expressed with quantitative or qualitative statements. The results should be summarized using tables or graphics. Reporting should be transparent, including all evidence considered, levels of confidence, uncertainties, data gaps identified, justification for the choice of methods used, detailed description of all the steps of the procedure and making clear where and how expert judgement has been used.

The ECHA template[6] is available to aid the CAs and applicants in the assessment and presenting the WoE approach/uncertainty. For further guidance, please refer to ECHA

[6] https://echa.europa.eu/documents/10162/992028/template_for_weight_of_evidence_en.docx/eb183c2e-c360-cbce-7a58-ad2d1270e5bd?t=1512025183895

Background document with examples[7], and on the use of the WoE approach in scientific assessments. Further guidance is also provided in *EFSA WoE Guidance*.

1.2.3 Representativeness of the information for the substance

According to BPR Annex II, point 3, it needs to be assessed that the studies conducted to support active substance approval are performed with representative batches:

> *Evidence should also be provided to demonstrate that the active substance upon which the tests have been carried out is the same as the substance for which the application has been submitted.*

Ideally, all toxicity studies would be performed with the highest impurity concentrations allowed in the specification, or slightly above these, while ensuring that also the concentration of the pure active substance is within the specification. As this would rarely be the case, further considerations are given in sections 1.2.3.1 to 1.2.3.3. These sections constitute an assessment similar to technical equivalence Tier II assessment.

Each study will need to be considered separately, comparing the composition of the tested batch and the specification of the active substance.

Please note that the guidance in section 1.2.3 should be considered as indicative, as it is not possible to establish clear rules for situations where information on the impurities may be insufficient for a comprehensive assessment. For reference, the CAs may also consider cases where such (confidential) comparison has already been performed. Expert judgment forms an integral part in this assessment.

1.2.3.1 Higher impurity concentrations were tested

Impurities that were present in the test batches at a concentration higher than in the specification are considered to cover the specification because these would represent a worst case. No further assessment is required for such impurities.

1.2.3.2 Lower impurity concentrations were tested

A test performed with a batch where one or more of the impurities were below the specification does not provide sufficient information to ensure that the active substance is adequately tested. In such a situation, it is necessary to consider each of the impurities for which an insufficient impurity concentration was tested and to assess the toxicological profile of the impurities.

No assessment is needed for those hazard properties for which the active substance has or is proposed to have the same or stricter classification than the impurities. An assessment of impurities for such hazard properties is necessary only if there is information on an impurity suggesting a particularly potent effect, e.g. extreme sensitiser or very potent carcinogen.

As first step, it is recommended to focus on the toxicity studies performed on the active substance that cover the most relevant hazard properties of the impurities. For instance, if an

[7] https://echa.europa.eu/documents/10162/992028/wo_eu_uncertainty_background_en.docx/4f2b49ab-ade0-6ee3-e977-8abe00c21c23?t=1512025184954

impurity is potentially genotoxic based on *in silico* data, and it has been tested at its maximum level in the batches used in the genotoxicity testing of the active substance, then there is no concern if its level is lower in other toxicological studies. Therefore, depending on the endpoints affected, information coming from other toxicity studies could be sufficient to allow concluding with sufficient certainty that the level of impurity does not have an impact on toxicity of the active substance.

Where the first step is not conclusive, all the available information on the impurity has to be considered in concluding whether the concentration at which it is present in the active substance (specification) affects the toxicity of the active substance. In this assessment, the *ECHA Guidance on Technical Equivalence* can be used as providing the guiding principles.

If it is necessary to generate further data, consideration could be given to the possibility of testing several impurities as a mixture, or using a batch spiked with impurities of interest. In such considerations, one must note that OECD TGs are not intended to be used for mixtures due to e.g. difficulties in top dose selection or possible interactions or chemical reactions of the impurities. Data generation with such methods require careful consideration and any such data must be evaluated with caution.

Where it is considered that the toxicity of the active substance would be impacted by an impurity that was not included in testing, the nature of the possible effect has to be considered in deciding on the impact on the assessment and the possible need to request further information. Depending on the situation, the options also include non-approval of the active substance as specified and reducing the concentration of the impurity to a level at which it would not impact the toxicity.

1.2.3.3 Information on impurity concentrations is missing

For an endpoint for which a study was performed using test batches without sufficient information on impurities, all the impurities in the active substance specification need to be considered.

The principles and steps are the same as in section 1.2.3.2.

1.2.4 Considerations on specific effects

This section provides some considerations on interpreting effects that are relevant for more than one of the following sections.

Reduced body weight gain

Reduced body weight gain should usually be considered as an adverse effect and as a basis for setting the NOAEL, unless it can be shown that there is a causal relationship between reduced palatability and reduced bodyweight gain or food consumption. If the effect is present also in e.g. gavage or inhalation studies, it cannot be explained by unpalatability.

Emesis

Emesis should be considered an adverse effect and as a basis for setting the NOAEL.

Liver effects

Liver cell hypertrophy and liver weight increase should be considered as potentially adverse effects. However, on a case-by-case basis, hepatocellular hypertrophy leading to ≤15% increased mean absolute or relative liver weight should not be regarded as adverse, and should not be used for the purpose of defining the LOAEL for that specific study, in the demonstrated absence of all of the following changes:

- other histopathological findings such as necrosis, inflammation, fibrosis, vacuolation, pigmentation, degeneration, hyperplasia, etc. but not limited to these;
- other effects that are indicative of specific liver toxicity, such as adverse clinical chemistry changes.

If relevant and comprehensive histopathological and clinical chemistry investigations have not been performed or where there is insufficient information to determine whether the observed increase in liver weight is an adaptive or an adverse response, it must be assumed that the effect is adverse. Mechanistic information such as enzyme induction can be used to support decision making.

Further considerations are provided in a document available at: https://webgate.ec.europa.eu/s-circabc/d/a/workspace/SpacesStore/3733c8dc-419c-4c58-ad1c-af18c4f333af/Interpretation%20of%20liver%20effects_annex.pdf.

1.3 Toxicokinetics

Toxicokinetic (TK) data of a substance are needed for the interpretation of toxicological findings and hence in the risk assessment. Information on the fate of a substance in the organism is required to relate exposure to effects. Route-to-route or interspecies extrapolations may be possible on the basis of internal exposure data, which may allow refinement of default interspecies AFs. This may also enable sensitive sub-populations who may be at particular risk to be taken into account in the risk assessment by evaluating interindividual differences.

TK information may be an important tool for extrapolation from high to low dose effects and can be used to make informed decisions on further testing and study design. In specific circumstances, valid toxicokinetic data may be used to support derogation statements. For example, proof that a substance is not systemically available can form a part in justifying that no further testing is needed. TK can also be essential in refining hazard characterisation, for example in deriving chemical specific adjustment factors and in investigating the mode of action.

Information on TK can be derived either from *in vitro* and *in vivo* experiments, or from the use of Physiologically Based Kinetic (PBK) modelling.

Table 3: Guidance to be considered together with the current guidance

Guidance	Section
ECHA Guidance Vol III Part A	1.8. Toxicokinetics and metabolism studies in mammals
REACH Guidance R.7c	R.7.12 Guidance on Toxicokinetics
EFSA Guidance on dermal absorption	
OECD GD 28	
OECD 156	
OECD GD 331	

1.3.1 Definitions

Toxicokinetics (TK) is used to describe the time-dependent fate of a substance within the body, including absorption, distribution, metabolism, and/or excretion (ADME).

Toxicodynamics (TD) means the process of interaction of chemical substances with target sites and the subsequent reactions leading to adverse effects. The concentration at the effect site(s) drives directly or indirectly the toxicodynamic effect, which may be reversed or modified by several factors (e.g. repair mechanisms for DNA damage, compensatory cell proliferation).

Disposition is the sum of processes following absorption of a chemical into the circulatory systems, distribution throughout the body, biotransformation, and excretion.

TK studies are designed to obtain species-, dose-, and route-dependent data on the concentration-time course of parent compound and its metabolites (e.g. in blood, urine, faeces, exhaled air, and organs).

The following information can be obtained from *in vivo*/ex-vivo TK studies:

(a) Primary information:

- the concentration-time profile of the substance/metabolites in blood (plasma), tissues, and other biological fluids (e.g. urine, bile, exhaled air), and the volume of the excreted fluids;
- protein binding and binding to erythrocytes (*in vitro*/*ex vivo* studies).

(b) Derived information:

- rate and extent of absorption and bioavailability;
- distribution of the substance in the body;
- biotransformation;
- rate and extent of pre-systemic (first pass) and systemic metabolism after oral and inhalation exposure;
- information on the formation of reactive metabolites and possible species differences;
- rate and extent of excretion in the urine, faeces, via exhalation, and other biological fluids (e.g. milk, bile, sweat, etc.);
- half-life and potential for accumulation under repeated or continuous exposure;
- information on enterohepatic circulation.

Enterohepatic circulation may pose particular problems for route-to-route extrapolation since oral administration may result in greater systemic availability than non-oral administration. This will result in an Area Under the Curve (AUC) which will reflect both absorption/systemic availability of the compound and the extent of circulation. As the relative extent of target organ exposure following different routes of exposure is often calculated from the ratio of AUCs by different routes, the target organ exposure after oral exposure may be overestimated when enterohepatic circulation takes place.

It is helpful to have TK information for the expected exposure routes in humans (oral, inhalation, dermal) at appropriate dosing levels. From the AUC profile and from the excretion over time, it can be calculated whether the substance will accumulate when given repeatedly or continuously. However, it is only possible to make this extrapolation for substances that have linear kinetics. If information on the accumulative potential is important for the risk assessment, it will be necessary to gather data from studies with repeated dosing regimes.

TK data from more than one species can enable the assessment of interspecies differences. In the absence of *in vivo* data, some data may be derived from *in vitro* experiments. These include parameters of metabolic steps, such as Vmax, Km, intrinsic metabolic clearance, as well as skin permeation rate and distribution coefficient. Physiologically based toxicokinetic modelling techniques may be used to simulate the concentration-time profile in blood and at the target site.

1.3.2 Main principles and uses of toxicokinetics

The expression of toxicity is a consequence of a chain of events that results in the affected tissues of an organism receiving the ultimate toxicant in amounts that cause an adverse effect. The factors that confer susceptibility in certain species and lead to major differences between animals and humans in their response to such chemical insults is based either on the nature and quantity of the ultimate toxicant that is presented to the sensitive tissue (TK) or in the sensitivity of those tissues to the ultimate toxicant, i.e. the TD response.

Prior to any animal study, it is crucial to identify the benefits that will be gained from conducting such a study, as overall one should avoid generating data that are unlikely to be used and that constitute an unnecessary use of animals, time, and resources.

The TK behaviour derived from available data might make further testing unnecessary in terms of predictability of other properties. The definition of actual TK studies on a case-by-case basis might further improve the knowledge about substance properties in terms of expanding knowledge on properties sufficiently to enable risk assessment. TK information can provide important information for the design of toxicity studies, for the application of read-across and building of categories. For the generation of new TK data this section should be used together with the *ECHA Guidance Vol III Part A*.

The aim of this section is to provide a general overview on the main principles of TK and to give guidance on the generation/use of TK information for the human health risk assessment of chemicals, and to make use of this information to support better testing strategies.

TK begins with exposure and depending on the ADME of the substance, results in a certain concentration of the ultimate toxicant at the target site (tissue dose). ADME describes the uptake of a substance into the body and its lifecycle within the body, including excretion (OECD TG 417):

- **absorption**: how, how much, and how fast the substance enters the body;
- **distribution**: reversible transfer of substances between various parts of the organism, i.e. body fluids or tissues;
- **metabolism**: the enzymatic or non-enzymatic transformation of the substance into a structurally different chemical (metabolite);
- **excretion**: the physical loss of the parent substance and/or its metabolite(s) via the urine, faeces (including bile), exhaled air and other routes of excretion including breast milk.

For consistency, and unless otherwise specified, metabolism does not include largely reversible chemical transformations resulting in an observable equilibrium between two chemical species (inter-conversion).

1.3.3 Absorption

Toxicants usually enter the body via lungs, GI tract (both having absorption surfaces by nature) and the skin. To be absorbed, substances must transverse across biological membranes, mostly by passive diffusion. As biological membranes consist of lipid layers as well as aqueous phases, a process like this requires the substance to be soluble both in lipid and water. For chemicals that do not meet these criteria, absorption may occur via facilitated diffusion, active transport or pinocytosis processes, which are more actively directed and require energy.

Absorption is a function of the potential for a substance to diffuse across biological membranes. In addition to molecular weight the most useful parameters providing information on this potential is the log P value and the water solubility. The log P value indicates the relative solubility of the substance in water and in the hydrophobic solvent octanol (used as a surrogate for lipids) and is a measure of lipophilicity. Log P values > 0 indicate that the substance is lipophilic and, therefore, more soluble in octanol than in water. Negative values of log P indicate that the substance is hydrophilic and hence more soluble in water than in octanol. In general, log P values between -1 and 4 are favourable for absorption. Solubility in water and lipids, and log P value should nevertheless be considered when assessing the potential of a substance to be absorbed.

1.3.3.1 Oral/GI absorption

Substances may undergo chemical changes in the GI fluids as a result of metabolism by GI flora, enzymes or hydrolysis, and predictions based upon the physico-chemical characteristics of the parent substance may not apply. For a detailed listing of physiological factors, data on stomach and intestine pH, data on transit time in the intestine, see Appendix R.7.12-1 in *REACH Guidance R.7c*.

One consideration that could influence the absorption of ionic substances (e.g. acids and bases) is the varying pH of the GI tract. Ionised substances generally do not readily diffuse across biological membranes, which is why pKa values of substances (pH at which 50% of the substance is ionised and 50% non-ionised) are informative. Absorption of acids is favoured when pH < pKa whereas absorption of bases is favoured when pH > pKa.

Substances can also be absorbed in the GI tract as small water soluble molecules (molecular weight up to around 200) can pass through aqueous pores, or be carried by such molecules across membranes with the bulk passage of water. The absorption of highly lipophilic substances (log P ≥ 4) may be limited by the inability to dissolve into GI fluids and make contact with the mucosal surface. However, the bile salts micellular solubilisation enhances the absorption of such substances. Substances absorbed as micelles (aggregate of surfactant molecules, lowering surface tension) enter the circulation via the lymphatic system, bypassing the liver. Although particles and large molecules (with molecular weights in the 1000's) would normally be considered too large to cross biological membranes, small amounts of such substances may be transported into epithelial cells by pinocytosis or persorption, and pass through gaps in membranes left when the tips of villi are sloughed off. Absorption of surfactants or irritants may be enhanced because of damage to cell membranes.

Absorption can occur at different sites and with different mechanisms along the GI tract.

In the mouth, minimal absorption occurs by passive diffusion, and substances enter directly the systemic circulation. Some enzymatic degradation may occur however.

Absorption is minimal also in the stomach, occurring only by passive diffusion. The acidic environment favours uptake of weak acids. There is potential for hydrolysis and, very rarely, metabolism by endogenous enzymes prior to uptake. Once absorbed at this point, substances will go to the liver before entering the systemic circulation, and first pass metabolism may then limit the systemic bioavailability of the parent compound.

The small intestine has a very large surface area and the transit time through this section is the longest, making this the predominant site of absorption within the GI tract. Most substances will be absorbed by passive diffusion. Gut microflora or enzymes in the GI mucosa may inhibit or limit the absorption of compounds by metabolising a part or total amount of them prior to absorption. Since substances that enter the blood at this point pass through the liver before entering the systemic circulation, hepatic first pass metabolism may limit the amount of parent compound that enters the systemic circulation.

In the large intestine, absorption occurs mainly by passive diffusion, but active transport mechanisms for electrolytes are also present. Compared to the small intestine, the rate and extent of absorption within the large intestine is very low. Most blood flow from the large intestine passes through the liver first.

Table 4 provides an overview of different types of data that can be considered for the estimation of oral/GI absorption.

Table 4: Interpretation of data regarding oral/GI absorption

Data source	What it tells us
Structure	It may be possible to identify ionisable groups within the structure of the molecule. Groups containing oxygen, sulphur or nitrogen atoms are all potentially ionisable, e.g. thiol (SH), sulphonate (SO_3H), hydroxyl (OH^-), carboxyl (COOH) or amine (NH_2).
Molecular weight	Generally the smaller the molecule the more easily it may be taken up. Molecular weights <500 are favourable for absorption; molecular weights >1,000 do not favour absorption.
Particle size	Generally, solids have to dissolve before they can be absorbed. It may be possible for particles in the nanometre size range to be taken up through pinocytosis. The absorption of very large particles, several hundreds of micrometres in diameter, that were administered dry (e.g. in the diet) or in a suspension may be reduced because of the time taken for the particle to dissolve. This would be particularly relevant for poorly water soluble substances.
Water solubility	Water soluble substances will readily dissolve into the GI fluids. Absorption of very hydrophilic substances via passive diffusion may be limited by the rate at which the substance partitions out of the GI fluid. However, if the molecular weight is low (<200) the substance may pass through aqueous pores or be carried through the epithelial barrier by the bulk passage of water.
Log P	Moderate log P values (between -1 and 4) are favourable for absorption by passive diffusion. Any lipophilic compound may be taken up by micellular solubilisation but this mechanism may be of particular importance for highly lipophilic compounds (log P >4), particularly those that are poorly soluble in water (≤1 mg/L) and would otherwise be poorly absorbed.

Dosing vehicle	If the substance has been dosed using a vehicle, the water solubility of the vehicle and the vehicle/water partition coefficient of the substance may affect the rate of uptake. Compounds delivered in aqueous media are likely to be absorbed more rapidly than those delivered in oils. Compounds delivered in oils that can be emulsified and digested, such as corn oil or arachis oil, are likely to be absorbed to a greater degree than those delivered in non-digestible mineral oil (liquid petrolatum) or in soil, the latter being an important vehicle for children.
Oral toxicity data	If signs of systemic toxicity are present and are not secondary to local effects, then absorption has occurred. Coloured urine and/or organ tissue can provide evidence that a coloured substance has been absorbed. This information will give no indication of the amount of substance that has been absorbed. Some clinical signs such as hunched posture could be due to discomfort caused by irritation, mishandling, or simply the presence of a large volume of test substance in the stomach, and reduced feed intake could be due to an unpalatable test substance. It must therefore be clear that the effects that are being cited as evidence of systemic absorption are genuinely due to absorbed test substance and not to local effects at the site of contact.
Hydrolysis test	The hydrolysis test (OECD TG 111) provides information on the half-life of the substance in water at 50°C and pH values of 4.0, 7.0, and 9.0. The test is conducted using a low concentration, 0.01 M or half the concentration of a saturated aqueous solution (whichever is lower). Since the temperature at which this test is conducted is much higher than that in the GI tract, this test will not provide an estimate of the actual hydrolysis half-life of the substance in the GI tract. However, it may give an indication that the parent compound may only be present in the GI tract for a limited period of time. Hence, TK predictions based on the characteristics of the parent compound may be of limited relevance.

1.3.3.2 Respiratory absorption – inhalation

For inhaled substances the deposition processes of the substance on the surface of the respiratory tract and the actual absorption have to be differentiated. The physico-chemical characteristics of the substance influence both processes.

Substances that can be inhaled include gases, vapours, liquid aerosols (liquid or solid substances in solution) and fine powders/dusts. Substances may be absorbed directly from the respiratory tract or through the action of clearance mechanisms and then being swallowed. This means that absorption from the GI tract will contribute to the total systemic burden of substances that are inhaled.

To be readily soluble in blood, a gas or vapour must be soluble in water while also being sufficiently lipophilic to cross the alveolar and capillary membranes. A log P value between -1 and 4 would be favourable for absorption. The deposition pattern of vapours in the form of readily soluble hydrophilic substances differs from lipophilic substances. Hydrophilic substances are effectively removed from the air in the upper respiratory tract, whereas lipophilic substances reach the deep lung and thus absorption through the huge gas exchange region may occur. The rate of systemic uptake of very hydrophilic gases or vapours may be limited by the rate at which they partition out of the aqueous fluids (mucus) lining the respiratory tract and into the blood. Such substances may be transported out of the deposition region with the mucus and swallowed or may pass across the respiratory epithelium via aqueous membrane pores. Highly reactive gases or vapours can react at the site of contact, reducing the amount available for absorption. Physical activity, such as exercise or heavy work, has a great impact on the amount absorbed and must also be addressed.

Precise deposition patterns for dusts will depend not only on the particle size of the dust but also the hygroscopicity, electrostatic properties and shape of the particles, and the respiratory dynamics of the individual.

Generally, liquids, solids in solution, and water soluble dusts would readily diffuse or dissolve into the mucus lining the respiratory tract. Lipophilic substances (log P > 0) would then have the potential to be absorbed directly across the respiratory tract epithelium. Very hydrophilic substances with molecular weights < ca. 200 might be absorbed through aqueous pores or be retained in the mucus and transported out of the respiratory tract. For poorly water soluble dusts, the rate at which the particles dissolve into the mucus will limit the amount that can be absorbed directly. Poorly water soluble dusts depositing in the nasopharyngeal region could be coughed or sneezed out of the body or swallowed. Such dusts depositing in the tracheo-bronchial region would mainly be cleared from the lungs by the mucocilliary mechanisms and swallowed. However, a small amount may be taken up by phagocytosis and transported to the blood via the lymphatic system. Poorly water soluble dusts depositing in the alveolar region would mainly be engulfed by alveolar macrophages. The macrophages will then either translocate particles to the ciliated airways or carry particles into the pulmonary interstitium and lymphoid tissues.

Table 5 provides an overview of the type of data that can be considered for the estimation of respiratory absorption.

Table 5: Interpretation of data regarding respiratory absorption

Data source	What it tells us
Vapour pressure	Indicates whether a substance may be available for inhalation as a vapour. As a general guide, highly volatile substances are those with a vapour pressure greater than 25 kPa (or a boiling point below 50°C). Substances with low volatility have a vapour pressure of less than 0.5 kPa (or a boiling point above 150°C).
Particle size	Indicates the presence of inhalable/respirable particles. In humans, particles with aerodynamic diameters below 100 µm have the potential to be inhaled. Particles with aerodynamic diameter below 50 µm may reach the thoracic region and those below 15 µm the alveolar region of the respiratory tract. These values are lower for experimental animals with smaller dimensions of the structures of the respiratory tract. Particles with aerodynamic diameters >1-5 µm have the greatest probability of settling in the nasopharyngeal region, whereas particles with aerodynamic diameters <1-5 µm are most likely to settle in the tracheo-bronchial or pulmonary regions.
Log P	Moderate log P values (between -1 and 4) are favourable for absorption directly across the respiratory tract epithelium by passive diffusion. Any lipophilic compound may be taken up by micellular solubilisation but this mechanism may be of particular importance for highly lipophilic compounds (log P > 4), particularly those that are poorly soluble in water (≤1 mg/L) that would otherwise be poorly absorbed.
Water solubility	Deposition: Vapours of very hydrophilic substances may be retained within the mucus. Low water solubility, like small particle size enhances penetration to the lower respiratory tract. For absorption of deposited material similar criteria as for GI absorption applies.
Inhalation toxicity data	If systemic toxicity is present then absorption has occurred. This cannot be used as a quantitative measure of absorption.
Oral toxicity data	If systemic toxicity is present in an oral toxicity study or there are other data indicating the potential for absorption following ingestion, the substance will likely be absorbed also when inhaled.

Hydrolysis test	The hydrolysis test (OECD TG 111) provides information on the half-life of the substance in water at 50°C and pH values of 4.0, 7.0 and 9.0. The test is conducted using a low concentration, 0.01 M or half the concentration of a saturated aqueous solution (whichever is lower). Since the temperature at which this test is conducted is much higher than that in the respiratory tract, this test will not provide an estimate of the actual hydrolysis half-life of the substance in the respiratory tract. However, it may give an indication that the parent compound may only be present in the respiratory tract for a limited period of time. Hence, TK predictions based on the characteristics of the parent compound may be of limited relevance.

1.3.3.3 Dermal absorption

The skin is a dynamic, living multilayered biomembrane and its permeability may vary as a result of changes in hydration, temperature, and occlusion. In order to cross the skin, a compound must first penetrate into the *stratum corneum* (non-viable layer of corneocytes forming a complex lipid membrane) and may subsequently reach the viable epidermis, the dermis and the vascular network. The *stratum corneum* provides its greatest barrier function against hydrophilic compounds, whereas the viable epidermis is the most resistant to penetration for highly lipophilic compounds.

Dermal absorption is influenced by e.g. physico-chemical properties of the substance, vehicle, concentration, and the exposure pattern (e.g. occlusion of the application site) as well as the skin site of the body. Substances that can potentially be taken up across the skin include gases and vapours, liquids, and particulates.

For the purpose of estimating dermal absorption for biocidal active substance and products, the principles described in the *EFSA Guidance on dermal absorption* should be followed, complemented with *OECD GD 28* and *OECD 156*. For anticoagulant rodenticides dermal absorption values have been harmonised[8] and additionally an alternative approach for the occupational setting was developed[9]. When test data is not available, default values can be used as a first step. See also the document[10] on dermal absorption of antifouling products (PT 21).

In vivo and/or *in vitro* studies can be used as standalone or in combination for estimation of dermal absorption percentage.

The 'triple pack' approach can be used when *in vivo* dermal penetration data are available for animals and *in vitro* information for the same animal species and humans. The *in vivo* dermal absorption in rats may be adjusted in light of the relative absorption through rat and human skin *in vitro* under comparable conditions (see the equation below). The latter adjustment may be done because the permeability of human skin is often lower than that of animal skin. A generally applicable correction factor for extrapolation to humans cannot be derived because the extent of overestimation depends on dose, substance, and species. For the correction factor based on *in vitro* data, preferably maximum flux values should be used, but percentage (receptor medium plus skin dose) may also be used. Because the permeation constant (K_P in cm/h) is, by definition,

[8] *Dermal absorption values for anticoagulant rodenticides*: https://webgate.ec.europa.eu/s-circabc/d/a/workspace/SpacesStore/f98f9676-4716-448a-80d6-50fa671f7860/Dermal_abs_anticoagulant_rodenticides.docx

[9] *Dermal absorption values for anticoagulant rodenticides: Alternative approach for the occupational setting*: https://webgate.ec.europa.eu/s-circabc/d/a/workspace/SpacesStore/c59f1958-3032-418c-a10b-0fa7ba93ffe8/Dermal_abs_anticoagulant_rodenticides_alternative.docx

[10] *Dermal absorption of PT 21 active substances* (https://webgate.ec.europa.eu/s-circabc/d/a/workspace/SpacesStore/c9893d6f-92c6-48ab-9bdb-ccc443e90a50/Dermal absorption_PT 21.pdf)

established at infinite dose levels, the usefulness of the K_P for dermal risk assessment is limited. See also *ECHA Guidance Vol III Part A*.

Using the 'triple pack' approach, human absorption can be calculated as follows:

$$in\ vivo \text{ human absorption} = \frac{in\ vivo \text{ animal absorption} \times in\ vitro \text{ human absorption}}{in\ vitro \text{ animal absorption}}$$

Table 6 provides an overview of the type of data to be considered for dermal absorption estimation.

Table 6: Interpretation of data regarding dermal absorption

Data source	What it tells us
Physical state	Liquids and substances in solution are taken up more readily than dry particulates. Dry particulates will have to dissolve into the surface moisture of the skin before uptake can begin. Absorption of volatile liquids across the skin may be limited by the rate at which the liquid evaporates off the skin surface.
Molecular weight	Molecular weight <100 favours dermal uptake, while molecules >500 may be too large.
Structure	As a result of binding to skin components the uptake of chemicals with the following groups can be slowed: certain metal ions, particularly: Ag^{+}, Cd^{2+}, Be^{2+} and Hg^{2+} acrylates quaternary ammonium ions, heterocyclic ammonium ions, sulphonium salts. A slight reduction in the dermal uptake of chemicals belonging to the following substance classes could also be anticipated for the same reason: quinines, dialkyl sulphides, acid chlorides, halotriazines, dinitro- or trinitro benzenes.
Water solubility	The substance must be sufficiently soluble in water to partition from the *stratum corneum* into the epidermis. Therefore, if the water solubility is <1 mg/L, dermal uptake is likely to be low. Between 1-100 mg/L absorption is anticipated to be low to moderate and between 100-10 000 mg/L moderate to high. If water solubility is above 10 000 mg/L the substance may be too hydrophilic to cross the lipid rich environment of the *stratum corneum* resulting in low dermal uptake.
Log P	For substances with log P values <0, poor lipophilicity will limit penetration into the *stratum corneum* and hence dermal absorption. Values <−1 suggest that a substance is not sufficiently lipophilic to cross the *stratum corneum* and dermal absorption is likely to be low. Log P values between 1 and 4 favour dermal absorption (values between 2 and 3 are optimal) particularly if water solubility is high. At log P values >4, the rate of penetration may be limited by the rate of transfer between the *stratum corneum* and the epidermis, but uptake into the *stratum corneum* will be high. At log P values >6, the rate of transfer between the *stratum corneum* and the epidermis will be slow and will limit absorption across the skin. Uptake into the *stratum corneum* itself may be slow.
Vapour pressure	The evaporation rate will offset the rate at which gases and vapours partition from the air into the *stratum corneum*. Therefore, although a substance may readily partition into the *stratum corneum*, it may be too volatile to penetrate

	further. This can be the case for substances with vapour pressures above 100-10 000 Pa (ca. 0.76-76 mmHg) at 25°C, though the extent of uptake would also depend on the degree of occlusion, ambient air currents, and the rate at which it is able to transfer across the skin. Vapours of substances with vapour pressures below 100 Pa are likely to be well absorbed and the amount absorbed dermally may be more than 10% of the amount that would be absorbed by inhalation.
Surface tension	If the surface tension of an aqueous solution is <10 mN/m, the substance is a surfactant and this will enhance the potential dermal uptake. Surfactants can also substantially enhance the absorption of other compounds, also in the absence of skin irritant effects.
Skin irritation/ Corrosivity	If the substance is a skin irritant or corrosive, damage to the skin surface may enhance penetration. For corrosive formulations/dilutions, 100% dermal absorption should be assumed unless there is data indicating lower dermal absorption.
Dermal toxicity data	Systemic toxicity indicates that absorption has occurred. However, if grooming was not prevented, the substance may have been ingested and systemic toxicity could be due to oral rather than dermal absorption.
Skin sensitisation data	If the substance has been identified as a skin sensitiser, some uptake must have occurred although it may only have been a small fraction of the applied dose.
Trace elements	If the substance is a cationic trace element, absorption is likely to be very low (<1%). Stable or radio isotopes should be used and background levels determined to prevent analytical problems and inaccurate recoveries.

While many of the factors in Table 6 are linked to the chemical itself, the final formulation or the use can influence both rate and extent of dermal absorption. For biocidal products, the approach in Chapter 6.2 in *EFSA Guidance on dermal absorption* should be followed.

For active substance approval, the List of Endpoints should indicate how the value(s) were derived and based on which information, the test material used, including the concentration of the active substance and the type of formulation where relevant. Where possible, the applicability of the derived values to (representative) product should be indicated, considering both the concentrate and in-use dilutions.

In case a leave-on biocidal product is applied directly on human skin, it is preferable to test the product at a concentration of the final product if technically feasible.

If a biocidal product is applied directly on human skin, other products should be considered if these may be applied on the skin at the same time. As an example, an insect repellent and sun lotion may be applied on skin, possibly resulting in enhanced dermal absorption of the biocidal product. If information of such interactions is available, enhanced dermal absorption due to simultaneous application of another (non-biocidal) product should be considered at product authorisation stage and not in active substance approval. This would normally not affect the approval of the active substance but the information should be included under *Elements to be taken into account by MSs when authorising products*.

The establishment of a value for dermal absorption may be performed by use of a tiered approach from a worst case to a more refined estimate. It is however recommended to proceed

to the highest refinement allowed by the available information, establishing the values for each product. This will ensure that the values can be used in assessing any new scenarios.

In following the *EFSA Guidance on dermal absorption* it is in some cases necessary to consider whether the product is a concentrate or a dilution. For this purpose, according to SANTE/2018/10591 rev.1[11], a biocidal product is considered:

1. a concentrate when the active substance is present in the biocidal product at a concentration higher than 50 g/L (or 50 g/kg or 5%);
2. a dilution when the active substance is present in the biocidal product at a concentration lower than or equal to 50 g/L (or 50 g/kg or 5%).

In considering dried dispersed residues, the appropriate dermal absorption value should be the higher of the values for the concentrate and the in-use dilution in line with *EFSA Guidance on dermal absorption*.

Where dermal absorption to animals such as livestock with fur or feathers is considered in risk assessment in the absence of data concerning that animal species, a practical approach has been to consider all the material arriving on the skin as absorbed, but only 50% of the material ending up on the skin due to the protective effect of fur and feathers. Therefore, overall, 50% of the material to which the animal is exposed would be considered systemically available. This value was chosen for pragmatic reasons, but it has no scientific basis. For pigs, there are indications that percutaneous absorption is very similar to humans (Jung and Maibach, 2015).

1.3.4 Distribution

Once the chemical has entered the blood stream, it may exert its toxic action directly in the blood or in any target tissue or organ to which the circulatory system transports or distributes it. The rate of distribution and the target tissues are determined by the blood flow through the organ, the ability of the substance to cross membranes and capillaries, its relative affinity for the various tissues, and possible reactive metabolites produced by the tissue. Regarding cross-membrane transfer, both passive transport and active transport by transport proteins (e.g. p-glycoprotein) must be considered. This is of particular importance for crossing the blood-brain barrier.

Distribution is a dynamic process involving multiple equilibria, and only the circulatory system is a distinct, closed compartment where chemicals are distributed rapidly. Distribution to the various tissues and organs is usually delayed. However, compounds may be rapidly distributed into the highly perfused tissues, such as liver, kidney, and lungs, with the result that kinetics cannot be distinguished from events in the blood. In this case, such organs are considered as part of the initial, central compartment, and peripheral compartment is reserved for slowly equilibrating tissues, e.g. muscle, skin, and adipose. There is an equilibrium of the free substance between the so-called rapid (or central) and the slow (or peripheral) compartment: as the free substance is eliminated, the substance from the peripheral compartment is slowly released back into the circulation.

PBK modelling uses the subdivision of body into different compartments. Based on available toxicological studies, tissue distribution is mathematically calculated using partition coefficients between blood or plasma and the tissue considered.

[11] https://food.ec.europa.eu/system/files/2018-11/pesticides_ppp_app-proc_guide_tox_dermal-absorp-2018-paff.pdf

The concentration of a chemical in blood or plasma (blood level) is dependent on the dose, absorption, distribution and elimination, as well as accumulation of the compound in certain tissue (e.g. adipose). Tissue affinity is usually described using a parameter known as the volume of distribution which is a proportionality factor between the amount of compound present in the body and the measured plasma or blood concentration. The larger the volume of distribution is, the lower the blood level will be for a given amount of compound in the body. A particularly useful volume term is the volume of distribution at steady state (Vdss). At steady state, all distribution phenomena are completed, the various compartments of the body are in equilibrium, and the rate of elimination is compensated by the rate of absorption. In non steady state situations the distribution volume varies with time except in the simplest case of a single-compartment model.

The rate at which highly water soluble molecules distribute may be limited by the rate at which they cross cell membranes. Access of such substances across physiological blood barriers, such as the blood-brain barrier and blood-testis barrier, is likely to be restricted. There are species differences in placentas, and trans-placental transfer may occur due to differing placental structure, metabolic capacity, and placental transporters.

Although protein binding can limit the amount of a substance available for distribution, it will generally not be possible to determine from the available data which substances will bind to proteins and how avidly. Furthermore, if a substance undergoes extensive first pass metabolism, predictions made on the basis of the parent substance are not valid.

Table 7 provides an overview of data that can be considered for estimation of distribution.

Table 7: Interpretation of data regarding distribution

Data source	What it tells us
Molecular weight	In general, the smaller the molecule, the wider the distribution.
Water solubility	Small water soluble molecules and ions will diffuse through aqueous channels and pores. The rate at which very hydrophilic molecules diffuse across membranes could limit their distribution.
Log P	If the molecule is lipophilic (log P > 0), it is likely to distribute into cells and the intracellular concentration may be higher than extracellular concentration particularly in fatty tissues.
Target organs	If the parent compound is toxicologically active, the target tissues provide some information on the distribution. If the substance is a dye, coloration of internal organs can inform of distribution but will not provide any quantitative information. Note that anything present in the blood will be accessible to the bone marrow.
Signs of toxicity	Clear signs of CNS effects indicate that the substance (and/or its metabolites) has distributed to the CNS. However, not all behavioural changes indicate that the substance has reached the CNS. The behavioural change may be due to discomfort caused by some other effect of the substance.
Skin sensitisation data	If the substance has been identified as a skin sensitiser, some uptake and distribution to lymph nodes must have occurred, although it may only have been a small fraction of the applied dose.
Trace elements	If the substance is a cationic trace element, absorption is likely to be very low (<1%). Stable or radio isotopes should be used and background levels determined to prevent analytical problems and inaccurate recoveries.

1.3.5 Metabolism or biotransformation

Biotransformation is one of the main factors which influence the fate of a chemical in the body, its toxicity, and its rate and route of elimination. Traditionally, biotransformation is divided into two main phases:

- ➔ **Phase I**, the so-called functionalisation phase, has a major impact on lipophilic molecules, rendering them more polar and more readily excreted.
- ➔ **Phase II** is often referred to as detoxification; functionalised moieties are conjugated with highly polar molecules or hydrolysed before they are excreted.

Both phases are catalysed by specific enzymes, which are either membrane bound (microsomal proteins) or present in the cytosol (cytosolic or soluble enzymes). It has been suggested that a **phase III** relates to the excretion of conjugates and involves ATP-dependent plasma membrane transporters.

Most chemicals are potentially susceptible to biotransformation, and all cells and tissues are potentially capable of biotransforming compounds. The major sites of such biotransformation are substrate- and route-dependent; generally, the liver and the entry portals of the body are the main biotransformation sites. The presence of metabolising enzymes varies in different tissues, and also between different cells in an organ. There are also marked intra- and interspecies differences in the expression and catalytic activities of many biotransforming enzymes. Information on metabolic differences may provide crucial insight in characterising the potential risk of chemicals to humans.

Differences in metabolism are the main reason for species and route specific toxicity. The liver has the greatest capacity for metabolism and is commonly causing route specific pre-systemic (first pass) effects especially following oral intake. Route specific toxicity may also result from hydrolysis within the GI or respiratory tract, metabolism by GI flora or within the GI tract epithelia (mainly in the small intestine), respiratory tract epithelia and skin.

It is difficult to predict the changes that a substance may undergo only on the basis of the physico-chemical information. Although it is possible to identify potential metabolites, these reactions might not occur *in vivo* (e.g. the molecule may not reach the necessary site for a particular reaction to take place). It is even more difficult to predict the extent of metabolism along different pathways and the existing species differences. Experimental data is therefore needed in assessing potential metabolic pathways.

1.3.6 Excretion

Chemicals can be excreted via various routes and mechanisms, and the relative importance of the excretion processes depends on the physical and chemical properties of the substance and its metabolites. Part of an oral dose might avoid absorption and biotransformation, being directly excreted to faeces.

Besides passive transportation (diffusion or filtration), there are carrier-mediated mechanisms to shuttle a substance through a biological membrane. There is a variety of pumps responsible for transportation of specific types of substances, such as sodium, potassium, magnesium, organic acids, and organic bases. Related compounds may compete for the same transport mechanism. Additional transport systems, phagocytosis and pinocytosis can also be of importance, for example in removing particulate matter from the alveoli by alveolar phagocytes and large molecules from the body by the reticulo-endothelial system in the liver and spleen.

The major routes of excretion for substances from the systemic circulation are the urine, faeces, and bile.

The kidney excretion processes involve passive glomerular filtration through membrane pores and active tubular secretion via carrier processes. Substances that are excreted in the urine tend to be water soluble and of low molecular weight (<300 in rats, mostly anionic and cationic) and generally, they are conjugated metabolites (e.g. glucuronides, sulphates, glycine conjugates) from Phase II biotransformation. Kidneys filter most of them out of the blood, though a small amount may enter the urine directly by passive diffusion and there is the potential for re-absorption into the systemic circulation across the tubular epithelium.

Biliary excretion involves active secretion. Substances that are excreted to bile tend to have higher molecular weights or may be conjugated as glucuronides or glutathione derivatives. In rats, substances with molecular weights < ca. 300 do not tend to be excreted to bile. Species differences and the nature of the substance also plays a role. Hepatic function influences the excretion of compounds to bile, as metabolites formed in the liver may be excreted directly to bile without entering the bloodstream. Blood flow also is a determining factor.

Substances in the bile pass through the intestines before they are excreted to faeces. As a result, the substances may have a longer biological half-life as they may undergo enterohepatic recycling, i.e. circulation of bile from the liver to the small intestine where it aids digestion of fats and other substances, and back to the liver. This is a particular problem for conjugated molecules that are hydrolysed by GI bacteria to form smaller, more lipid soluble molecules that can then be reabsorbed from the GI tract. Substances with strong polarity and high molecular weight are less likely to re-circulate. Other substances excreted to faeces are those that have diffused out of the systemic circulation into the GI tract directly, substances which have been removed from the GI mucosa by efflux mechanisms, and non-absorbed substances that have been ingested or inhaled and subsequently swallowed. Depending on the possible metabolic changes, the compound that is finally excreted may not have the physico-chemical characteristics of the parent compound.

Table 8 provides an overview of the data that can be used for estimation of excretion.

Table 8: Interpretation of data regarding excretion

Route	Favourable physico-chemical characteristics
Urine	Characteristics favourable for urinary excretion are low molecular weight (<300 in rats), good water solubility, and ionisation of the molecule at the pH of urine.
Exhaled air	Vapours and gases are likely to be excreted to exhaled air. Volatile liquids and metabolites may be excreted as vapours to exhaled air.
Bile	In rats, molecules that are excreted in the bile are amphipathic (containing both polar and nonpolar regions), hydrophobic/strongly polar, and have a high molecular weight. In rats, it is unlikely that more than 5-10% of organic cations with a molecular weight <300 will be excreted in the bile, and for organic anions (e.g. quaternary ammonium ions) this cut off may be even lower. Substances excreted in bile may potentially undergo enterohepatic circulation. This is particularly a problem for conjugated molecules that are hydrolysed by GI bacteria to form smaller, more lipid soluble molecules that can then be reabsorbed from the GI tract. Substances with strong polarity and high molecular weight are less likely to re-circulate. Little is known about the determinants of biliary excretion in humans.

Breast milk	Substances present in plasma may be found in breast milk. The concentration of lipid soluble substances may be higher in milk than in blood/plasma. Although lactation is a minor route of excretion, for some chemicals exposure of neonates via nursing to mother's milk has toxicological significance.
Saliva/sweat	Non-ionised and lipid soluble molecules may be excreted to saliva or sweat. In saliva the molecules may be repeatedly swallowed.
Hair/nails	Metal ions may be incorporated into hair and nails.
Exfoliation	Highly lipophilic substances that have penetrated the *stratum corneum* but did not penetrate the viable epidermis may be sloughed off with dead skin cells.

1.3.7 Accumulative potential

The potential of a substance to accumulate or to be retained within the body must be considered. Gradual build up with successive exposures can maintain the body burden for long periods of time.

Although there is no direct correlation between the lipophilicity of a substance and its biological half-life, substances with high log P values tend to have longer half-lives unless high clearance counterbalances their large volume of distribution. On this basis, there is the potential for highly lipophilic substances (log P > 4) to accumulate in individuals that are frequently exposed to the substance. Once the exposure stops, the concentration within the body will decline at a rate determined by the half-life of the substance. Other substances that can accumulate within the body include poorly soluble particulates deposited in the alveolar region of the lungs, substances that bind irreversibly to endogenous proteins, and certain metals and ions that interact with the matrix of the bone.

Table 9 provides an overview of data that can be considered for the estimation of accumulation.

Table 9: Interpretation of data regarding accumulation

Site	Characteristics of substances of concern
Lung	Poorly water and lipid soluble particles (i.e. log P is ca. 0 and water solubility ca. 1 mg/L or less) with aerodynamic diameters ≤1 µm have the potential to deposit in the alveolar region of the lung and are likely to undergo phagocytosis by alveolar macrophages. The macrophages will then either translocate particles to the ciliated airways or carry particles into the pulmonary interstitium and lymphoid tissues. Particles can also migrate directly to the pulmonary interstitium; this is likely to occur to the greatest extent where the particle is toxic to alveolar macrophages or inhaled in sufficient quantities to overwhelm the phagocytic capabilities of alveolar macrophages. Within the pulmonary interstitium, clearance depends on solubilisation alone, with possible long-term retention.
Adipose tissue	Lipophilic substances tend to accumulate in adipose tissue if exposure is repeated. Generally, substances with high log P values have long biological half-lives. Daily exposure to a substance with a log P value of around 4 or higher could result in build up of the substance within the body. Substances with log P ≤ 3 would be unlikely to accumulate with the repeated intermittent exposure patterns normally encountered in the workplace but may accumulate if exposures are continuous. If fat reserves are mobilized more rapidly than normal, e.g. under stress or during lactation, there is the potential for large quantities of the parent compound to be released into the blood and excreted to milk.

Site	Characteristics of substances of concern
Lung	Poorly water and lipid soluble particles (i.e. log P is ca. 0 and water solubility ca. 1 mg/L or less) with aerodynamic diameters ≤1 µm have the potential to deposit in the alveolar region of the lung and are likely to undergo phagocytosis by alveolar macrophages. The macrophages will then either translocate particles to the ciliated airways or carry particles into the pulmonary interstitium and lymphoid tissues. Particles can also migrate directly to the pulmonary interstitium; this is likely to occur to the greatest extent where the particle is toxic to alveolar macrophages or inhaled in sufficient quantities to overwhelm the phagocytic capabilities of alveolar macrophages. Within the pulmonary interstitium, clearance depends on solubilisation alone, with possible long-term retention.
Bone	Certain metals (e.g. lead) and small ions (e.g. fluoride) can mimic essential minerals and interact with ions in the matrix of bone. This interaction can displace the normal constituents of the bone, leading to retention of the metal or the ion.
Stratum corneum	Highly lipophilic substances (log P between 4 and 6) that come in contact with skin can readily penetrate the lipid rich stratum corneum but are not well absorbed systemically. Although they may persist in the stratum corneum, they will eventually be cleared as the stratum corneum is sloughed off.

1.3.8 Bioavailability, saturation, non-linearity and accumulation

The most critical factor influencing toxicity is the concentration of the ultimate toxicant at the actual target site (tissue dose). In this context bioavailability is a relevant parameter for the assessment of the toxicity profile of a test substance. It links dose and concentration of a substance with the mode of action which covers the key events within a complete sequence of events leading to toxicity.

1.3.8.1 Bioavailability

Bioavailability is usually considered as systemic bioavailability, describing the passage of a substance from the site of absorption into the blood of the general (systemic) circulation.

Systemic bioavailability is not necessarily equivalent to the amount of substance absorbed: in many cases excretion or metabolization may take place before reaching the systemic circulation, for example in the gut. Conversely, substances absorbed from the intestine can be partly eliminated by the liver at their first passage through that organ (first pass effect). The amount of substance that becomes systemically bioavailable is referred to as systemic exposure.

For liver effects upon oral administration, the oral absorption suffices. For other effects than at the portal of entry, bioavailability is generally more reliable for use in risk assessment.

1.3.8.2 Linearity, non-linearity, saturation, accumulation

When all transfer rates between the different compartments of the body are proportional to the amounts or concentrations present, a steady state is reached. For a xenobiotic this means that the amount eliminated equals the amount of substance input and the concentration in the body is (relatively) constant. If the input of a substance to an organism is greater than the maximum rate at which the substance is lost, the organism is accumulating the substance. This applies to both linear (first order) and non-linear (zero order) processes.

The process is called linear when a constant half-life can be calculated. This implies that the amounts of a substance cleared and distributed depend on the concentration of the substance

and are proportional to the exposure. Most substances in a biological system have a biological half-life, determining how long half of the substance will stay in the system until it is lost by mainly excretion, degradation or metabolism. Elimination thus depends on the concentration and is always half of the concentration in one half-life.

The process is called non-linear when elimination takes place at a rate that does not depend on the concentration. Clearance is thus a constant value that is characteristic for a substance, and no half-life can be calculated. Non-linear processes are more easily saturated, and a substance eliminated through these processes is more likely to reach toxic concentrations. The possibility of non-linear kinetics should always be considered (if not already shown by TK studies), especially when interpreting results from repeated dose studies.

When a kinetic process is saturated e.g. as a result of high exposure, it becomes non-linear as a result of key factors being inhibited or reaching their maximum capacity. These factors can be enzymes involved in biotransformation processes or transporters involved in distribution or elimination, or binding proteins (i.e. receptors). From that point on, clearance starts to follow zero order kinetics, becoming constant, which results in concentration or dose-dependency, or time-dependency of some of the kinetic characteristics. Clearance stays constant until the excess amount of the xenobiotic is eliminated, after which the normal half-life will apply again. The extent of accumulation reflects the relationship between the body burden compared with the steady state condition at maximum concentration. Species differences in clearance will determine the difference in steady state body burden between experimental animals and humans.

When relying on the half-life to assess bio-accumulative potential, please note that in non-steady state situations for multicompartmental models the observed kinetics are still influenced by the dynamic distribution of the xenobiotic across compartments. As a result, the half-life i.e. the net result from distribution and elimination processes, is not constant but varies with time. Especially when there is limited data available, e.g. because of not measuring long enough or when methods are used that are not sensitive enough to detect the lowest concentrations, it might be difficult to determine the terminal elimination half-life. However, in the disposition time course of the substance and at a distributional pseudo-equilibrium, the clearance of the substance can be assumed to be rate limited by the slowest relative redistribution from a tissue with highest permeation/affinity for the substance. In such situations, the longest half-life should then be selected, assuming this would represent the terminal phase. However, it must be noted that even if there is marked sparsity in the sampling data, there should be some data available through the TK profile, especially towards the end of the disposition kinetics profile, to give some idea how the terminal elimination might look like, and to allow for the calculation of a half-life.

1.3.9 Generating and integrating toxicokinetic information

The strategies for generating TK information are described in the *ECHA Guidance Vol III Part A*. The possible activity profile of a substance should be considered on the basis of physico-chemical and other data, as well as structurally related substances. This might help in the argumentation on waiving or triggering further testing and may provide a basis for understanding the mode of action of a substance.

In vivo studies provide an integrated perspective on the relative importance of different processes in an intact biological system, which can be used for comparison with the results of the toxicity studies. To ensure a valid set of TK data, an *in vivo* study has to consist of several experiments that include blood/plasma-kinetics, mass balances and excretion experiments, as well as tissue distribution experiments. Depending on the problem to be solved, particular

experiments (e.g. plasma-kinetics) may be sufficient to provide needed data for further assessments (e.g. bioavailability).

The high dose level administered in an ADME study should be linked to the levels that cause adverse effects in toxicity studies. Ideally a dose without toxic effects is included, which should be in the range of expected human exposure. A comparison between toxic dose levels and those that are likely to represent human exposure values may provide valuable information for the interpretation of adverse effects, as well as for extrapolation and risk assessment.

In an *in vivo* study the systemic bioavailability is usually estimated by comparing dose-corrected amounts excreted, or dose-corrected AUC of plasma/blood/serum kinetic profiles after extra- and intravascular administration. The systemic bioavailability is the dose-corrected amount excreted or AUC determined after an extravascular substance administration, divided by the dose-corrected amount excreted or AUC determined after an intravascular substance application, which corresponds by definition to 100% bioavailability. This is only valid if the kinetics of the compound is linear (i.e. dose-proportional) and relies upon the assumption that the clearance is constant between experiments. If the kinetics is not linear, the experiment has to be planned on a case-by-case basis, depending on the type of non-linearity involved (e.g. saturated protein binding or metabolism).

Generally, *in vitro* studies provide data on specific aspects of pharmacokinetics, such as metabolism or dermal absorption after metabolism. A major advantage of *in vitro* studies is that it is possible to carry out parallel tests on samples from the species used in toxicity tests and samples from humans, thus facilitating interspecies comparisons (e.g. metabolite profile, metabolic rate constants). *In vitro* comparative metabolism studies may also help in deciding which animal model, with regard to a particular compound, is the most relevant for humans. They can also provide additional information of all metabolites of concern as well as of species specific metabolism and may allow identifying unique and/or disproportionate human metabolites (see *EFSA Opinion on comparative in vitro metabolism studies*). In recent years, methods have been developed to use the appropriate physiologically based kinetic models to integrate a number of *in vitro* results into a prediction of ADME *in vivo*. Such methods allow both the prediction of *in vivo* kinetics at early stages of development and the progressive integration of all available data into a predictive model of ADME. The uncertainty associated with the prediction depends largely on the amount of available data.

In addition to the predictive approaches described earlier and to the test methods described in Section 8.8 in the *ECHA Guidance Vol III Part A*, kinetic modelling should also be considered for the generation of ADME data. In particular, generation of TK data should aim at providing essential information for the building of PBK models, to enable more accurate estimation of internal exposure, where relevant. The following section provides an overview of *in silico* methods for use in TK assessment.

1.3.9.1 *In silico* methods - kinetic modelling

In silico methods for TK can be defined as mathematical models which can be used to understand physiological phenomena of ADME in the body. These methods include, for example, (Q)SAR models, compartmental models, or allometric equations. Their main advantages compared to classical (*in vitro*, *in vivo*) methods are that they are quicker and cheaper, and reduce the use of experimental animals. However, they are currently not able to replace *in vivo* and *in vitro* methods but provide additional information and support experimental data. For a detailed discussion of the approaches that integrate information generated *in silico* and *in vitro*, see Appendix R.7.12-2 of *REACH Guidance R.7c*.

When using kinetic *in silico* models, two opposite situations can be schematically described:

- Fitting situation, where values of some or all parameters are unknown and the model is adjusted (fitted) to data to extract from the dataset these parameter values;
- Simulation situation, where the parameter values are considered as known and the model is used to generate simulated datasets.

Appropriate algorithms implemented in validated suitable software are available to perform fitting and simulation operations. Only adequately trained scientists can perform the model fitting or the simulation operations with uncertainty estimations. Simulation is an extremely useful tool because it is the only way to predict situations for which it is not possible to generate or collect real data.

The TK information collected from *in vitro* and *in vivo* experiments can also be analysed on the basis of *in silico* models. The purpose of the TK *in silico* models is to describe or predict the concentrations, and to define the internal dose of the parent chemical or its active metabolite. This is important because internal doses provide a better basis than external exposure for predicting toxic effects. The combined use of pharmacokinetic models (describing the relationships between dose/exposure and concentrations within the body), with pharmacodynamic models (describing the relationship between concentrations or concentration-derived internal dose descriptors and effects), is referred to as pharmacokinetic/pharmacodynamic modelling. The term toxicokinetic/toxicodynamic modelling covers the same concept.

TK models fall into two main classes: empirical models and physiologically based kinetic models. All these models subdivide the body into compartments within which the toxic agent is assumed to be homogeneously distributed, thus simplifying the complex physiology. Empirical TK models represent the body by one or two (rarely more than three) compartments not reflecting the anatomy of the species. These models are simple and with few parameters, allow describing many kinds of kinetics, and can easily be fitted to experimental data.

Experimental as well as observational datasets essentially determine the structure and parameter values of empirical kinetic models. Datasets generally consist of concentration versus time curves in various fluids or tissues, after dosing or exposure by various routes, at various dose or exposure levels, in various individuals of various species. Classic kinetic models describe the body as a small number of compartments (usually 1 or 2, rarely 3 or more per compound or metabolite) where ADME occurs. The virtual volume terms and transfer rates are the parameters of the models, which describe the phenomena. The function of the volume parameters are to relate the concentrations measured (e.g. in plasma) to the amounts of xenobiotic present in the body. The volumes described in the model usually have no physiological counterpart.

The datasets largely determine the structure of the respective models. Therefore, the models often are said to be data-driven or top to bottom. Compared to physiologically based models, classic kinetic models are usually better adapted to fitting the model to data in order to extract parameter values.

A physiologically based kinetic model is an independent structural mathematical model, comprising the tissues and organs of the body perfused by, and connected via, the blood/lymphatic circulatory system. Physiologically based kinetic models comprise four main parameter types: physiological, anatomical, biochemical and physico-chemical.

Physiological and anatomical parameters include tissue masses and blood perfusion rates, estimates of cardiac output and alveolar ventilation rates. Biochemical parameters include enzyme metabolic rates and polymorphisms, enzyme synthesis and inactivation rates, receptor and protein binding constants, etc. Physico-chemical parameters refer to partition coefficients. A partition coefficient is a ratio of the solubility of a chemical in a biological medium, usually

blood-air and tissue-blood. Anatomical and physiological parameters are readily available and many have been obtained by measurements. Biochemical and physico-chemical parameters are compound specific. When parameters are measured and used to construct an a priori model that qualitatively describes a dataset, confidence in such a model should be high. In the absence of measured data, such as partition coefficients, these may be estimated using tissue-composition based algorithms. Metabolic rate constants may be fitted using a physiologically based kinetic model, although this practice should only be undertaken if there are no other alternatives. A sensitivity analysis (see 1.3.9.2) of these models should be performed for identifying which parameters are important within a model. It helps prioritising and focusing on those parameters which have a significant impact on the risk assessment and to identify sensitive populations. For a discussion on the applicability of physiologically based kinetic modelling for the development of AFs in risk assessment, see Appendix R.7.12-3 of *REACH Guidance R.7c*.

The potential of physiologically based kinetic models to generate predictions from *in vitro* or *in vivo* information makes them useful in the risk assessment of chemicals. The degree of later refinement of the predictions depends on the particular purpose for which kinetic information is generated and on the feasibility of generating additional data. When new information becomes available, the physiologically based kinetic model should be calibrated using e.g. Bayesian techniques.

Physiologically based kinetic models are very useful when the kinetic process of interest cannot be directly observed and also when extrapolations are needed. Interspecies, interindividual, inter-dose or inter-route extrapolations are more robust when they are based on physiologically based kinetic models rather than on empirical ones. The intrinsic capacity for extrapolation makes physiologically based kinetic models particularly useful for assessing the risk of chemicals because it is usually impossible to gather kinetic data by all relevant exposure schemes or on all the species of interest, particularly on human. Physiologically based kinetic models also allow evaluating TK in reprotoxicity, developmental and multi-generational toxicological studies. A model can be developed to depict internal disposition of a chemical during pregnancy in the mother and in the embryo/foetus. Lactation transfer of toxicant from mother to newborn can also be quantified using physiologically based kinetic models. Physiologically based kinetics can also be used to check complex hypothesis, such as the existence of an unknown metabolism pathway or site, and to give predictions on internal doses which are not always observable in human. They also allow estimation of kinetic parameter (e.g. metabolism constant) and dose reconstruction from biomarkers.

The rationale for using physiologically based kinetic models in risk assessment is that they provide a documentable, scientifically defensible means of bridging the gap between animal bioassays, *in vitro* assays and human risk estimates. In particular, they explicitly describe the relationships of the administered dose to a dose more closely associated with the toxic effect, as a function of dose, species, route, and exposure scenario. Any risk assessment using the physiologically based kinetic models must counter-balance the increased complexity and data demand by increased accuracy, biological plausibility and scientific justifiability. Hence, physiologically based kinetic models are more likely to be used for chemicals of high concern.

As more and more data become available from *in vitro* assays, in the future it may be possible to use physiologically based kinetic models to extrapolate from *in vitro* effective concentrations to *in vivo* doses, i.e. (quantitative) *in vitro* to *in vivo* extrapolation, (Q)IVIVE. While this is an area of active research, it is currently not possible to provide detailed guidance. Guidance on the characterisation and reporting of Physiologically Based Kinetic (PBK) models used in the regulatory assessment of chemicals, with emphasis on the use of *in vitro* and *in silico* (non-animal) approaches for toxicity testing can be found in *OECD GD 331*.

1.3.9.2 Sensitivity analysis

The increasing understanding of physiological systems allows more complex mathematical models that exhibit more complex non-linear behaviour. Although the governing equations of these models can be solved usually with relative ease using a generic numerical technique, often the real strength of the model is not the predictions it produces but how they were produced. Sensitivity analysis techniques that give a measure of the effects on model output caused by variation in its input can be used to determine:

- Whether a model sufficiently emulates the studied organism;
- Which parameters require additional research to strengthen knowledge;
- The influence of structures such as *in vitro* scalings;
- Physiological characteristics or compound specific parameters that have an insignificant effect on the output and may be eliminated from the model;
- Feasible combinations of parameters for which the model variation is the greatest;
- The most appropriate regions within the space of input parameters for use in parameter optimization;
- Whether the interaction between parameters occurs and which of them interact.

Predictions from a complex mathematical model require a detailed sensitivity analysis in order to assess the limitations of the model predictions. A thorough understanding of the model can greatly reduce the efforts in collating physiological and compound specific data, and lead to more refined and focused simulations that more accurately predict human variability across a population and identify groups susceptible to toxic effects of a given compound.

1.3.10 Variability and uncertainty in toxicokinetics

Uncertainty and variability are inherent to a TK study and affect potentially the conclusion of the study. It is necessary to minimise uncertainty to assess the variability that may exist between individuals so that there is confidence in the TK results.

Variability typically refers to differences in the physiological characteristics among individuals (inter-individual variability) or across time within a given individual (intra-individual variability). It may stem from genetic differences, activity level, lifestyle, physiological status, age, sex, etc. Variability is characteristic for animal and human populations. It can be observed and registered but not reduced. An important feature of variability is that it does not tend to decrease when larger samples of a population are examined.

Variability in the population should be taken into account in TK studies. The application of probability distributions on the parameters representing the distribution of physiological characteristics in the population may introduce variability into physiologically based kinetic models. The propagation of the variability to model predictions may be evaluated using Monte Carlo simulation methods.[12]

[12] Monte Carlo simulation methods consist of specifying a probability distribution for each model parameter, sampling randomly each model parameter from its specified distribution, running the model using the sampled parameter values, and computing various model predictions of interest. Instead of specifying independent distributions for parameters, a joint probability distribution may be assigned to a group of parameters to describe their correlation.

Uncertainty can be defined as inability to make precise and unbiased statements. It is essentially due to a lack of knowledge and can be reduced with the size of the sample studied. Further optimised experiments and better understanding of the process under study can theoretically eliminate or at least reduce the uncertainty.

Uncertainty may be related to:

- **The experimental nature of the data**. Uncertainty comes from errors in experimental data. Experimental data are typically known with finite precision dependent of the apparatus and methodology used. Such uncertainties may be easily assessed with quality measurement data and can be modelled with probability distributions (e.g. the measured quantity is distributed normally with the mean, the actual quantity and the given standard deviation). The data gathering process and errors made at this stage (reading errors, systematic measurement errors, etc.) may also generate uncertainty.
- **The modelling procedure**. Uncertainty is most of the time inevitable due to the complexity and unknown nature of the phenomena involved (model specification). The source of uncertainty in the model structure (and more particularly in physiologically based kinetic models) is primarily a lack of theoretical knowledge to correctly describe the phenomenon of interest on all scales. A massive amount of information in a model can also be a technical challenge. An organism may be viewed as an integrated system whose components' correlations are both strong and multiple (e.g. a large liver volume might be expected to be associated with a large blood flow). Given the complexity of an organism, it is necessary to simplify as it is not feasible to integrate all interactions between its components in the development of a model. Such assumptions will however introduce uncertainty. A general approach to quantify model uncertainty is first to evaluate the accuracy of the model when predicting some datasets. Models based on different assumptions may be tested and statistical criteria (such as the Akaike criterion[13]) may be used to discriminate between models.
- **The high inherent variability of biological systems**. The variability itself is a source of uncertainty. In some cases it is possible to fully know variability, for example by exhaustive enumeration, with no uncertainty attached. However, variability may be a source of uncertainty in predictions if not fully understood and attributed to randomness.

1.3.11 Human data

Human biological monitoring and biomarker measurement studies provide dosimetric means for establishing aggregate and/or cumulative absorbed doses of chemicals following specific situations or exposure scenarios or for establishing baseline, population-based background levels. The results from these studies, e.g. temporal situational biological monitoring, provide a realistic description of human exposure.

Biomonitoring, the analysis of human tissues or excreta for direct or indirect evidence of human exposures to substances, can provide insights into the relationship between dose and putative toxicity thresholds established in experimental animals, usually rats. Urine is the most frequently used biological specimen, due to its easy non-invasive collection and importance as a route of excretion for most analytes. The analyte to be monitored should be selected depending on the metabolism of the compound and the biological relevance and feasibility, to maximise the relevance of the information obtained.

[13] Akaike criterion is a measure of the relative quality of a statistical model for a given set of data.

1.3.12 Using toxicokinetic information

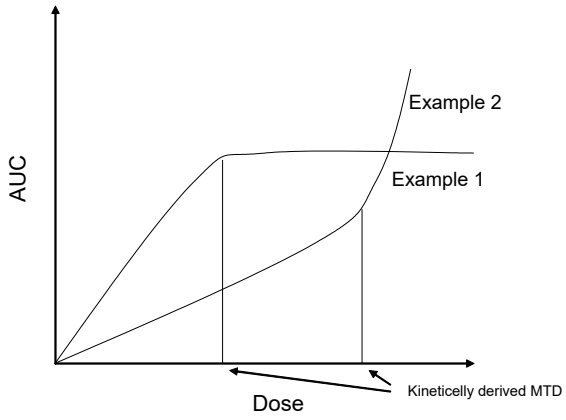
1.3.12.1 Dose setting for repeated dose studies

TK data, especially information on absorption, metabolism, and elimination, are useful in designing repeated dose toxicity studies. The highest dose level in such studies should induce toxicity but not death or severe suffering in the test animals. The OECD/EU guidelines suggest to test up to the standardised limit dose level called maximum tolerated dose (MTD). In certain cases, such doses may cause saturation of metabolism. Therefore, the obtained results need to be carefully evaluated when eventually assessing the exposure risk posed at levels where a substance can readily be metabolised and cleared from the body. When designing repeated dose toxicity studies, appropriate dose levels can be selected on the basis of metabolic and TK information.

If it can be demonstrated that a substance is not absorbed and cannot induce direct systemic effects, in principle there is no need for further repeated dose testing. If the substance is absorbed and there is a linear relationship between the administered dose and the AUC in the blood but the substance is not metabolised, there is no kinetic argument against testing at the MTD.

The dose/AUC relationship often deviates from linearity above a certain dose, as illustrated in Figure 1. For both Example 1 and Example 2, the dose level corresponding to the inflexion point can be regarded as the kinetically derived MTD. If this information is available, it might be considered setting the highest dose level for repeated doses studies according to the kinetically derived MTD.

Figure 1: Departure from linearity at certain doses. *In example 1 the AUC does not increase beyond a certain dose level. This is the case when absorption becomes saturated above a certain dose level. The dose/AUC relationship presented in example 2 can be obtained when elimination or metabolism becomes saturated above a certain dose level, resulting in an over proportional increase in the AUC.*



1.3.12.2 Chemical categories/grouping

Information on kinetics *in vivo* can be used in setting categories. Candidate category substances for performing *in vitro* or *in vivo* tests can be identified, which makes extrapolation of toxicological findings between substances more relevant. In case of uncertainty or contradictory information within a category, the category can be verified using kinetics information.

1.3.12.3 Internal dose considerations

Biotransformation of a substance produces metabolites that may have different toxicological properties than the parent compound. Although metabolism is generally referred to as having a detoxification purpose, there are many examples where metabolites have higher intrinsic toxicity than the parent compound itself (metabolic activation). Therefore, it is necessary to know if the test substance is metabolised and to which metabolites.

If the test substance is not metabolised, the parent compound is the relevant marker for the measurement and the definition of the internal dose. If the test substance is metabolised, the knowledge on metabolites is essential for the assessment. When this information is not available, it can be investigated by appropriate *in vitro* and/or *in vivo* metabolism studies, considering possible species differences. Metabolites may also show a high degree of isomeric specificity, which should be kept in mind when designing and interpreting mixtures of isomers, including racemates. If the metabolites are known and toxicity studies are available for them, risk assessment and internal dose assessment may be carried out based on the data. If the toxicity profile of the metabolites is unknown, toxicity studies that address the metabolites may be performed, also considering potential group approaches (e.g. carboxylic acid as a metabolite of different esters).

TK information can be helpful in bridging various gaps in the risk assessment, from toxicity study design and biomonitoring setup to the derivation of the threshold levels and various extrapolations (cross-dose, cross-species including human, cross-exposure regimens, cross-routes, and cross-substances). Internal dose is the central output parameter of TK studies.

Biomonitoring information should be seen as equivalent to other forms of exposure data, having neither greater nor less importance. Biomonitoring results reflect an individual's total exposure to a substance from any route, including consumer products, environment and occupational exposure.

Exposure should normally be understood as external exposure, which can be defined as the amount of substance ingested, the total amount in contact with the skin, or either the amount inhaled or the concentration of the substance present in the atmosphere combined with the exposure duration. For systemic risk characterisation, the total body burden has to be estimated and expressed as an internal dose that may come from various routes of exposure.

Determination of the level of systemic exposure is considered synonymous to determination of the bioavailability to the general circulation. Depending on the problem considered and other information such as exposure scenarios, this could be expressed as a fraction bioavailable, a mass bioavailable, a concentration profile, an average concentration, or AUC. It is usually not possible to show that the amount of a substance bioavailable is zero, apart from some cases where the substance is absorbed via the dermal route, considering only intact skin. It should be assessed whether the bioavailability of a substance is predicted to be below a certain threshold. The degree of certainty of the prediction will depend on each case. Important factors include the accuracy and reliability of the *in vivo*, *in vitro* or *in silico* model used, the performance of the methods used to assay the substance or its metabolites, and the estimated variability in the target population.

The compound's tissue distribution characteristics can be an important determinant of its potential to cause toxicity in specific tissues. Tissue distribution may also be an important determinant of the ability of a compound to accumulate upon repeated exposure. Correlation of tissue distribution with target tissues in toxicity studies should be accomplished while substantial amounts of the chemical remain present in the body, e.g. one or more times around the peak blood concentration following oral absorption. Such data should quantify the parent compound and the metabolites to the extent feasible. If the metabolites are unknown or difficult to quantify,

subtracting parent compound from total radioactivity will estimate the behaviour of the total metabolites formed.

1.3.12.4 Extrapolation

Extrapolation of information is needed when data are poor, sparse, or do not concern human populations. TK data are usually gathered for few concentrations (<5) and limited number of different exposure times, while risk assessment should cover the different doses, concentrations and times. Extrapolation is a common way to satisfy this demand, using mathematical methods such as linear regression. The non-linear kinetic behaviour of chemicals in a biological organism is the result of a number of mechanisms, including saturable metabolism and depletion of cofactor reserves. High-dose-low-dose extrapolation of tissue dose is accomplished via physiologically based kinetic modelling accounting for such mechanisms.

Where human data is available, extrapolation is needed to cover all populations in terms of e.g. gender, age, and ethnic groups. From animal data, interspecies extrapolation is needed. Extrapolation from one exposure route to another is needed when the administration route in experimental study is different from the most likely exposure route, or there are several relevant exposure routes.

Default values have been derived to match the extrapolation idea in a general way. Quantitative data on interspecies differences or human variability in TK and TD can be considered by setting chemical specific AFs (see 2.3.4). Information is often limited to address interspecies differences in TD and interindividual variability in TK and TD. Useful TK information includes the rate and extent of absorption, the extent of systemic availability, the rate and extent of pre-systemic (first pass) and systemic metabolism, the extent of enterohepatic circulation, formation of reactive metabolites including species differences, and knowledge of the half-life and potential for accumulation under repeated exposure.

Physiologically based kinetic models facilitate the required extrapolations as these models are transposable from rat to human by changing anatomical parameters, such as organ volumes or blood flows.

Interspecies extrapolation

When information is available on both animals and humans, chemical-specific interspecies extrapolation factors can be defined. In allometric scaling, extrapolation is based on different body sizes, while more complex approaches collect various types of data and includes these in the physiologically based kinetic modelling.

Allometric scaling is a commonly employed extrapolation approach. It is based on the principle that biological diversity is largely explained by body size and the proportion of body surface area. Allometric scaling captures the correlations of physiological parameters or TK with body size. More precisely, allometric equations relate the quantity of interest (e.g. a tissue dose) to a power function of body mass fitted across species:

$$Y = a\ BM^{b}$$

In the above equation:

Y: quantity of interest
a: species-independent scaling coefficient (fitting data points to form a curve)
BM: body mass
b: allometric exponent

Values of b depend upon whether the quantity of interest scales approximately with body mass (b=1), metabolic rate in terms of oxygen consumption (b=0.75), or body surface area (b=0.67). It is easy to apply allometric scaling but it is very approximate and may not hold for the chemical of interest.

For a chemical that demonstrates significant interspecies variation in animal toxicity experiments, the most susceptible species are generally used as the reference point for extrapolation. Uncertainty factors ≥10 may be applied where necessary. Whereas the metabolic rate estimated may be used in a physiologically based kinetic model, it is preferable to determine such parameters *in vitro* using tissue subcellular fractions or estimate them by fitting a physiologically based kinetic model to an appropriate dataset.

To better estimate tissue exposure across species, physiologically based kinetic models may be used, accounting for transport mechanisms and metabolism within the body. The same equation set then models the processes for all species, assuming species differences due to different physiological, chemical, and metabolic parameter values. When parameter values of physiologically based kinetic model are not known for the considered species, it is possible to use *in vitro* data, *in silico* predictions or allometric scaling of those parameters. For population variability in the extrapolation, probability distributions of parameters may be used rather than single parameter values. Physiologically based kinetic models can be particularly useful where data are extrapolated to population subgroups for which little information is available, such as pregnant women or infants.

Route-to-route extrapolation

Route-to-route extrapolation is used to predict the total amount of a substance that needs to be administered by a specific route to produce the same systemic exposure and toxic response as that obtained for a known amount administered by another route. More guidance is given in *REACH Guidance R.8* and ECHA Practical Guide 14[14] which also contains default values for route-to-route extrapolation (table 2).

Route-to-route extrapolation is generally a poor substitute for toxicity data obtained using the appropriate route of exposure. Uncertainties increase when toxicity data was obtained by an administration route which does not correspond to the human route of exposure. In extrapolation, internal doses after absorption are used and all predictions are based on the internal dose instead of the administered dose or concentration.

Route-to-route extrapolation only applies for systemic effects. For local effects, results from toxicity studies performed with the relevant route should be used.

The major factors responsible for differences in toxicity due to route of exposure include differences in absorption, bioavailability, metabolism (first pass effects) and internal exposure pattern (internal dose).

In the absence of relevant kinetic data, route-to-route extrapolation is only possible if:

- Absorption can be quantified;
- The compound is relatively soluble in body fluids, therefore systemically bioavailable, and internal dose can be estimated;

[14] Practical guides available at https://echa.europa.eu/practical-guides; direct link: https://www.echa.europa.eu/documents/10162/17250/pg_14_on_hazard_endpoint_en.pdf/8a85bb85-f4da-49b1-a28a-bfdf269c68b4

- First pass effects are minimal.

Default values must normally be used in route-to-route extrapolation. If an internal N(O)AEL/starting point needs to be derived to assess exposure from several routes, information on the extent of absorption for the different routes of exposure should be used to modify the starting point. Case-by-case judgment is needed on whether the experimentally determined extent of absorption can be used for the starting point of interest. Special attention should be given to the dose ranges in the absorption studies compared to those used to determine the starting point.

Consideration should also be given to the age of the animals in the absorption studies (e.g. adult), compared to the age of the animals used to determine the starting point (e.g. pups during lactation). For substances that undergo first pass metabolism by one or more routes of administration, information on the extent of the pre-systemic metabolism and systemic availability should also be considered. This could require additional modification of the starting point.

The estimation of oral absorption efficiency and its use in adjusting the factor from administered to absorbed dose introduces uncertainty. Part of this uncertainty relates to distinctions between the terms absorption and bioavailability. Typically, the term absorption refers to the disappearance of chemical from the gastrointestinal lumen, while oral bioavailability refers to the rate and amount of chemical reaching the systemic circulation. Bioavailability thus accounts for both absorption and pre-systemic metabolism. The pre-systemic metabolism includes both gut wall and liver metabolism, including the liver first pass effect.

In the absence of metabolic activation or detoxification, toxicity adjustment should be based on bioavailability rather than absorption. Simple adjustment of the oral toxicity factor based on oral absorption does not account for metabolic by-products that might occur in the gut wall but not in the skin, or vice versa.

The efficiency of first pass metabolism determines the impact on route-to-route extrapolation. An adjusted dermal toxicity factor may overestimate the dose-response relationship when based on the amount of parent compound in systemic circulation rather than on the toxic metabolite. Additionally, percutaneous absorption may not generate a toxic metabolite in the same rate and extent as the GI route.

An adjustment in oral toxicity factor may be needed to account for absorbed dose in the dermal exposure pathway. This would be the case if the toxicity value derived from the critical study is based on an administered dose (e.g. dose delivered in diet or by gavage), and it can be concluded that the GI absorption from a medium (e.g. water, feed) similar to the one employed in the critical study is significantly less than 100%. If these conditions are not met, a default 100% oral absorption may be assumed. Note also that 100% oral absorption should be applied for the derivation of AELs and internal exposure levels when oral absorption rate exceeds 80%.

Extrapolation of the kinetic behaviour from one exposure route to another can also be performed using physiologically based kinetic models. Appropriate model equations for the exposure routes of interest is the basis of the extrapolation. Once the chemical is in the systemic circulation, its biodistribution is independent of the exposure route.

Oral exposure of a chemical may be modelled by a first order or a zero order uptake rate constant. For dermal absorption, a diffusion-limited compartment model may represent skin as a portal of entry. Inhalation route is often represented with a simple pulmonary compartment, and the uptake is controlled by the blood over air partition coefficient. With equations describing the route specific entry of chemicals into systemic circulation in the model, it is possible to conduct extrapolations of TK and dose metrics.

1.4 Acute toxicity

Table 10: Guidance to be considered together with the current guidance

Guidance	Section
ECHA Guidance Vol III Part A	1.7 Acute toxicity

1.4.1 Definition of acute toxicity

The term acute toxicity is used to describe adverse effects that may result from a single exposure or multiple exposures within 24 h to a substance. In the context of this guidance, exposure relates to the oral, dermal, or inhalation routes. The adverse effects can be seen as clinical signs of toxicity, abnormal body weight changes, and/or pathological changes in organs and tissues, which in some cases may result in death.

In addition to acute systemic effects, some substances may have the potential to cause local irritation or corrosion of the GI tract, skin, or respiratory tract following a single exposure. Acute irritant or corrosive effects due to the direct action of the chemical on the exposed tissue are not specifically covered in this section, although their occurrence may contribute to the acute toxicity of the chemical and must be reported.

At the cellular level, acute toxicity can be related to three main types of toxic effects:

(i) general basal cytotoxicity;

(ii) selective cytotoxicity, and

(iii) cell-specific function toxicity.

Acute toxicity may also result from chemicals interfering with extracellular processes. Toxicity to the whole organism also depends on the degree of dependence of the whole organism on the specific function affected.

Generally, the objectives of investigating acute toxicity are to find out:

- whether single exposures of humans to the substance of interest could be associated with adverse effects on health;
- in studies in animals, the lethal potency of the substance based on the LD50 or LC50, the discriminating dose, and/or the acute toxic class;
- what toxic effects are induced following a single exposure to a substance, their time of onset, duration and severity (all to be related to dose);
- when possible, the slope of the dose-response curve;
- when possible, whether there are marked sex differences in response;
- and obtain information necessary for the classification and labelling of the substance for acute toxicity.

The indices of LD_{50} and LC_{50} are statistically derived values relating to the dose that is expected to cause death in 50% of treated animals in a given period. These values do not provide information on all aspects of acute toxicity. Information on lethality is not a requirement for the classification decision or risk assessment. Other parameters and observations and the type of dose-response may provide valuable information.

There is an overriding obligation to minimise the use of animals in any assessment of acute toxicity. The potential to apply read-across or other non-testing methods should be explored. Old LD_{50} results can be used for assessment when available. Further considerations on the nature and reversibility of the toxic effects are necessary in risk assessment.

1.4.2 Data to be used in the effects assessment

Whichever approach is used in determining acute toxicity, critical information needs to be derived from the data used in risk assessment. It is important to identify dose levels that cause toxic signs, as well as the relationship of the severity of the toxic signs with the dose and the dose level at which toxicity is not observed (i.e. NOAEL). Although it is possible to use information from physico-chemical properties and modelling in a WoE approach for the assessment of acute toxicity (as described below), in principle, *in vivo* data are always needed for the derivation of acute threshold levels. A NOAEL is not usually determined in acute toxicity studies, partly because of the limitations in study design.

1.4.2.1 Non-human data for acute toxicity

1.4.2.1.1 Non-testing data for acute toxicity

(a) Physico-chemical properties

It may be possible to conclude from the physico-chemical characteristics of a substance whether it is likely to be corrosive or absorbed by a particular route and produce acute toxic effects after exposure. Physico-chemical properties may be important for the inhalation route (vapour pressure, MMAD, log Kow), determining the technical feasibility of the testing and acting upon the distribution in the airways in particular for 'local-acting substances'. Some physico-chemical properties of the substance or mixture could be the basis to omit testing. In particular, this should be considered for substances having vapour pressures $<1 \times 10^{-5}$ kPa (7.5×10^{-5} mmHg) for indoor uses, and $<1 \times 10^{-4}$ kPa (7.5×10^{-4} mmHg) for outdoor uses. Furthermore, inhalable particles are capable of entering the respiratory tract via nose and/or mouth and are generally smaller than 50 µm in diameter. Particles larger than 50 µm are less likely to be inhalable. For aerosols, particle size determination is important.

In particular, the particle size of the substances in powder form strongly influences the deposition behaviour in the respiratory tract and potential toxic effects. Particle size considerations (determined by e.g. granulometry testing, OECD TG 110) can contribute to:

- selecting a representative sample for acute inhalation toxicity testing;
- assessing the respirable and inhalable fractions, preferably based on aerodynamic particle size;
- justifying derogations from testing, for instance when read-cross or chemical grouping data can be associated with results from particle size distribution analyses (see *REACH Guidance R.6).*

Physico-chemical properties (log Kow, molecular weight and volume, molar refraction, degree of hydrogen bonding, melting point) are also important in determining the potential of exposure through the skin.

(b) Read-across to structurally or mechanistically similar substances

According to *RAAF*, substances that have physicochemical, toxicological and ecotoxicological properties that are likely to be similar or follow a regular pattern as a result of structural similarity may be considered as a group, or 'category' of substances. Regarding the assessment of read-across, see Section 1.2.2.4.3.

(c) (Q)SAR systems

Several (Q)SAR systems are available for making predictions on e.g. dermal penetration or metabolic pathways. However, such systems may have limitations regarding validation against appropriate experimental data. That is why the modelled data can be used for hazard identification and risk assessment purposes only as part of a WoE approach.

The possibility of multiple mechanisms for acute toxicity is one of the reasons for limited availability and predictivity of (Q)SAR models. In the absence of complete validation information, available models could be used as a part of the WoE approach for hazard identification and risk assessment purposes after precise evaluation of the information derived from the model.

For acute oral toxicity, examples of available software tools are available in the *REACH Guidance R.7a*, Section R.7.4.3.1.1.

In grouping approaches, adequacy should be assessed and documented according to guidance described in the *REACH Guidance R.6*.

1.4.2.1.2 Test data for acute toxicity

(a) In vitro data

The currently available *in vitro* tests provide supplementary information to determine starting doses for *in vivo* studies, to assist evaluation of data from animal studies and identification of species differences, or to increase understanding of the toxicological mechanism of action of the substance. Currently they cannot be used to replace testing on animals completely.

In vitro data may be useful for predicting acute toxicity in humans, provided that the domain of applicability for the test method is appropriate for the class of chemical under evaluation and a range of test concentrations that permits calculation of an IC50 (inhibitory concentration 50%) value have been investigated.

Generic guidance is given in *REACH Guidance R.4* for judging the applicability and validity of the outcome of various study methods, assessing the quality of the conduct of a study (including how to establish whether the substance falls within the applicability domain of the method and the validation status for the given domain) and aspects such as vehicle, number of duplicates, exposure/incubation time, GLP compliance or comparable quality description.

(b) Animal data

Before initiating any new testing for acute toxicity, already existing data must be considered. These may be available from a wide variety of animal studies, including the following:

- OECD TG 420 Acute oral toxicity – Fixed dose procedure;
- OECD TG 423 Acute oral toxicity – Acute toxic class method;
- OECD TG 425 Acute oral toxicity – Up-and-down procedure;

- OECD TG 401 Acute Oral Toxicity (method deleted from the OECD TGs and EU test methods);
- OECD TG 402 Acute dermal toxicity;
- OECD TG 403 Acute inhalation toxicity;
- OECD TG 433 "Acute Inhalation Toxicity, Fixed Concentration Method";
- OECD TG 436 "Acute Inhalation Toxicity, Acute Toxic Class Method";
- International Conference on Harmonisation (ICH) compliant studies;
- mechanistic and toxicokinetic studies;
- studies in non-rodent species;
- single dose studies for genotoxicity (e.g. a micronucleus test);
- sighting studies conducted as preliminary/dose-ranging studies for e.g. repeated dose studies;
- studies using other acute toxicity test protocols (e.g. simple lethality studies; dermal or inhalation tests in which the periods of exposure are different from those specified in test guidelines; tests to study effects on particular organs/systems such as the cardiovascular system).

Unreferenced data reported in secondary sources (e.g. toxicology handbooks) may also be considered when no other data is available.

Traditionally, acute toxicity tests on animals have used mortality as the main observational endpoint, usually in order to determine LD_{50} or LC_{50} values. These values were earlier regarded as key information for hazard assessment and supportive information for risk assessment, but derivation of a precise LD_{50} or LC_{50} value is no longer considered essential. Some of the current standard acute toxicity test guidelines, such as the fixed dose procedures (OECD TG 420 and OECD TG 433), use signs of non-lethal toxicity and have animal welfare advantages over other guidelines.

Existing OECD TG 401 data would normally be acceptable, but testing should not be performed using this obsolete method. In addition to current regulatory methods, acute toxicity data on animals may be obtained by conducting a literature search and reviewing all available published and unpublished toxicological or general data, and the official/existing acute toxicological reference values. For more extensive general guidance see *REACH Guidance R.3*, Section R.3.1. Utilising all the available information from sources such as those above, WoE approach should be taken to maximise use of existing data and minimise the commissioning of new testing. When several sets of data are available, a hierarchal strategy should be used to focus on the most relevant.

In many cases, there will be little information on the cause of death or mechanism underlying the toxicity, and only limited information on pathological changes in specific tissues or clinical signs, such as behavioural or activity changes.

Many acute toxicity studies on chemicals of low toxicity are performed as limit tests. For more harmful chemicals, the choice of optimal starting dose will minimise the use of animals. When multiple dose levels are assessed, characterisation of the dose-response relationship may be possible, and signs of toxicity identified at lower dose levels may be useful in estimating LOAELs or NOAELs for acute toxicity. For locally acting substances, mortality after inhalation may occur due to tissue damage in the respiratory tract. In these cases, the severity of local effects may be related to the dose or concentration level and therefore, it might be possible to identify a

LOAEL/LOAEC or NOAEL/NOAEC. For systemic toxicity, there could be some evidence of target organ toxicity or signs of toxicity based on clinical observations.

Whichever approach is used in determining acute toxicity, critical information needs to be derived from the data to be used in risk assessment. The dose levels producing signs of toxicity must be identified, as well as the severity of these toxicity signs and their relationship with the dose and the level at which the toxicity is not observed (NOAEL).

Whichever test is used to evaluate acute toxicity on animals, the evaluation of studies should take into account the reliability, the relevance and the adequacy of the data (see Section 1.2.2) for the purposes of evaluating the given hazard from acute exposure. Most useful information comes from studies that give a precise description of the nature and reversibility of the toxic effects, the number of subjects, sex, the number of animals affected by the observed effects and the exposure conditions (atmosphere generation for inhalation, duration and concentration or dose).

When several study results are available, the most relevant should be selected; data from other studies that have been evaluated should be considered as supportive data for the full evaluation of the substance.

The classification criteria for acute inhalation toxicity relate to a 4-hour experimental exposure period. If data for a 4-hour period are not available, then extrapolation of the results to 4 hours are often achieved using Haber's Law ($C \times t = k$), where:

C: concentration
t: exposure time
k: constant

However, there are limits to the validity of such extrapolations, and it is recommended that the Haber's Law approach should not be applied to experimental exposure durations of less than 30 minutes or greater than 8 hours in determining the 4-hour LC_{50}.

Nowadays, a modification of Haber's Law is used ($C^n \times t = k$), as for many substances it has been shown that the regression coefficient *n* is not equal to 1. In case extrapolation of exposure duration is required, the *n* value should be considered. If this *n* value is not available from literature, a default value of 3 may be used for extrapolation to shorter duration than the duration for which the LC_{50} or EC50 was observed and a default value of 1 for extrapolation to longer duration, also taking the range of approximately 30 minutes to 8 hours into account.

Experimentally, when concentration-response data are needed for specific purposes, OECD TG 403 features two study types:

- traditional LC_{50} protocol resulting in a concentration-response curve at a single exposure duration;
- concentration × time ($C \times t$) protocol resulting in a concentration-time-response curve, taking different exposure durations into account.

The $C \times t$ approach uses two animals per $C \times t$ combination and exposure durations may vary from about 15 minutes up to approximately 6 hours. This approach may provide detailed information on the concentration-time-response relationship in particular for risk assessment and determination of NOAEL/LOAEL.

1.4.2.2 Human data for acute toxicity

When available, epidemiological studies, case reports, information from medical surveillance or volunteer studies may be crucial for acute toxicity and can provide evidence of effects that are undetectable in animal studies (e.g. symptoms like nausea or headache).

Acute toxicity data on humans may be available from:

- Epidemiological data identifying hazardous properties and dose-response relationships;
- Routine data collection, poisons data, adverse event notification schemes, coroner's report;
- Biological monitoring/personal sampling;
- Human kinetic studies – observational clinical studies;
- Published and unpublished industry studies;
- National poison centres.

Available human data could also be useful to identify particularly sensitive sub-populations like newborns, children, and patients with diseases, in particular with chronic respiratory conditions, such as asthma or chronic obstructive pulmonary disease.

Additional guidance on the reliability and the relevance of human studies is provided in the *REACH Guidance R.4*, as there are no standardised guidelines for such studies and they are normally not conducted according to GLP. Poor reporting and potential confounding factors compromise the usefulness of reports on the effects arising from accidents or abuse, or the effects of short-term exposures in the workplace. Suspected subjective reporting of symptoms by the exposed people may complicate the evaluation. Accidents, abuse and use of the substance as or in a medicinal agent may involve exposure routes different from those of concern in normal use, and though the latter may have very good exposure data, possible differences in TK parameters need to be taken into account. It may be possible to derive a minimum lethal dose from reports of human accidents or abuse.

1.4.3 Remaining uncertainty on acute toxicity

Data from studies on animals will often give very good information on the acute toxicity of the substance in the test species, and in general, it can be assumed that substances which are highly toxic to animals will be toxic to humans. However, there are subjective effects (e.g. nausea, CNS depression) experienced by humans exposed to substances which may not be detected in standard studies conducted in the usual laboratory animal species. Therefore, it is not certain that substances of low toxicity in single exposure studies in animals will not cause adverse effects in humans.

1.4.4 Concluding on suitability for Classification and Labelling

In concluding on classification and labelling, all the available information needs to be considered. The *CLP Guidance* should be followed. If the data available is not sufficient, additional testing may be required as described in the *ECHA Guidance Vol III Part A*.

1.4.5 Concluding on suitability for risk assessment

It may sometimes be possible to derive reliable NOAEL values for specific sub-populations from well-documented human data.

It is not usual to derive "acute NOAELs" for acute toxicity in animals, but often the only numerical value derived is the LD_{50} or LC_{50} value. Care should be taken when using LD_{50} or LC_{50} values from dermal or inhalation acute toxicity tests in which the duration of exposure was different from that specified in the OECD Test Guidelines.

Information on acute toxicity is normally not limited to availability of a LD_{50} or LC_{50} value. Additional information for risk assessment can be both qualitative and quantitative and may include parameters such as the nature and severity of the clinical signs of toxicity, local irritant effects, time of onset and reversibility of the toxic effects, the occurrence of delayed signs of toxicity, body weight effects, dose-response relationships (the slope of the dose-response curve), sex-related effects, specific organs and tissues affected, highest non-toxic and lowest lethal dose.

Information on toxic signs and the dose levels at which they occur (if available from test reports or the literature) can help in the subsequent risk characterisation for acute toxicity. Equally, dose levels leading to no effect can provide useful information.

The slope of the dose-response curve is a particularly useful parameter as it indicates the extent to which reduction of exposure will reduce the response: the steeper the slope, the greater the reduction in response for a particular finite reduction in exposure.

For risk assessment, the standard OECD test guideline data performed under GLP are considered reliable and relevant and thus should be used. A quantitative rather than qualitative assessment is preferred to conclude on the risk with regards to acute toxicity dependent on the data available and the potential exposure to the substance during the use pattern/lifecycle of the substance. If quantitative data are not available, the nature and severity of the specific acute toxic effects can be used to make specific recommendations with respect to handling and use of the substance.

If a NOAEL can be identified, this can be used in determination of a threshold level. However, while data from an OECD method may permit calculation of an LD_{50}/LC_{50} value or identification of the range of exposure where lethality is expected, or the dose at which evident toxicity is observed, it may not provide information on the dose level at which no adverse effects on health are observed. It may nevertheless be possible to derive a NOAEL if a dose-response curve is available.

When a limit test has been conducted and no adverse effects on health have been observed, then the limit dose can be regarded as the NOAEL. If adverse effects on health are seen at the limit dose and lower dose levels have not been investigated, the identification of a NOAEL will not be possible. If data is available for several species, the most sensitive species should be chosen for the purposes of the risk assessment, provided it is relevant to humans.

If human data on acute toxicity is available, it is unlikely that this will be derived from carefully controlled studies or from a significant number of individuals. It may not be appropriate to determine a threshold level from such data alone, but the information should be considered in the WoE and may be used to confirm the validity of animal data. In addition, human data should be used in the risk assessment to determine threshold levels for particularly sensitive sub-populations like newborns, children, or those in poor health.

The anticipated effects from physico-chemical properties and bioavailability data on the acute toxicity profile of the substance must also be considered in the risk assessment.

1.5 Irritation and corrosivity

Irrespective of whether a substance can become systemically available, it may cause changes at the site of first contact (skin, eye, mucous membrane in the GI tract, mucous membrane in the respiratory tract). These changes are considered local effects. Substances causing local effects after single exposure can be further distinguished as irritant or corrosive substances, depending on the magnitude and (ir)reversibility of the effects observed. A further distinction can be made between effects observed after single and after repeated or prolonged exposure.

This section concerns local effects after single ocular, dermal or inhalation exposure. For the assessment of local effects after repeated or prolonged exposure, see Section 4.4.2.

The elements described in this section should be considered together with other guidance presented in Table 11.

Table 11: Guidance to be considered together with the current guidance

Effect	Guidance	Section
Skin corrosion or irritation	*ECHA Guidance Vol III Part A*	1.1. Skin corrosion or irritation
	REACH Guidance R.7a	R.7.2.6 Testing and assessment strategy for skin corrosion/irritation
	CLP Guidance	3.2. Skin corrosion/irritation
	OECD GD 203	
Serious eye damage or eye irritation	*ECHA Guidance Vol III Part A*	1.2. Serious eye damage or eye irritation
	REACH Guidance R.7a	R.7.2.7 Information requirements for serious eye damage/eye irritation
	CLP Guidance	3.3. Serious eye damage/eye irritation
	OECD GD 263	

The general objectives in the assessment of skin corrosion/irritation are to find out:

- whether the substance is (or is likely to be) corrosive;
- whether there is evidence of significant skin, eye or respiratory irritation in animal or *in vitro* studies;
- whether there are indications from human experience with the substance of skin, eye, mucous membrane or respiratory irritation;
- the time of onset, the extent and severity of the responses and information on reversibility.

The likelihood of an acute corrosive or irritant response of humans exposed to the substance is assessed by considering the route, pattern and extent of the expected human exposure and taking into account the severity of the effect, as far as it can be judged from the information available.

1.5.1 Definitions

Irritant substances are non-corrosive substances which may cause inflammation through contact with tissue.

Corrosive substances may destroy living tissues with which they come into contact.

The criteria for classification of irritant and corrosive substances are given in Annex I of the CLP Regulation.

The following definitions are taken from the *CLP Guidance*:

- **Dermal irritation** means the production of reversible damage to the skin following the application of a test substance for up to 4 hours.
- **Repeated exposure may cause skin dryness or cracking** is warranted for substances and mixtures which may cause concern as a result of skin dryness, flaking or cracking but which do not meet the classification criteria for skin irritancy based on either:
    - practical observations; or
    - relevant evidence concerning their predicted effects on the skin.
- **Skin corrosion** means the production of irreversible damage to the skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to 4 hours.

  Corrosive reactions are typified by ulcers, bleeding, bloody scabs, and, by the end of observation at 14 days, by discolouration due to blanching of the skin, complete areas of alopecia, and scars. Histopathology shall be considered to evaluate questionable lesions.
- **Eye irritation** means the production of changes in the eye following application of a test substance to the anterior surface of the eye, which are fully reversible within 21 days of application.
- **Serious eye damage** means the production of tissue damage in the eye, or serious physical decay of vision, following application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application.
- **Respiratory tract irritation** characterized by effects that include localized redness, oedema, pruritus and/or pain and impair function causing symptoms such as cough, pain, choking, and breathing difficulties. The evaluation is based primarily on human data, but if available, also on inhalation studies in animals after repeated exposure.

Some of the above definitions are based on animal models, while the testing requirements have shifted towards non-animal testing.

1.5.2 Mechanisms of corrosion and irritation

For the mechanisms of corrosion/irritation, see the Appendix R.7.2–1 of *REACH Guidance R.7a*, including a critical review of the mechanisms of:

- Skin corrosion and irritation
- Serious eye damage and eye irritation
- Respiratory tract corrosion and irritation

1.5.3 Identification and evaluation of data to be used in the effects assessment

The testing strategy described in *ECHA Guidance Vol III Part A* should be considered together with the following sections in the *CLP Guidance*:

- Section 3.2.2 *Classification of substances for skin corrosion/irritation*
- Section 3.3.2 *Classification of substances for serious eye damage/eye irritation*
- Section 3.8.2 *Classification of substances for STOT-SE* (for respiratory irritation).

For irritation and corrosivity, examples are available in *REACH Guidance R.7a*, Appendix R.7.2–2 and Appendix R.7.2–3; as well as R.7.2–1 and Table R.7.2–3.

Specific considerations for respiratory irritation

All available data should be evaluated to estimate the potential of a substance to induce respiratory tract irritation. Sources of information could be:

1. *Human data*

   Human data may consist of:

   - Experience from occupational exposure
   - Published data on volunteers (objective measurements, psychophysical methods, and subjective reporting)
   - Other data (e.g. from nasal lavage)

   Consideration should be given to real-life human observational experience if properly collected and documented, e.g. data from well-designed workplace surveys and worker health monitoring programmes. For substances with an array of industrial uses and abundant human evidence, the symptoms of respiratory irritation can sometimes be associated with certain concentrations of the irritants in the workplace air and might allow derivation of AECs. However, the exposure information has to be well documented, and due consideration should be given to possible confounding factors.

   Sensory irritation of the airways is described as unpleasant sensation such as pain, burning or tingling. Data on such effects may be available from volunteer studies including objective measurements of respiratory tract irritation such as electrophysiological responses, data from lateralization threshold testing, and biomarkers of inflammation in nasal or bronchoalveolar lavage fluids.

   Including anosmics as subjects could exclude odour as a bias. Good quality and relevant human data have precedence over other data. However, absence of positive findings in humans does not necessarily overrule good quality animal data that are positive.

2. *Animal data:*

   Animal data may consist of:

   - Alarie assay

     Although the Alarie test is not an OECD TG, results of the Alarie assay can be used for hazard identification of sensory irritation. Additional considerations for the

evaluation of the Alarie test are provided under *Mechanisms of respiratory tract corrosion and irritation* in Appendix R.7.2-1 of *REACH Guidance R.7a*.

- Data from other inhalation studies (acute, repeated exposure)
- Clinical symptoms of dyspnoea or breathing difficulties
- Histomorphology of the respiratory tract
- Lavage examination (nasal, bronchoalveolar)
- Data from other toxicological studies on the substance, in which local responses of respiratory system have been reported. Such studies may provide useful information particularly if it can be related to exposure levels.

Data indicating cytotoxic respiratory irritation, which could be mainly gained from histopathological examinations of tissues, should be considered in deriving reference values (see section 4.4.2 on risk characterisation for local effects).

For sensory irritation of the respiratory tract, the evidence from all sources has to be considered in qualitative or (semi-) quantitative risk assessment. Detailed guidance on sensory irritation is provided in Appendix R.7.2-1 of *REACH Guidance R.7a*.

1.5.4 Remaining uncertainty on irritation/corrosion

It is usually possible to unequivocally identify a substance as being corrosive in nature, whatever type of study provides the information. For further details and specific considerations regarding classification as corrosive, see *CLP Guidance*.

According to *ECHA Guidance Vol III Part A*, the testing and assessment strategy aims at identifying skin corrosion/irritation and serious eye damage/irritation by using all the information available. The results of one study or information source are evaluated before another study is initiated, aiming for the most efficient and humane approach and minimising animal usage and costs.

In vitro test methods have been developed on the basis of animal data that already have recognised limitations. For example, the Draize rabbit eye test has long been criticised for a) differences between species (e.g. compared to human eyes, rabbit eyes have a nictitating membrane, a thinner cornea and lack significant tearing), b) subjective scoring and c) high variability between experiments (Curren et al., 2002). For certain substances and mixtures, depending on the applicability domain of the method, full replacement of animal models by NAMs adopted by OECD is possible (*OECD GD 263*). However, the different *in vitro* methods have specific limitations as outlined e.g. for eye irritation[15]. It should be verified that the test substance is within the applicability domain of the test methods. With regard to using non-animal methods for assessing skin corrosion/irritation and eye damage/irritation in accordance with the CLP Regulation, see also chapters 3.2 and 3.3 of Revision 10 of GHS[16]. The changes to GHS are currently being incorporated into the CLP Regulation.

Data from animal studies in accordance with internationally accepted test methods will usually give good information on the skin or eye irritancy of a substance in the test species. Data of

[15] https://echa.europa.eu/documents/10162/21650280/oecd_test_guidelines_eye_irritation_en.pdf/0b39b86e-a3b5-4022-a43c-26d35bd45b75
[16] https://unece.org/transport/dangerous-goods/ghs-rev10-2023

good quality can be obtained on respiratory and mucous membrane irritation from well-designed and well-reported inhalation studies in animals, often clearly related to exposure levels.

There may be a significant level of uncertainty in human data on irritant effects. This may be due to e.g. poor reporting, lack of specific information on exposure, subjective or anecdotal reporting of effects, and small numbers of subjects. The uncertainties linked to the information and/or methods used should be considered in the WoE when deciding on the hazard properties of the substance.

1.5.5 Concluding on suitability for Classification and Labelling

In concluding on classification and labelling, all the available information needs to be considered. The *CLP Guidance* should be followed. If the data available is not sufficient, additional testing may be required as described in the *ECHA Guidance Vol III Part A*.

1.5.6 Concluding on suitability for risk assessment

Assessing dose-response is challenging for irritation and corrosion because most of the data have been produced with undiluted chemicals in accordance with test guidelines. From a risk characterisation perspective, it is therefore advisable to generally rely on the classification: a substance that is classified is assumed to be sufficiently characterised. For substances that are not classified, special attention should be paid to effects occurring after repeated or prolonged exposure (see Section 4.4.2).

A quantitative or semi-quantitative risk assessment requires information on both hazard and dose-response. If dose-response information is available, it should be taken into account. For instance, dose-response information might be available from sub-acute or repeated dose dermal/inhalation toxicity studies as well as from human experience.

For respiratory irritation, special consideration is needed whether dose-response information in animal tests can be extrapolated to humans.

1.6 Sensitisation

This section provides brief guidance for the assessment of sensitisation, and it should be considered together with Section 4.4.2 and the guidance listed in Table 12.

Table 12: Guidance to be considered together with the current guidance

Guidance	Section
ECHA Guidance Vol III Part A	1.3. Skin sensitisation
REACH Guidance R.7a	R.7.3 Skin and respiratory sensitisation
CLP Guidance	3.4. Respiratory or skin sensitisation
OECD 497	

1.6.1 Definitions and mechanisms of skin and respiratory sensitisation

A number of diseases are recognised as being, or presumed to be, allergic in nature. These include asthma, rhinitis, conjunctivitis, allergic contact dermatitis, urticaria and food allergies. The endpoints discussed in this Section are those traditionally associated with occupational and consumer exposure. Photosensitisation is potentially important but is not discussed in detail because its mechanism of action is poorly understood.

A sensitiser is an agent that can cause an allergic response in susceptible individuals. As a consequence, following subsequent exposure via the skin or by inhalation, the characteristic adverse health effects of allergic contact dermatitis or asthma (and related respiratory symptoms such as rhinitis) may be provoked.

According to the definitions in the *CLP Guidance*:

- **Respiratory sensitiser** means a substance that will lead to hypersensitivity of the airways following inhalation of the substance.
- **Skin sensitiser** means a substance that will lead to an allergic response following skin contact.

Asthma and rhinitis are generally thought to be a result of an allergic reaction; however, other non-immunological mechanisms may occur, making it more appropriate to use a term based on disease rather than mechanism. Respiratory hypersensitivity is a term that is used to describe asthma and other related respiratory conditions, irrespective of the mechanism causing them. When directly considering human data in this document, the clinical diagnostic terms asthma, rhinitis and alveolitis have been retained. In this guidance, the term skin sensitisation specifies an allergic mechanism of action, while respiratory hypersensitivity does not.

The first phase of the pathogenesis of skin and respiratory allergy is the sensitisation phase. In this phase, upon first contact with an allergen, induction of specialised immunological memory in an individual takes place. The second phase is elicitation, i.e. production of a cell-mediated or antibody mediated allergic response by exposure of a sensitised individual to an allergen.

For both skin and respiratory sensitisation, lower exposure levels are usually necessary for elicitation than for induction.

For skin sensitisation, an induction phase is required in which the immune system learns to react; clinical symptoms can then arise when subsequent exposure is sufficient to elicit a visible skin reaction (elicitation phase). Predictive tests usually follow this pattern in which there is an induction phase, the response to which is measured by a standardised elicitation phase, typically involving a patch test. The local lymph node assay is the exception, directly measuring the induction response.

In assessing the sensitising potential of a substance, the general objectives are to find out whether there are indications from human experience of skin allergy or respiratory hypersensitivity following exposure to the agent, and whether the agent has skin sensitisation potential based on tests in animals.

The likelihood that an agent will induce skin sensitisation or respiratory hypersensitivity in humans is determined by several factors including the route, duration and magnitude of exposure and the potency of the substance.

For further information on the sensitisation mechanisms, see the following sections of *REACH Guidance R.7a*:

- R.7.3.2 Mechanisms of skin sensitisation
- R.7.3.8 Mechanisms of respiratory sensitisation

Software tools that may be used to predict skin sensitisation properties are provided in *REACH Guidance R.7a*, Table R.7.3-1 for skin sensitisation.

1.6.2 Identification and evaluation of data to be used in the effects assessment

The testing strategy described in *ECHA Guidance Vol III Part A* should be considered together with the following sections in the *CLP Guidance*:

- Section 3.4.2.1 Classification of substances for respiratory sensitisation
- Section 3.4.2.2 Classification of substances for skin sensitisation

In these sections, guidance is included on the assessment of WoE and potency of skin and respiratory sensitisation.

1.6.3 Remaining uncertainty on sensitisation

The following situations may increase the uncertainty in the assessment of sensitisation:

- The Local Lymph Node Assay (LLNA) can occasionally give false positive results with irritants. For irritating substances, consideration on ear thickness is necessary as explained in the OECD TG 429.
- An existing guinea pig maximisation test (GPMT) may have included the use of adjuvant that may have lowered the threshold for irritation and lead to false positive reactions. Helpful information could in this case come if a pre-test was performed with Freund's Complete Adjuvant.
- An existing Buehler test should be considered less sensitive than GPMT and therefore may provide false negative results, especially for low potency sensitisers.
- Due to the individually described test designs in the guinea pig test (GPMT/Buehler), it is often not possible to conclude whether the substance is a strong/extreme (i.e. Cat 1A of CLP) skin sensitiser or not.
- Careful consideration should be given to circumstances where exposure may be sub-optimal, which could be due to difficulties in achieving a good solution and/or a solution of sufficient concentration.
- For existing human data, consideration must be given to interindividual variability and whether it is scientifically sound to generalise from a limited test panel.
- Substances inducing symptoms of asthma by irritation only in people with bronchial hyperreactivity should not be considered respiratory sensitisers.
- For *in chemico/in vitro* methods, substance specific limitations need to be considered as described in OECD TGs 442C to E and *OECD 497*.

1.6.4 Additional considerations

Chemical allergy is commonly designated as being associated with skin sensitisation (allergic contact dermatitis), or with sensitisation of the respiratory tract (asthma and rhinitis). In view of this it is sometimes assumed that allergic sensitisation of the respiratory tract will result only

from inhalation exposure to the causative chemical, and that skin sensitisation necessarily results only from dermal exposure. This is misleading, and it is important for the purposes of risk management to acknowledge that sensitisation may be acquired by other routes of exposure.

Since adaptive immune responses are essentially systemic in nature, sensitisation of skin surfaces may theoretically develop from encounter with contact allergens via routes of exposure other than dermal contact, although in practice this appears to be uncommon. Similarly, there is evidence from both experimental and human studies indicating that effective sensitisation of the respiratory tract can result from dermal contact.

Effective prevention of respiratory sensitisation therefore requires protection of both skin and respiratory tracts. This includes the cautious use of known contact allergens in products to which consumers are (or may be) exposed via inhalation, such as sprays.

Overall, minimising the risk of sensitisation to chemical allergens will require protection for all relevant routes of exposure.

For skin and respiratory sensitisers, the risk assessment is qualitative and is based on the classification of substances and products (see section 4.4.2).

1.6.5 Concluding on suitability for Classification and Labelling

In concluding on classification and labelling, all the available information needs to be considered. The *CLP Guidance* should be followed. If the data available is not sufficient, additional testing may be required as described in the *ECHA Guidance Vol III Part A*.

1.7 Repeated dose toxicity

The *ECHA Guidance Vol III Part A* should be considered together with the elements described in this section for the assessment of repeated dose toxicity. Information from experimental and non-test approaches with regard to other endpoints (e.g. TK, genotoxicity) should be assessed in a WoE approach in the assessment of toxicological findings following repeated dose administration; the ultimate goal is to identify the potential mode of action and underlying key events.

Table 13: Guidance to be considered together with the current guidance

Guidance	Section
ECHA Guidance Vol III Part A	1.9 Repeated dose toxicity
REACH Guidance R.7a	R.7.5 Repeated dose toxicity
CLP Guidance	

1.7.1 Definition of repeated dose toxicity

The aim of repeated dose toxicity studies is to characterise the toxicological profile of a chemical following repeated daily administration for different periods of the expected lifespan (sub-acute, sub-chronic or chronic exposure). This includes identification of potential target organs of toxicity, dose-response relationships and potential reversibility of toxic effects. Repeated dose toxicity studies also examine parameters that have the potential to identify specific

manifestations of toxicity such as neurotoxicity, immunotoxicity, endocrine mediated effects, reproductive toxicity and carcinogenicity. For further guidance on such effects, please refer to the corresponding sections within this Guidance.

For further insight on mechanistically informative endpoints that can help in interpreting and evaluating toxicological parameters used in animal studies, in particular organ-specific key characteristics of the liver, kidney, lung and cardiovascular system, see (Jennings et al., 2023).

The term general toxicological effects (often referred to as *general toxicity*) includes effects on:

- body weight and/or body weight gain,
- absolute and/or relative organ and tissue weights,
- alterations in clinical chemistry,
- urinalysis and/or haematological parameters,
- functional disturbances in organs and tissues in general
- functional disturbances in the nervous or hormonal system
- pathological alterations in organs and tissues as examined macroscopically and microscopically.

An adverse effect is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system, or (sub) population that results in an impairment of functional capacity, or an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences.

A chemical substance may induce systemic and/or local effects. A local effect is an effect that is observed at the site of first contact, caused irrespective of whether a substance is systemically available. A systemic effect is an effect that is normally observed distant from the site of first contact, i.e., after having passed through a physiological barrier (mucous membrane of the GI tract or of the respiratory tract, or the skin) and becomes systemically available.

It should be noted that toxic effects on surface epithelia may reflect indirect or secondary effects taking place due to systemic distribution of the substance or its metabolite(s).

Repeated dose toxicity tests provide information on possible adverse effects likely to arise from repeated exposure of target organs, and on dose-response relationships. The determination of the dose-response relationship should allow setting a NOAEL or BMD. If this is not possible, a LOAEL should be set. As part of the risk assessment, data on the adverse effects and the dose levels at which the effects occur are evaluated.

The objectives of assessing repeated dose toxicity are to evaluate:

- whether exposure of humans has been associated with adverse toxicological effects occurring as a result of repeated daily exposure; these human studies may also potentially identify populations that have higher susceptibility;
- whether administration of a substance to experimental animals causes adverse toxicological effects as a result of repeated daily exposure; effects that are predictive of possible adverse human health effects;
- the target organs, effects occurring over time including effects due to accumulation and the reversibility of the adverse toxicological effects;

- the dose-response relationship and threshold for any of the adverse toxicological effects observed in the repeated dose toxicity studies;
- the basis for hazard characterisation and classification and labelling of substances for repeated dose toxicity.

1.7.2 Data to be used in the effects assessment

1.7.2.1 Non-human data for repeated dose toxicity

1.7.2.1.1. Non-testing data for repeated dose toxicity

(a) Physico-chemical data

The physico-chemical properties of a chemical substance are essential elements in deciding on the appropriate administration route to be applied in experimental *in vivo* repeated dose toxicity studies (see also Section 1.3). The physico-chemical properties of a substance can indicate whether it is likely that the substance can be absorbed following exposure to a particular route and whether it, or an active metabolite, is likely to reach the target organs and tissues.

The physico-chemical properties are also important in judging whether testing is technically possible. Testing for repeated dose toxicity may be omitted if it is technically not possible to conduct the study because of the properties of the substance, for example being very volatile, highly reactive or unstable, or mixing of the substance with water may cause danger of fire or explosion.

(b) Read-across

Regarding the assessment of read-across, see Section 1.2.2.4.3.

Regarding the use of read-across for repeated dose toxicity, there are no formal criteria to identify structural alerts for repeated dose toxicity or for read-across to closely related substances.

(c) (Q)SAR systems

A (Q)SAR analysis for a substance may give indications of a specific mechanism occurring and identify possible organ or systemic toxicity upon repeated exposure.

Overall, (Q)SAR approaches are currently not well validated for repeated dose toxicity and no firm recommendations can be made concerning their routine use in a testing strategy in this area. There are a large number of potential targets/mechanisms associated with repeated dose toxicity that currently cannot be adequately covered by a battery of (Q)SAR models. A negative result from (Q)SAR models without other supporting evidence does not demonstrate a lack of a toxicological hazard or a need for hazard classification.

Another limitation of (Q)SAR modelling is that dose-response information, including NOAEL, is not provided. Similarly, a validated (Q)SAR model might identify a potential toxicological hazard, but because of limited confidence, such a result would not be adequate to support hazard classification.

In some cases, (Q)SAR models could be used as part of a WoE approach, when considered alongside other data, provided the applicability domain is appropriate. Also, (Q)SARs can be used as supporting evidence when assessing the toxicological properties by read-across within a substance grouping approach, provided that the applicability domain is appropriate. Positive and negative (Q)SAR modelling results can be of value in a read-across assessment and for classification purposes.

1.7.2.1.2. Testing data for repeated dose toxicity – in vitro

Available *in vitro* data alone is currently not useful for regulatory decisions such as risk assessment and C&L for repeated dose toxicity. However, such data may be helpful in the assessment, for instance to detect local target organ effects and/or to clarify the mechanisms of action. The quality of each of these studies and the adequacy of the data provided should be carefully evaluated.

For detailed information on the use and assessment of *in vitro* studies, see *OECD GD 286*. Mechanistic information can be obtained and interpreted by AOPs that explicitly capture the linkage of measurable, biological effects (key events) to an adverse outcome (AO). See AOP wiki for potentially relevant AOPs for different organs (https://aopwiki.org/).

1.7.2.1.3. Testing data for repeated dose toxicity – animal data

The most appropriate data on repeated dose toxicity for use in hazard characterisation and risk assessment are obtained from experimental animal studies conforming to internationally agreed test guidelines. In some circumstances repeated dose toxicity studies not conforming to conventional test guidelines may also provide relevant information.

The information that can be obtained from the available OECD test guideline studies for repeated dose toxicity is briefly summarised below.

a) Repeated dose 28-day toxicity studies:

Separate guidelines are available for studies using:

- oral administration (EU B.7/OECD TG 407);
- dermal application (EU B.9/OECD TG 410); and
- inhalation (EU B.8/OECD TG 412).

Apart from the standard parameters investigated in these protocols, additional parameters are also recommended to enable the identification of a neurotoxic potential, immunological effects or reproductive organ toxicity. If interim euthanasia are planned, the number of animals should be increased accordingly. Additional animals may also be considered if the 28-day study is intended as a definite study and not only as a range-finding study. Consideration should be given to an additional satellite group in the control and in the top dose groups for observation of reversibility, persistence, or delayed occurrence of toxic effects, for at least 14 days post treatment.

b) Repeated dose 90-day toxicity studies:

Separate guidelines are available:

- OECD TG 408/EU B.26 (oral administration in rodent);

- OECD TG 409/EU B.27 (oral administration in non-rodent species);
- OECD TG 411/EU B.28 (dermal application); and
- OECD TG 413/EU B.29 (inhalation).

The test guidelines recommend additional optional investigations, such as toxicokinetics and/or systemic toxicity evaluations (e.g., immune, hepatic, neurologic and/or cardiovascular effects evaluations) to better characterise the overall toxicity.

The 90-day studies provide information on the general toxicological effects arising from subchronic exposure covering post-weaning maturation and growth well into adulthood, on target organs and on potential accumulation of the substance.

c) Chronic toxicity studies:

Chronic toxicity studies (OECD TG 452/EU B.30) provide information on the toxicological effects arising from repeated exposure over a prolonged period of time covering the major part of the animal's life span. The duration of the chronic toxicity studies should be at least 12 months.

Combined chronic toxicity/carcinogenicity studies (OECD TG 453/EU B.33) include an additional high-dose satellite group for evaluation of pathology other than neoplasia. The satellite group should be exposed for at least 12 months and the animals in the carcinogenicity part of the study should be retained in the study for the majority of the normal life span of the animals.

Ideally, chronic studies should allow for the detection of general toxicity effects (physiological, biochemical and haematological effects etc.) but could also inform on neurotoxic, immunotoxic, reproductive and carcinogenic effects of the substance. However, in 12-month studies, non-specific life shortening effects that require a long latent period or are cumulative may possibly not be detected. The combined study will allow for detection of neoplastic effects and determination of carcinogenic potential and life-shortening effects.

d) Combined repeated dose toxicity study with the reproduction / developmental toxicity screening test:

The combined repeated dose toxicity / reproductive screening study (OECD TG 422) provides information on the toxicological effects arising from repeated exposure (generally oral exposure) over a period of about 6 weeks for males and approximately 54 days for females (a relatively limited period of the animal's life span) as well as on reproductive toxicity. For repeated dose toxicity, the OECD TG 422 is in concordance with the OECD TG 407/EU B.7 except for use of pregnant females and longer exposure duration in the OECD TG 422 compared to the OECD TG 407/EU B.7.

e) Neurotoxicity studies:

The neurotoxicity study in rodents (OECD TG 424/EU B.43) has been designed to further characterise potential neurotoxicity observed in repeated dose systemic toxicity studies. It will provide detailed information on major neuro-behavioural and neuro-pathological effects in adult rodents. Findings in the DNT study (OECD TG 426) can also provide relevant information for the characterisation of neurotoxic effects.

f) Delayed neurotoxicity studies of organophosphorus substances:

The delayed neurotoxicity study (OECD TG 419/EU Annex B.38) is specifically designed to be used in the assessment and evaluation of the neurotoxic effects of organophosphorus

substances. This study provides information on delayed neurotoxicity arising from repeated exposure over a relatively limited period of the animal's life span.

g) Other studies providing information on repeated dose toxicity:

Consideration of *in vitro* data as well as TK data is essential during the evaluation of the repeated dose toxicity information as they can assist in the correct derivation of internal exposure values, the correct application of AFs in deriving threshold levels and in the design of new tests if the data is not sufficient.

Although not aiming at investigating repeated dose toxicity per se, other available OECD/EU test guideline studies involving repeated exposure of experimental animals may provide useful information on repeated dose toxicity.

The one-generation, two-generation or extended one generation reproductive toxicity studies (OECD TG 415/416/443; EU B.34/B.35) may provide information on the general toxicological effects arising from repeated exposure over a prolonged period of time (about 90 days for parental animals) as clinical signs of toxicity, body weight, selected organ weights, and gross and microscopic changes of selected organs are recorded.

The prenatal developmental toxicity study (OECD TG 414/EU B.31), the reproduction/developmental toxicity screening study (OECD TG 421) and the developmental neurotoxicity study (OECD TG 426) may give indications of general toxicological effects arising from repeated exposure over a relatively limited period of the animals' life span as clinical signs of toxicity and body weight are recorded.

The carcinogenicity study (OECD TG 451/EU B.32) will, in addition to information on neoplastic lesions, provide information on the general toxicological effects arising from repeated exposure over a major portion of the animal's life span as clinical signs of toxicity, body weight, and gross and microscopic changes of organs and tissues are recorded.

In addition, other studies performed in experimental animals may provide useful information on repeated dose toxicity.

1.7.2.1.4. General guidance for evaluating repeated dose toxicity data and weight of evidence

The following general suggestions are made for considering the weight of studies:

- Studies on the most sensitive animal species have a greater weight, unless toxicokinetic and toxicodynamic data show that this species is less relevant for human risk assessment.
- Studies using an appropriate route, duration and frequency of exposure in relation to the expected routes, frequency and duration of human exposure have greater weight.
- Studies enabling the identification of NOAEL and robust hazard identification have a greater weight.
- Studies of a longer duration have generally greater weight. However, studies of different durations are used to derive separate AEL values for different time frames.

The information is sufficient when, based on a WoE analysis, the critical effects and target organs and tissues can be identified, the dose-response relationships and NOAELs/LOAELs for the critical effects can be established, and the relevance for humans can be assessed.

Where the existing data as a whole is deemed inadequate to provide a clear assessment, further testing should be considered in view of all available information on the substance, including physico-chemical properties and structural alerts, as well as the use pattern and the potential for human exposure.

Potential effects in certain target organs (e.g. thyroid) following repeated exposure may not be observed within the span of the 28-day study.

The protocols for the oral 28-day and 90-day studies include additional parameters (e.g. determination of thyroid hormones, examination of muscle and bone) compared to those for the 28-day and 90-day dermal and inhalation protocols.

Oral 28-day and 90-day toxicity studies include endpoints capable of detecting effects on neurotoxicity and immunotoxicity. Indicators of neurotoxicity include clinical observations, a functional observational battery, motor activity assessment and histopathological examination of spinal cord and sciatic nerve. Indicators of immunotoxicity include changes in haematological parameters, serum globulin levels, alterations in immune system organ weights such as spleen and thymus, and histopathological changes in immune organs such as spleen, thymus, lymph nodes and bone marrow. Where data from oral 28-day and 90-day studies identify evidence of neurotoxicity or immunotoxicity, other studies may be necessary to further investigate the effects. It should be noted that endpoints capable of detecting neurotoxicity and immunotoxicity are not examined in the standard 28-day and 90-day dermal or inhalation repeated dose toxicity studies.

In general, results from toxicological studies requiring repeated administration of a test substance can contribute to the assessment of repeated dose toxicity, e.g. studies on reproduction, developmental toxicity and carcinogenicity. However, such studies rarely provide the information obtained from a standard repeated dose toxicity study and are not sufficient for addressing repeated dose toxicity.

Studies on acute toxicity, irritation and *in vivo* genotoxicity contribute limited information to the overall assessment of the repeated dose toxicity but may be useful in deciding on the dose levels for repeated dose toxicity testing.

Guidance on the dose selection for repeated dose toxicity testing is provided in detail in the EU and OECD test guidelines. Unless limited by the physico-chemical nature or biological effects of the test substance, the highest dose level should be chosen with the aim to induce toxicity but not death or severe suffering. Further guidance on dose level selection is available in Advice on dose-level selection for the conduct of sub-acute and sub-chronic assays under REACH[17].

Toxicokinetic studies may be helpful in the evaluation and interpretation of repeated dose toxicity data, for example in relation to accumulation of a substance or its metabolites in certain tissues or organs as well as in relation to mechanistic aspects of repeated dose toxicity and species differences. Toxicokinetic information can also assist in the selection of the dose levels. When conducting repeated dose toxicity studies, it is necessary to ensure that the observed toxicity is not associated with the administration of excessively high doses causing saturation of absorption and detoxification mechanisms. Results obtained with excessive doses causing saturation of metabolism are often of limited value in defining the risk posed at more relevant and realistic exposures where a substance can be readily metabolised and cleared from the body.

[17] https://echa.europa.eu/documents/10162/17220/211221_echa_advice_dose_rdt_en.pdf

Table 14: List of *in vivo* repeated dose toxicity test guideline studies

Duration	Test guideline	Route	Name
Subacute	OECD TG 407 EU B.7	Oral	Repeated dose 28-day oral toxicity study in rodents
	OECD TG 410 EU B.9	Dermal	Repeated dose dermal toxicity: 21/28-day study
	OECD TG 412 EU B.8	Inhalation	Repeated dose inhalation toxicity: 28-day study
Subchronic	OECD TG 408 EU B.26	Oral	Repeated dose 90-day oral toxicity study in rodents
	OECD TG 409 EU B.27	Oral	Repeated dose 90-day oral toxicity study in non-rodents
	OECD TG 411 EU B.28	Dermal	Subchronic dermal toxicity: 90-day study
	OECD TG 413 EU B.29	Inhalation	Subchronic inhalation toxicity: 90-day study
Long-term	OECD TG 452 EU B.30	Oral, dermal or inhalation	Chronic toxicity studies
	OECD TG 453 EU B.30	Oral, dermal or inhalation	Combined chronic toxicity/carcinogenicity Studies

Table 15: Overview of other *in vivo* test guideline studies giving information on repeated dose toxicity

Test	Design	Endpoints (general toxicity)
OECD TG 424 Neurotoxicity study in rodents	Exposure for at least 28 days Dose levels: not specified At least 10 males and females per group Preferred rodent species: rat Generally oral route of administration	Detailed clinical observations Functional observations (sensory reactivity to stimuli of different types, grip strength, motor activity, more specialised tests on indication) Ophthalmological examination Body weight and food/water consumption Haematology (haematocrit, haemoglobin, erythrocyte count, total and differential leukocyte count, platelet count, blood clotting time/potential) Clinical biochemistry Histopathology: at least 5 animals/sex/group) for neuropathological examinations (brain, spinal cord, and peripheral nerves); remaining animals to be used either for specific neurobehavioural, neuropathological, neurochemical or electrophysiological procedures that may supplement the histopathology or alternatively, for routine pathological evaluations according to the guidelines for standard repeated dose toxicity studies
OECD TG 419 (EU B.38)	Exposure for 28 days	Detailed clinical observations Body weight and food/water consumption

Test	Design	Endpoints (general toxicity)
Delayed neurotoxicity of organophosphorus substances: 28-day repeated dose study	At least 3 dose levels plus control At least 12 birds per group Species: domestic laying hen	Clinical biochemistry (NTE activity, acetylcholinesterase activity Gross necropsy (all animals) Histopathology (neural tissue)
OECD TG 416 (EU B.35) Two-generation reproduction toxicity study	Exposure before mating for at least one spermatogenic cycle until weaning of 2nd generation At least 3 dose levels plus control At least 20 parental males and females per group	Clinical observations Body weight and food/water consumption Gross necropsy (all parental animals) Organ weights (reproductive organs, brain, liver, kidneys, spleen, pituitary, thyroid, adrenal glands, and known target organs) Histopathology (reproductive organs, previously identified target organ(s) - at least control and high-dose groups
OECD TG 415 (EU B.34) One-generation reproduction toxicity study	Exposure before mating for at least one spermatogenic cycle until weaning of 1st generation At least 3 dose levels plus control At least 20 parental males and females per group	As in OECD TG 416
OECD TG 443 Extended one generation reproductive toxicity study	As described in OECD TG 443	Clinical observations Body weight and food/water consumption Clinical chemistry Haematology Thyroid hormones (T4 and TSH) Clinical biochemistry Urinalysis Sperm parameters Gross necropsy (adults) Splenic lymphocyte subpopulation analysis Organ weights Histopathology (Specific investigation on developmental neurotoxicity and in cases of a particular concern, developmental immunotoxicity)
OECD TG 414 (EU B.31)	Exposure at least from implantation to one or two days before expected birth	Clinical observations Body weight and food/water consumption

Test	Design	Endpoints (general toxicity)
Prenatal developmental toxicity study	At least 3 dose levels plus control At least 20 pregnant females per group	Macroscopical examination of all dams for any structural abnormalities or pathological changes, which may have influenced the pregnancy
OECD TG 421 Reproduction/ developmental toxicity screening test	Exposure from 2 weeks prior to mating until at least post-natal day 4 At least 3 dose levels plus control At least 8-10 parental males and females per group	Clinical observations Body weight and food/water consumption Gross necropsy (adult animals, special attention to reproductive organs) Organ weights (all adult males: testes, epididymides) Histopathology (reproductive organs in at least control and high-dose groups)
OECD TG 422 Combined repeated dose toxicity study with the reproduction/devel opmental toxicity screening test	Exposure for a minimum of 4 weeks (males) or from 2 weeks prior to mating until at least post-natal day 4 (females – at least 6 weeks of exposure) At least 3 dose levels plus control At least 10 males and females per group	Clinical observations as in OECD TG 407 Functional observations as in OECD TG 407 Body weight and food/water consumption Haematology as in OECD TG 407 Clinical biochemistry Urinalysis (optional) Gross necropsy (full, detailed, all adult animals) Organ weights (testes and epididymides - all males; liver, kidneys, adrenals, thymus, spleen, brain, heart - in 5 animals of each sex per group, i.e. as in OECD TG 407) Histopathology (ovaries, testes, epididymides, accessory sex organs, all gross lesions - all animals in at least control and high-dose groups; brain, spinal cord, stomach, small and large intestines, liver, kidneys, adrenals, spleen, heart, thymus, thyroid, trachea and lungs, urinary bladder, lymph nodes, peripheral nerve, a section of bone marrow - in 5 animals of each sex in at least control and high-dose groups, i.e. as in OECD TG 407)
OECD TG 426 Developmental neurotoxicity study (draft)	Exposure at least from implantation throughout lactation (PND 20) At least 3 dose levels plus control At least 20 pregnant females per group	Clinical observations Body weight and food/water consumption
OECD TG 451 (EU B.32) Carcinogenicity studies	Exposure for majority of normal life span At least 3 dose levels plus control At least 50 males and females per group	Clinical observations (special attention to tumour development) Body weight and food consumption Gross necropsy Histopathology (all groups - all grossly visible tumours or lesions suspected of being tumours; at least control and

Test	Design	Endpoints (general toxicity)
		high-dose groups - brain, pituitary, thyroid, parathyroid, thymus, lungs, heart, salivary glands, liver, spleen, kidneys, adrenals, oesophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, uterus, urinary bladder, lymph nodes, pancreas, gonads, accessory sex organs, female mammary gland, skin, musculature, peripheral nerve, spinal cord, sternum with bone marrow and femur, eyes)

1.7.2.2 Human data for repeated dose toxicity

Human data adequate to serve as the sole basis for the hazard and dose-response assessment are rare. When available, reliable and relevant human data are preferable over animal data and can contribute to the overall WoE. Human volunteer studies shall not be performed for the purposes of the BPR.

The following types of human data may be available:

- Analytical epidemiology studies on exposed populations may be useful for identifying a relationship between human exposure and effects such as biological effect markers, early signs of chronic effects, disease occurrence, or long-term specific mortality risks. Study designs include case control studies, cohort studies and cross-sectional studies.
- Descriptive or correlation epidemiology studies examine differences in disease rates among human populations in relation to age, gender, race, and differences in temporal or environmental conditions. These studies do not normally provide dose-response information.
- Case reports describe a particular effect in an individual or a group of individuals exposed to a substance. Generally case reports are of limited value for hazard identification, especially if the exposure represents single exposures, abuse or misuse of substances. They can however provide insight for the assessment of human relevance of effects observed in animal studies.
- Existing controlled studies in human volunteers performed in accordance with internationally accepted ethical standards, including low exposure toxicokinetic studies, may be of use in risk assessment.
- Meta-analysis combines and analyses data from multiple studies in one overall assessment of the relative risk or dose-response curve.

Human epidemiological studies or case reports can contribute to the hazard identification as well as to the risk assessment. Criteria for assessing the adequacy of epidemiology studies include an adequate research design, proper selection and characterisation of the exposed and control groups, adequate characterisation of exposure, sufficient length of follow-up for the effect to develop, valid ascertainment of effect, proper consideration of bias and confounding factors, proper statistical analysis and a reasonable statistical power to detect an effect.

The results from human experimental studies are often limited by a number of factors, such as a relatively small number of subjects, short duration of exposure, and low dose levels resulting in poor sensitivity in detecting effects.

In hazard identification, the relative lack of sensitivity of human data may cause particular difficulty. Therefore, negative human data cannot be used to override positive findings in animals, unless the mode of action observed in animals is not relevant for humans.

1.7.3 Neurotoxicity, immunotoxicity and pulmonary fibrosis

1.7.3.1 General aspects

For some specific system/organ effects the OECD/EU test methods may not provide adequate characterisation of the toxicity. There may be indications of such effects in the standard studies for systemic toxicity, or from SAR. For adequate characterisation of the toxicity and the risk to human health, it may be necessary to conduct studies using other published test methods, "in-house" methods or specially designed tests.

Some specific investigation of organ/systemic toxicity (e.g. hepatotoxicity and nephrotoxicity) is undertaken as part of the OECD/EU repeated dose toxicity tests. Specific investigation of any organ/system toxicity (e.g. kidney, cardiac, adrenal, thyroid) may be necessary and should be addressed on a case-by-case basis. Guidance on specific investigation of neurotoxicity and immunotoxicity forms a part of this testing strategy.

1.7.3.2 Neurotoxicity

1.7.3.2.1 Definition of neurotoxicity

Neurotoxicity (or a neurotoxic effect) is an adverse change in the structure or function of the nervous system that results from exposure to a chemical. Irrespective of whether the adverse change in the structure or function of the nervous system is the consequence of direct or indirect chemical insult (i.e. the molecular initiating event of the neurotoxic mode of action/mechanism is respectively inside or outside nervous system), the effect should be considered as a neurotoxic effect.

1.7.3.2.2 Introduction

Neurotoxicity (which is assessed separately from developmental neurotoxicity (DNT), which is addressed under reproductive toxicity) is ADS, which may be triggered by observations from e.g. the standard 28-day and 90-day tests, in vitro data, effects of structurally related substances or for substance classes with an alert for neurotoxicity. Only where concerns arise, additional investigations such as OECD TG 424, OECD TG 418 or OECD TG 419 are required. Substance classes with an alert for neurotoxicity may include organic solvents (e.g. for chronic toxic encephalopathy); organophosphorus compounds (for delayed neurotoxicity), carbamates (for cholinergic effects), pyrethroids (sodium channel inhibitors) and neonicotinoids (disruptors of neural transmission).

Where data acquired from the standard systemic toxicity tests are inadequate or provide indications of neurotoxicity which are not adequate for risk characterisation, guidance on the appropriate testing strategy is provided in the *ECHA Guidance Vol III Part A*.

If additional studies are to be performed to investigate neurotoxicity further, these studies should provide sufficient information to assess the neurotoxic potential of the active substance after both single and repeated exposure. In addition, the data should also be considered for classification and labelling in accordance with CLP. Note that under CLP, neurotoxicity (i.e. neurotoxicity observed following exposure after sexual maturation) is currently divided into neurotoxicity after single and repeated exposure and addressed under hazard class of specific target organ toxicity-single exposure (STOT SE) and specific target organ toxicity- repeated

exposure (STOT RE), respectively. If neurotoxicity is caused by endocrine activity, it is also relevant for the identification of endocrine disruption. More guidance on classification aspects is given in *CLP Guidance*.

1.7.3.2.3 Assessment of available information

In many cases, no dedicated and sensitive neurobehavioral tests have been performed in standard acute and repeated dose studies, or at least not initially. Consequently, neurotoxicity hazard identification (including STOT SE/RE classification) may rely solely on clinical observations and/or neurohistopathological findings, in some cases supplemented by mechanistic data. In acute toxicity studies where high doses are administered, clinical signs are often observed which are suggestive of effects on the nervous system (e.g. observations of lethargy, postural or behavioural changes). However, these signs of neurotoxicity in standard acute or repeated dose toxicity tests may be primary neurotoxic effects or secondary to other organ toxicity.

For classification under CLP, attempts have to be made to determine the primary target organ of toxicity. The data shall be carefully evaluated secondary target organ effects should not be included when it is clear that neurotoxicity is solely secondary to other target organ toxicity (i.e. not a co-occurring primary effect) and the identification of the secondary target organ does not have an added value for human health protection. An adverse neurotoxic effect secondary to an effect in another organ that is not in itself considered adverse or does not lead e.g. to classification of that organ should be considered as a neurotoxic effect and lead to classification if fulfilling the criteria. More guidance on classification aspects (including target organ toxicity at lethal doses) is given in *CLP Guidance*.

As discrete neural structures and networks have been associated with many specific neural functions, several dedicated test methods have been developed for different neural functions (see also *OECD GD 20*). Therefore, lack of response in one neurobehavioral test does not reduce the concern on effect seen by other neurobehavioral test if they investigate different neural functions mediated via different neural pathways. In addition, performance impairment detected in a neurobehavioural test may not be reflected in the outcome of brain weight measurements, brain pathology or brain morphometry, and vice versa. Neurobehavioural effects may reflect e.g. effects on specific ion channels or neurotransmitters affecting nerve cell communication and such effects are not observable via standard histopathological staining procedures or morphometry. However, if the behaviour of the animal is affected solely due to discomfort from physical effects such as a distended or blocked GI tract, the effect is not neurotoxic.

Nervous system stimulants are substances that increase the activity of the nervous system. With low nervous system activation, stimulants produce an increase in wakefulness, attention and vigilance. As the neural activation increases, hyperlocomotion, mania and euphoria are typical effects. When the neuronal stimulation is increased further, deficits in cognition and disturbed thinking are usually observed followed by agitation, confusion, and psychosis and subsequently by convulsions, coma, circulatory collapse, and ultimately death, depending on the magnitude of nervous system activation.

Nervous system depressants are substances that decrease the activity of the nervous system. The effect depends on the magnitude of nervous system depression and can vary from reduction of anxiety to drowsiness, further to anaesthesia, depression of respiratory and vasomotor centres in medulla, coma and ultimately death. Depending on the steepness of the dose-response curve of the neurotoxic compound and the sensitivity of the neurotoxicity test methods available, the neurotoxic effects may be detected only at doses that are lethal.

When the identification of neurotoxicity relies on clinical observations, the neurobehavioural effects must be rather obvious to be detectable by visual inspection. For example, effects related to low or medium nervous system stimulation or depression may not be detected without a specific sensitive and dedicated test (e.g. automated activity recording apparatus). When the only visually detectable neurotoxic effects are e.g. coma or convulsions close to lethal doses, they may incorrectly not be considered as signs of neurotoxicity even though it would not have been shown that nervous system is not the primary target organ. Recent reports on increased reporting of neuronal diseases (Steinmetz et al., 2024) and the association of exposure to certain pesticides with Parkinson's disease highlight the need for a careful assessment of available data and proper and scientifically valid justification if effects that are indicative of neurotoxicity are considered solely secondary to other target organ toxicity.

It is important to note that both reversible and irreversible neurotoxic effects are relevant for neurotoxicity hazard and risk characterisation[18], though irreversible or long-lasting neurotoxic effects are often considered of higher concern. Narcotic effects, though transient, might still be also of high concern in humans depending on the setting in which they occur, e.g. sleepiness in relation to operation of machinery. In addition, even though a single exposure to a substance may cause "only" a narcotic effect, repeated exposure to such substance may cause a different type of neurotoxicity and this should also be considered. For example, neurotoxic effects caused by long-term exposure to organic solvents are known as chronic solvent encephalopathy. The condition is characterised by symptoms such as fatigue, memory loss, difficulty in concentration, loss of initiative, headache, sustained personality or mood change and impairment in intellectual function. Unlike the acute single dose transient neurotoxic (narcotic) effects, these symptoms do not disappear but continue after cessation of long-term exposure to organic solvents.

While the nervous system possesses some reserve capacity which may compensate for damage, the resulting reduction in the reserve capacity should still be considered an adverse effect. Compensation may be suspected if a neurotoxic effect slowly resolves during the lifespan, which could be the case for developmental neurotoxicants (addressed under reproductive toxicity/developmental toxicity and endocrine activity mediated neurotoxicity). Even though compensation would happen later, the neurotoxic effects at the time of occurrence are of high concern and should not be dismissed. Many long-lasting or permanent functional effects (e.g. depression, involuntary motor tremor) are suspected to occur as a result of neurotoxicant exposure without necessarily morphological abnormalities.

Specific guidance on neurotoxicity testing in general, including the evaluation of data obtained from testing, is provided in *OECD GD 20*. Additional guidance on specific aspects of neurotoxicity testing, including the evaluation of the auditory startle response and the Morris Water Maze test, is provided in *NAFTA DNT Guidance*. See also (EFSA, 2018), where Appendix D contains information on some specific substances and can be used as supportive information. For additional guidance on the assessment of neurotoxicity for STOT SE and STOT RE, see the *CLP Guidance*.

[18] CLP criteria for STOT SE and STOT RE cover reversible and irreversible, immediate and/or delayed neurotoxic effects. STOT SE category 3, H336 is reserved for transient central nervous system depression after a single dose, i.e. for narcotic effects

1.7.3.3 Immunotoxicity

1.7.3.3.1 Definition of immunotoxicity

Immunotoxicity is defined as any adverse effect on the immune system that can result from exposure to a range of environmental agents, including chemicals (*WHO/IPCS Immunotoxicity guidance*).

Immunotoxic responses may occur when the immune system is the target of the chemical insult; this in turn can result in either immunosuppression and a subsequent decreased resistance to infection and certain forms of neoplasia, or immune dysregulation which exacerbates allergy or autoimmunity. Toxicity may also arise when the immune system responds to an antigenic specificity of the chemical as part of a specific immune response (i.e. allergy or autoimmunity). Changes of immunological parameters may also be a secondary response to stress resulting from effects on other organ systems. Therefore, it must be recognised that in principle all chemical substances may be able to influence parameters of the immune system if administered at sufficiently high doses. An immunotoxic effect should not be disregarded without a thorough investigation.

The *WHO/IPCS Immunotoxicity guidance* should be consulted together with this Guidance in assessing this endpoint.

1.7.3.3.2 Introduction

Immunotoxicity is of particular concern for test substances that induce toxicity on the immune system at dose levels below those causing toxicity at other target sites. If immunotoxicity is the critical effect, it should be assessed in the risk assessment as any other toxic effect. The test methods EU B.7 / OECD TG 407 and B.26 / OECD TG 408 are intended as a first screening for immunotoxicity, and depending on the results further testing may be needed.

General mechanistic information is available in AOP Wiki (https://aopwiki.org/). Immune dysregulation may also occur via endocrine disruption or inhibition of esterase activity and can also be linked to cancer (Dhouib et al., 2016; Semwal et al., 2022).

The following key characteristics of immunotoxic agents were identified by (Germolec et al., 2022):

1) covalently binds to proteins to form novel antigens;
2) affects antigen processing and presentation;
3) alters immune cell signalling;
4) alters immune cell proliferation;
5) modifies cellular differentiation;
6) alters immune cell–cell communication;
7) alters effector function of specific cell types;
8) alters immune cell trafficking;
9) alters cell death processes; and
10) breaks down immune tolerance.

1.7.3.3.3 Hypersensitivity

Skin and respiratory sensitisation to substances are examples of hypersensitivity. For further discussion on this topic, see Section 1.6.

1.7.3.3.4 Immunosuppression

The basis to assess immunotoxicity potential of a substance starts with standard tests for systemic toxicity, particularly if the relevant additional measures of the 28-day and 90-day test guidelines are used. In general, standard toxicological tests provide only indications of immunotoxicity. To properly assess immunotoxicity, functional test(s) are needed, such as T-cell dependent antibody response assay (TDAR), or host resistant assays. Special studies to characterise effects of concern for immunotoxicity are used only when necessary for adequate risk characterisation. The nature of special studies, and when they should be conducted, need to be decided on a case-by-case basis. In particular, the use of *in vivo* tests should not be undertaken without detailed consideration of the need for such studies.

The protocols of both the 28-day and 90-day studies include the measurement of thymus and spleen weights and histopathological examination of certain lymphoid tissues (thymus, draining and distant lymph nodes, Peyer's patches, bone marrow section) in addition to the total and differential white blood cell counts and spleen histopathology. These tissues all have immunological function and changes to them can be indicative of adverse effects on the immune system. However, tests on functional parameters of the immune system are lacking in the standard data set.

Indications of immunotoxicity from standard repeated dose studies may be:

- morphological changes of lymphoid organs and tissues including bone marrow (e.g. altered cellularity/size of major compartments);
- weight changes of lymphoid organs;
- changes in haematology parameters (e.g. white blood cell number, differential cell counts of lymphocytic, monocytic and granulocytic cells);
- changes in clinical chemistry parameters (e.g. serum protein levels).

Further testing to investigate immune function (e.g. a T-cell function test for substances which cause histopathological changes in the thymus, host resistance models) should be conducted only if the results of such studies can be interpreted in relation to the risk assessment for the substance. In many cases, the observation of the morphological changes or changes in haematology and clinical chemistry parameters, together with a NOAEL for those changes, will be sufficient for screening. Functional assays may give valuable information to identify immunotoxic effects and, in some cases, they can be more sensitive than non-functional assays. However, the observation of the immunological changes discussed above may not necessarily reflect a primary immunotoxic effect but may be secondary to other effects.

The methods for specific investigation of immunotoxic effects are listed in section 1.13.4 of *ECHA Guidance Vol III Part A*.

When assessing data obtained from functional immunotoxicology tests such as TDAR, it is important to carefully assess that the test facility has properly established the methodology for the identification of immunotoxic substances noting the lack of internationally adopted test guideline for the assay. The OECD TG 443, including cohort 3 for developmental immunotoxicity is currently being revised, including a proposal to harmonise the TDAR protocol.

1.7.3.4 Overload phenomena and pulmonary fibrosis

Substances which can be inhaled, are sparingly soluble in water and fat, and are of low systemic toxicity may cause adverse effects in the lung (irreversible impairment of lung clearance, lung fibrosis and lung tumour formation) which can be explained by 'overload phenomena'.

The available data on insoluble dusts indicate that overload related effects can be avoided in the workplace by maintaining the atmospheric concentration of the substance below the specific gravity (relative density) value of the substance expressed as mg × m^{-3}. For example, for a substance with a specific gravity of 1.6, the atmospheric concentration should be <1.6 mg × m^{-3}.

The principle outlined in the paragraph above does not apply to substances which are cytotoxic at concentrations below those leading to overload: such substances may induce fibrosis at lower concentrations. Therefore, it is recommended that inhalable, sparingly soluble substances with low systemic toxicity are examined immediately after the initial repeated dose toxicity testing, using an appropriate test for cytotoxicity (e.g. using primary macrophage cultures or epithelial cell lines *in vitro*; or analysis of broncho-alveolar lavage fluid). Positive (e.g. silica) and negative (e.g. TiO_2) control substances should be included in the test. If the cytotoxicity test is negative, no further testing in relation to pulmonary fibrosis is necessary.

If the substance is cytotoxic, a repeated dose inhalation study of sufficient duration to detect fibrotic changes may be necessary to establish the NOAEL. If a 28-day study has been conducted using the inhalation route of exposure, early indications of fibrotic change may have been detected, and a NOAEL identified. When inhalation testing for a longer period is required to establish a NOAEL, its timing will be influenced by the potential for human exposure as well as the amount of information available on the dose-response relationship. If human exposure is not well controlled (e.g. the substance is used as a consumer product) and/or there is insufficient information on the inhalation concentration-response from toxicity test data already available, further testing may be required.

The need for such repeated dose inhalation testing would have to be established on a case-by-case basis, taking into account all the relevant information available on the substance and the criteria discussed above.

1.7.4 Remaining uncertainty

The following elements contribute to the uncertainty in the determination of a threshold for the critical effects and the selection of the AF (see also Sections 2.3 and 1.2.2.5).

In the determination of the overall threshold for repeated dose toxicity, all relevant information is evaluated to determine the lowest dose that induces an adverse effect (LOAEL/LOAEC) and the highest level with no biologically or statically significant adverse effects (NOAEL/NOAEC). In this assessment all toxicological responses are taken into account and the critical effect is identified. The uncertainty in the threshold depends on the strength of the data and is largely determined by the design of the underlying experimental data. Parameters such as group size, study type/duration or the methodology need to be taken into account in assessing the uncertainty.

When testing is not technically possible, approaches such as (Q)SAR, category formation and read-across may be helpful in the hazard characterisation; they should also be considered for information that might be suitable as a surrogate for a PoD. Alternatively, generic threshold approaches such as TTC might be considered for the starting point of a risk characterisation (see Section 4.2.4).

1.7.5 Conclusions on repeated dose toxicity

Potentially relevant studies should be judged for quality and studies of high quality given more weight than those of lower quality. When both epidemiological and experimental data are available, similarity of effects between humans and animals is given more weight. If the mechanism or mode of action is well characterised, this information is used in the interpretation of observed effects in either human or animal studies. The study or studies used for the starting point are identified by an informed and expert evaluation of all the available evidence.

The available repeated dose toxicity data should be evaluated in detail for a characterisation of health hazards upon repeated exposure. In this process an assessment of all toxicological effects, their dose-response relationships and possible thresholds are taken into account. The evaluation should include an assessment of the severity of the effect, whether the observed effects are adverse or adaptive, if the effect is irreversible or not or if it is a precursor to a more significant effect or secondary to general toxicity. Correlations between changes in several parameters, e.g. between clinical or biochemical measurements, organ weights and (histo-) pathological effects, will be helpful in the evaluation of the nature of effects.

The effects data are also analysed for indications of potential serious toxicity of target organs or specific organ systems (e.g. neurotoxicity or immunotoxicity), delayed effects or cumulative toxicity. The evaluation should take into account the study details and determine if the exposure conditions and duration and the parameters studied are appropriate for an adequate characterisation of the toxicological effects.

If an evaluation allows the conclusion that the information of the repeated dose toxicity is adequate for a robust characterisation of the toxicological hazards, including an estimate of a PoD (NOAEL/LOAEL/BMD), and the data are adequate for risk assessment and classification and labelling, no further testing is necessary unless there are indications for further risk.

Another consideration is whether the study duration has been appropriate for an adequate expression of the toxicological effects. If the critical effect involves serious specific system or target organ toxicity (e.g. haemolytic anaemia, neurotoxicity or immunotoxicity), delayed effects or cumulative toxicity and a threshold has not been established, dose extrapolation may not be appropriate and further studies are required. In this case a specialised study is likely to be more appropriate for an improved hazard characterisation and should be considered instead of a standard short-term rodent or sub-chronic toxicity test.

In the identification of the NOAEL, other factors need to be considered such as the severity of the effect, the presence or absence of a dose- and time-effect relationship, biological relevance, reversibility, and the normal biological variation of an effect that may be shown by representative historical control values (see *ECHA Guidance Vol III Part A* for more information on historical control data).

1.7.6 Concluding on suitability for Classification and Labelling

In concluding on classification and labelling, all the available information needs to be considered. The *CLP Guidance* should be followed. If the data available is not sufficient, additional testing may be required as described in the *ECHA Guidance Vol III Part A*.

1.7.7 Concluding on suitability for risk assessment

For risk assessment, it is necessary to identify a PoD, i.e. a threshold dose for the critical effect as the starting point for deriving the reference values (AEL, ADI). If a NOAEL cannot be identified, the LOAEL may be used instead, provided the data are adequate for a robust hazard assessment.

The PoD should be route specific. In case only animal data with oral exposure are available and humans are exposed mainly via skin and/or inhalation, route-to-route extrapolation is needed, and if not possible, route specific information may be required (see *ECHA Guidance Vol III Part A*). More guidance is given in *REACH Guidance R.8* and ECHA Practical Guide 14[19] which also contains default values for route-to-route extrapolation (table 2).

1.8 Mutagenicity

Table 16: Guidance to be considered together with the current guidance

Guidance	Section
ECHA Guidance Vol III Part A	1.5 Mutagenicity 1.6 In vivo genotoxicity study (ADS)
REACH Guidance R.7a	R.7.7 Mutagenicity and carcinogenicity
CLP Guidance	

1.8.1 Definitions and objectives

Mutagenicity refers to the induction of permanent transmissible changes in the amount or structure of the genetic material of cells or organisms. These changes may involve a single gene or gene segment, a block of genes, or one or more chromosomes. Each of these changes can result in different mutagenic effects and affect one or more mutagenicity endpoints, i.e. gene mutation, clastogenicity or aneugenicity.

- **Gene mutation** refers to permanent changes in the DNA base sequence of a gene or gene segment.
- **Clastogenicity** is used for substances giving rise to structural chromosome aberrations. A clastogen can cause breaks in chromosomes that result in the loss or rearrangements of chromosome segments.
- **Aneugenicity** (aneuploidy induction) refers to the effects of substances that give rise to a change (gain or loss) in chromosome number in cells. An aneugen can cause loss or gain of chromosomes resulting in cells that have not an exact diploid or haploid number. For example, three number 21 chromosomes or trisomy 21 (characteristic of Down syndrome) is a form of aneuploidy.

Genotoxicity is a broader term. It covers mutagenicity and also processes that alter the structure, information content or segregation of DNA. These processes may be reversed by DNA repair or other cellular processes and are not necessarily associated with mutagenicity.

[19] Practical guides available at https://echa.europa.eu/practical-guides; direct link: https://www.echa.europa.eu/documents/10162/17250/pg_14_on_hazard_endpoint_en.pdf/8a85bb85-f4da-49b1-a28a-bfdf269c68b4

Under BPR, the information requirements for 'genotoxicity studies' include:

- Genotoxicity tests for DNA damage, i.e. indicator tests investigating induced damage to DNA but not providing direct evidence of induced mutations, and
- Mutagenicity tests, i.e. tests investigating induced mutations in specific target genes (and their regulatory regions) or growth regulating genes in somatic or germ cells.

The aim of testing for genotoxicity is to assess the potential of substances to induce mutagenic effects and/or DNA damage, which may lead to cancer or cause heritable DNA damage in humans.

While the induction of DNA damage does not provide direct evidence of mutagenicity, it may potentially lead to mutations. Therefore, both mutagenicity data and genotoxicity data linked to DNA damage are used in risk characterisation and classification of substances.

1.8.2 Data to be used in the effects assessment

Genotoxicity is a complex endpoint and requires evaluation by expert judgement. The reliability and relevance of the available data should be assessed as outlined in section 1.2.2. The completeness of the data is assessed on the basis of BPR information requirements (see also *ECHA Guidance Vol III Part A*); further information may be needed on genotoxicity endpoints for which the data is considered insufficient, unreliable or not relevant.

To evaluate the mutagenic potential of a substance in a comprehensive way, information is required on its ability to induce gene mutations, structural chromosome aberrations (clastogenicity) and numerical chromosome aberrations (aneugenicity). Many test methods are available by which such information can be obtained. Non-testing methods, such as (Q)SAR and read-across approaches, may also provide information on the mutagenic potential of a substance.

Typically, *in vitro* tests are performed with cultured bacterial cells, human or other mammalian cells. The applicability of these tests will vary with different classes of substances and can guide the selection of the most appropriate test systems to be used. Some substances need to be metabolically activated to become mutagenic and, to detect the mutagenic effects of such substances, an exogenous metabolic activation system is usually added in *in vitro* test systems. For this purpose, the post-mitochondrial 9000 × g supernatant (S9 fraction) of whole liver tissue homogenate is most commonly employed, containing a high concentration of metabolising enzymes and extracted from animals (usually rats) that have been induced to raise the oxidative cytochrome P450 levels. Alternatives such as human-derived metabolic activation systems or metabolically competent cells, like primary human liver cells or HepaRG cells, have also been developed (Brendt *et al.*, 2021; Reichstein *et al.*, 2023). Under OECD, there is an ongoing project[20] '*In vitro* genotoxicity testing for dermal exposure using 3D skin models: reconstructed skin micronucleus test and reconstructed skin comet assay'. Once adopted by OECD, both guidelines can be used in the *in vitro* genotoxicity testing. Note however that these studies cannot replace *in vivo* micronucleus and comet assays when these are required in accordance with the BPR Annex II.

When information is required on the mutagenic potential of a substance, several test methods are available. In *in vivo* tests, metabolism of the substance and its toxicokinetic properties can

[20] Project 4.139 in https://www.oecd.org/content/dam/oecd/en/topics/policy-sub-issues/testing-of-chemicals/work-plan-test-guidelines-2024.pdf

determine the genotoxic response of the test animal. Species-specific differences in metabolism and toxicokinetics are known, and therefore, different genotoxic responses may be obtained using different species. Care should be taken in the interpretation of results obtained in species other than the ones for which a specific test method has been developed and optimised. Some *in vivo* genotoxicity tests, such as the transgenic rodent (TGR) somatic and germ cell gene mutation assays and the *in vivo* alkaline comet assay, employ methods by which virtually any tissue (containing nucleated cells) of an animal can in theory be examined for effects on the genetic material. This gives the possibility to examine distant target tissues (including male germ cells) and site-of-contact tissues, i.e. skin and the epithelium of the respiratory or gastro-intestinal tract. However, differences can exist regarding the number and type of tissues for which the use of a specific test has been scientifically validated. For instance, the TGR assays can be used to examine male germ cells whereas the comet assay as described in the corresponding OECD TG 489 is, at present, not recommended for that purpose.

Some test methods, but not all, have an adopted EU and/or OECD TG. Where these are not available, established protocols should be followed, such as those defined by internationally recognised groups of experts like the International Workshop on Genotoxicity Testing (IWGT), under the umbrella of the International Association of Environmental Mutagen Societies. Furthermore, modifications to OECD TGs have been developed for some classes of substances, for instance the use of the Prival modification of the OECD TG 471 (i.e. in reductive metabolic activation conditions with uninduced hamster liver S9) for azo-dyes and diazo compounds. These modifications may enhance the accuracy of test results. Use of such modified protocols is a matter of expert judgement and will vary as a function of the chemical and physical properties of the substance to be evaluated. Similarly, the use of standard test methods for the testing of tissue(s) not covered by those standard test methods should be scientifically justified and validity of the results will depend on the appropriateness of the acceptability criteria, which should have been specifically developed for these tissues based on laboratory proficiency and historical data.

1.8.2.1 Non-human data for mutagenicity

1.8.2.1.1 Non-testing data for mutagenicity

Non-testing data can include:

- WoE assessment,
- read-across justification,
- (Q)SAR data.

Detailed guidance on the assessment of non-testing data for mutagenicity is provided in *REACH Guidance R.7a*[21]. Software tools that may be used to predict different mutagenicity endpoints are provided in *REACH Guidance R.7a*, and in a review article evaluating the applicability of existing (Q)SAR models for predicting genotoxicity (Benigni *et al.*, 2019).

[21] At the time of publishing the current guidance, the revision of *REACH guidance R.7a* is still ongoing. Until publication, the draft is available at https://echa.europa.eu/support/guidance/consultation-procedure/ongoing-reach. In the draft guidance, assessment of non-testing data is covered in Section R.7.7.4.1 and information on software tools is provided in Table R.7.7-2.

1.8.2.1.2 Testing data for mutagenicity

Test methods preferred for use are listed in the Tables below. Some of these have adopted EU/OECD guidelines, and others are regarded as scientifically acceptable for genotoxicity testing.

(a) *In vitro* data

Table 17: *In vitro* test methods

Test method	Genotoxic endpoint measured Principle of test method and special considerations	EU/OECD guideline
Bacterial reverse mutation test	Gene mutations The test uses amino acid requiring strains of bacteria to detect (reverse) gene mutations (point mutations and frameshifts).	OECD TG 471 EU B.12/13
In vitro mammalian cell gene mutation tests – HPRT and XPRT genes	Gene mutations The test identifies substances that induce gene mutations in the *hprt* and *xprt* genes of established cell lines.	OECD TG 476 EU B.17
In vitro mammalian cell gene mutation tests – Thymidine kinase gene (Mouse lymphoma MLA and TK6 assays)	Gene mutations and structural chromosome aberrations The test identifies substances that induce gene mutations in the *tk* gene of the L5178Y mouse lymphoma cell line and TK6 human lymphoblastoid cell line. If colonies in a *tk* mutation test are scored using the criteria of normal growth and slow growth colonies, gross structural chromosome aberrations (*i.e.* clastogenic effect) may be measured, since mutant cells that have suffered damage to both the *tk* gene and growth regulating genes situated close to the *tk* gene have prolonged doubling times. The 'normal growing' and 'slow growing' mutants are recognised as 'large colony' and 'small colony' mutants in the MLA and as 'early appearing colony' mutants and 'late appearing colony' mutants in the TK6 assay.	OECD TG 490 EU B.67
In vitro mammalian cell micronucleus test	Structural and numerical chromosome aberrations The test identifies substances that induce micronuclei in the cytoplasm of interphase cells and is considered as the most appropriate *in vitro* cytogenicity test. The micronuclei may originate from acentric fragments or whole chromosomes, and the test thus has the potential to detect both clastogenic and aneugenic substances. If the result of the *in vitro* micronucleus test is positive, the aneugenic potential of the substance must be assessed by using one of the centromere labelling or hybridisation procedures described in OECD TG 487 to determine whether the increase in the number of micronuclei is the result of clastogenic events (i.e. micronuclei contain chromosome fragments) and/or aneugenic events (i.e. micronuclei contain whole chromosomes).	OECD TG 487 EU B.49
In vitro mammalian chromosome aberration test	Structural (and some numerical) chromosome aberrations The test identifies substances that induce structural chromosome aberrations in cultured mammalian established cell lines, cell strains or primary cell cultures. An increase in polyploidy may indicate that a substance has the potential to induce numerical chromosome aberrations, but this test is not optimal to measure numerical aberrations and is not recommended for that purpose.	OECD TG 473 EU B.10

Accepted modifications to the standard test guidelines/methods have been developed to enhance test sensitivity to specific classes of substances and are described in the corresponding test guidelines. Expert judgement should be applied to determine whether any of these modifications are appropriate for a given substance being registered. For example, protocol modifications for the bacterial reverse mutation test (Ames test) might be appropriate for substances regarded as special cases such as gases, volatile liquids, azo-dyes, diazo compounds, glycosides, and petroleum oil derived products.

In addition, some new *in vitro* test methods have been included in the OECD work programme[22], with the aim to develop detailed review papers on the test protocols and performances and potentially OECD TGs:

- ToxTracker assay: a stem cell based reporter assay for mechanistic carcinogenicity hazard assessment;
- *in vitro* genotoxicity testing for dermal exposure using 3D skin models: reconstructed skin micronucleus test and reconstructed skin comet assay;
- *in vitro* γH2AX/phospho-Histone H3 assay: a multiplexed biomarker approach that provides information on genotoxic mode of action.

Other methods may be included in the OECD programme in the future (e.g., MultiFlow, Next Generation Sequencing techniques).

(b) Animal data

Table 18: Somatic cells - *in vivo* test methods

Test method	Genotoxic endpoints measured Principle of the test method	EU/OECD guideline
In vivo mammalian erythrocyte micronucleus test	Structural and numerical chromosome aberrations The test identifies substances that cause micronuclei in erythroblasts sampled from bone marrow and/or peripheral blood cells of animals, usually rodents. These micronuclei originate from acentric fragments or whole chromosomes, and the test thus has the potential to detect both clastogenic and aneugenic substances.	OECD TG 474 EU B.12
In vivo mammalian bone marrow chromosome aberration test	Structural chromosome aberrations The test identifies substances that induce structural chromosome aberrations in the bone marrow cells of animals, usually rodents. An increase in polyploidy may indicate that a substance has the potential to induce numerical chromosome aberrations, but this test is not optimal to measure numerical aberrations and is not recommended for that purpose.	OECD TG 475 EU B.11
Transgenic rodent (TGR) somatic and germ cell gene mutation assays	Gene mutations and chromosomal rearrangements (the latter specifically in the LacZ plasmid mouse model) Since the transgene is present in every cell, gene mutations and/or chromosomal rearrangements can be detected in virtually all tissues of an animal, including target tissues, male germ cells and specific site of contact tissues.	OECD TG 488 EU B.58

[22] https://www.oecd.org/chemicalsafety/testing/oecd-guidelines-testing-chemicals-related-documents.htm

In vivo mammalian alkaline comet assay	DNA strand breaks The DNA strand breaks may result from direct interactions with DNA, alkali labile sites or as a consequence of incomplete excision repair. Therefore, the alkaline comet assay recognises primary DNA damage that may lead to gene mutations and/or chromosome aberrations, but also detects DNA damage that may be effectively repaired or lead to cell death. The comet assay can be applied to almost every tissue of an animal from which single cell or nuclei suspensions can be made, including specific site of contact tissues.	OECD TG 489 EU B.62
Mammalian erythrocyte Pig-a gene mutation assay	Gene mutations The erythrocyte Pig-a assay uses an endogenous mammalian gene, the phosphatidylinositol glycan class A gene (Pig-a), as a reporter of somatic-cell gene mutations in erythroid precursor cells, primarily found in the bone marrow. The test can identify substances that cause gene mutations in these precursor cells, which are reflected in erythrocytes sampled from peripheral blood cells of animals, usually rodents. The test can be conducted without killing the animals, which facilitates integration of the Pig-a assay into many *in vivo* rodent testing protocols.	OECD TG 470 EU: none at present
Unscheduled DNA synthesis (UDS) test with mammalian liver cells *in vivo*	DNA repair The test identifies substances that induce DNA damage followed by DNA repair (measured as unscheduled "DNA" synthesis) in liver cells of animals, commonly rats. The test is usually based on the incorporation of tritium labelled thymidine into the DNA by repair synthesis after excision and removal of a stretch of DNA containing a region of damage. The test has been removed from the information requirements of BPR and is not part of the genotoxicity testing strategy in BPR.	OECD TG 486 EU: obsolete

Table 19: Germ cells - *in vivo* test methods

Test method	Genotoxic endpoints measured Principle of the test method	EU/OECD guideline
Mammalian spermatogonial chromosome aberration test	Structural chromosome aberrations The test identifies substances that induce structural chromosome aberrations in mammalian, usually rodent, spermatogonial cells and is therefore expected to be predictive of induction of heritable mutations in germ cells. An increase in polyploidy may indicate that a substance has the potential to induce numerical chromosome aberrations, but this test is not designed to measure numerical aberrations and is not routinely used for that purpose.	OECD TG 483 EU B.23
Transgenic rodent (TGR) somatic and germ cell gene mutation assays	Gene mutations and chromosomal rearrangements (the latter specifically in the LacZ plasmid mouse model) Since the reporter transgene is present in every nucleated cell of every tissue, gene mutations and/or chromosomal rearrangements can be detected in virtually all tissues of an animal, including target tissues, site of contact tissues and male germ cells. Appropriate sampling times must be considered in order to detect mutations in different stages of spermatogenesis.	OECD TG 488 EU B.58

Test method	Genotoxic endpoints measured Principle of the test method	EU/OECD guideline
Rodent dominant lethal test	Structural and numerical chromosome aberrations The test identifies substances that induce dominant lethal effects causing embryonic or foetal death resulting from inherited dominant lethal mutations induced in germ cells of an exposed parent, usually the male. It is generally accepted that dominant lethal effects are due to structural and numerical chromosome aberrations. Rats or mice are recommended as the test species. This test is no longer considered appropriate to generate new information under BPR.	OECD TG 478 EU: obsolete

A project has been included in the OECD work programme for the update of OECD TG 489 to study germ cell specific genotoxic effects in the *in vivo* comet assay for gonadal cells[23].

Evaluation of testing data on mutagenicity

Each test guideline contains **criteria for the acceptability** of the study based on important parameters related to the study design and test conditions (e.g. acceptable cell type or animal species, number of cells used and scored or animals tested per group, dose/concentrations levels and the number of test dose/concentrations, recommended negative and positive controls, treatment schedule, exposure and sampling time(s), acceptable levels of (cyto)toxicity, evidence of target tissue exposure, laboratory proficiency demonstration) and **criteria for the evaluation** and interpretation of results (definition of clearly positive and clearly negative responses based on e.g. statistical analysis or threshold values, comparison with historical control ranges for the negative controls).

Further description of these criteria as well as general recommendations to address some of the issues faced when conducting *in vitro* and *in vivo* genotoxicity studies can be found in *OECD 238*.

In particular, the following aspects need to be considered in concluding on the study results and their validity:

- Regarding *positive* findings:
  - Are the testing conditions in *in vitro* mammalian cell assays relevant to the conditions *in vivo*? For instance, marked changes in pH or osmolality can produce artefactual positive responses and should be avoided (e.g. with a pH buffer).
  - For *in vitro* mammalian cell assays, factors such as the cell line used, the top concentration tested, the toxicity measure used or the metabolic activation system used, should be taken into consideration as they are known to influence the specificity of these assays.
  - Responses generated only at highly toxic/cytotoxic doses or concentrations, or at precipitating concentrations should be interpreted with caution, taking into account the criteria in OECD test guidelines, as excessive toxicity/cytotoxicity or precipitation may lead to artefactual positive results.

[23] https://www.oecd.org/chemicalsafety/testing/oecd-guidelines-testing-chemicals-related-documents.htm

- The presence or absence of a dose (concentration) response relationship should be considered to determine whether the response is clearly positive or not (based on the criteria in OECD test guidelines).

- Regarding *negative* test results:
  - Were the doses or concentrations of test substance high enough? Was the maximum test dose or concentration recommended by the test guideline used or was a sufficient level of toxicity or cytotoxicity reached? Could the negative effects be related to dose spacing?
  - Does the batch used cover the representative technical active substance containing impurities at adequate levels?
  - Was the test system sensitive to the nature of the genotoxic changes that might have been expected? (e.g. point mutations or large deletions)? Was there an adequate response of the positive control?
  - The volatility of the test substance: were concentrations maintained in tests conducted *in vitro*?
  - For studies *in vitro*, the possibility of metabolism not being appropriate in the test system including studies in extra-hepatic organs.
  - Was the test substance taken up by the test system used for *in vitro* studies?
  - Was the number of cells and samples/technical replicates scored appropriate according to the OECD test guideline and sufficient to support statistical significance of the negative result obtained?
  - For studies *in vivo*, were the most relevant tissues (i.e. target tissues and/or exposed tissues) sampled? Did the substance reach the organs investigated by the test method? Or was the substance only expected to act at the site of contact due to its high reactivity or insufficient systemic availability (toxicokinetic data, *e.g.* rate of hydrolysis and electrophilicity, may need to be considered in the assessment)?
  - For studies *in vivo*, was sampling appropriate? Was a sufficient number of animals used? Were sufficient sampling times used? Was a sufficient number of cells scored/sampled?
  - In a negative micronucleus test conducted in accordance with OECD TG 474 (1997), scoring 2000 immature erythrocytes per animal (TG of 2016 recommends 4000) and dosing by intraperitoneal injection are limitations that do not render the test unacceptable. As long as there is clear evidence of bioavailability of the test substance in the bone marrow (e.g. from ADME data) and robust performance of the concurrent positive control, a negative result in a micronucleus test conducted with OECD 474 (1997) can be considered as reliable. In case of low confidence on the reliability of the negative result, requesting the following studies can be considered:
    - *in vitro* micronucleus assay in case of limited reliability of *in vitro* clastogenicity/aneugenicity testing, or
    - new *in vivo* micronucleus test in case of poor quality of the existing test, or
    - combined comet/micronucleus test in case of lacking confidence that the test substance reaches the bone marrow.

Different results between different test systems should be evaluated with respect to their individual significance. Examples of points to be considered:

- Different results obtained in non-mammalian vs. mammalian cell tests may be addressed by considering possible differences in substance uptake and metabolism, or in genetic material organisation and ability to repair. Although the results of mammalian tests may be considered of higher relevance for hazard conclusion, additional data may be needed to explain differences.
- If the results of indicator tests detecting DNA lesions (e.g. DNA binding, DNA damage, DNA repair, sister chromatid exchange, comet) are not in agreement with results obtained in tests for mutagenicity, the results of mutagenicity tests are generally of higher significance provided that appropriate mutagenicity tests have been conducted. This is subject to expert judgement.
- If different findings are obtained *in vitro* and *in vivo*, the results of *in vivo* tests generally have precedence over *in vitro* tests, as these have a higher relevance for the safety assessment in humans. However, for the evaluation of *negative* results *in vivo*, it should be considered whether the most appropriate tissues were sampled and whether there is evidence of target tissue exposure.
- The sensitivity and specificity of different test systems may vary for different classes of substances. If available testing data for other related substances permit assessment of the performance of different assays for the class of substance under evaluation, the result from the test system known to produce more accurate responses is given higher priority.

Different results may also be available from the same test, performed by different laboratories or on different occasions. Expert judgement should be used to evaluate the data and reach an overall conclusion. The quality of each of the studies and of the data provided should be evaluated, with special consideration of the study design, reproducibility of data, dose (concentration) response relationships, concurrent control values, historical control data, and biological relevance of the findings. The identity and purity of the test substance must also be taken into account. Where an EU/OECD test guideline is available for a test method, the quality of a study is considered higher if it was conducted in compliance with the requirements in the test guideline, unless convincing scientific evidence justifies deviations for the specific substance evaluated. Compared to non-GLP studies, studies compliant with GLP for the same assay generally provide more documentation and details of the study, which are important factors to consider when assessing study reliability/quality. Klimisch criteria take into account the above factors and the corresponding Klimisch scores give an overall indication of data reliability.

When assessing the potential mutagenicity of a substance or considering the need for further testing, data from various tests and genotoxic endpoints may be found. Both the strength and the weight of the evidence should be taken into account. The strongest evidence will be provided by modern, well-conducted studies in line with internationally established test guidelines/methods. Genotoxicity test battery should examine all three genotoxicity endpoints (gene mutations, clastogenicity and aneugenicity) and cover all core information requirements with at least three *in vitro* tests. For non-GLP and/or non-guideline studies, for each genotoxic endpoint and each core information requirement a separate WoE assessment is needed to demonstrate that the endpoint and the information requirement have been adequately investigated. It is not unusual to have positive evidence in just one test type or for only one endpoint. In such cases the positive and negative results for different endpoints are not conflicting but illustrate the advantage of using test methods for a variety of genetic alterations to increase the probability of identifying substances with mutagenic potential. Hence, results from methods testing different genotoxic endpoints should not be combined in an overall WoE analysis but should be subjected to such analysis separately for each endpoint. Based on the whole data set one has to consider whether concluding is possible or whether there are data

gaps. If there are data gaps after analysis of all available evidence, further testing should be considered.

1.8.2.2 Human data on mutagenicity

Occasionally, studies of genotoxic effects in humans exposed by, for example, accident, occupation or participation in clinical studies (e.g. from case reports or epidemiological studies) may be available. Generally, first contact tissues (e.g. stomach, duodenum for oral route) along with liver and bone marrow cells circulating in blood are investigated for the occurrence of various types of genetic alterations.

1.8.3 Integrated Testing Strategy on mutagenicity

The Integrated Testing Strategy describes a flexible, stepwise approach for hazard identification with regard to the mutagenic potential of substances, so that sufficient data may be obtained for adequate risk characterisation and classification and labelling. It serves to minimise the use of animals and costs as far as it is consistent with scientific rigour. Deviations from this strategy may be considered if existing data indicate that alternative testing strategies would yield results with greater sensitivity and specificity for mutagenicity *in vivo*.

A key concept of the strategy is that initial genotoxicity tests and test methods should be selected with full consideration of existing data to establish the most appropriate testing strategy for the class of substances under evaluation. Even then, initial testing may not always give adequate information, and further testing may be considered necessary in light of all available relevant information on the substance.

Already available, adequately performed *in vivo* data can be used as an alternative to the first *in vitro* mammalian cell test. For instance, if an *in vivo* micronucleus test is already available, it may be used to adapt the information requirement for the *in vitro* cytogenicity study in mammalian cells. In specific cases *in vitro* mammalian cell test may still be justified even though *in vivo* cytogenicity data exist. For example, in the *in vivo* micronucleus test, certain substances may not reach the bone marrow due to low bioavailability or specific tissue/organ distribution. Even if bioavailability of the parent compound in the bone marrow can be demonstrated, a clastogen requiring liver metabolism and for which the reactive metabolites formed are too short-lived to reach the bone marrow, could give a negative result in the *in vivo* micronucleus test. In these cases, *in vitro* testing could provide useful information on the mode of action of the substance, e.g. to understand whether the substance is clastogenic (or aneugenic) *in vitro*, and whether it requires specific metabolism to be genotoxic. Justification of *in vitro* testing when reliable *in vivo* data already exist should be considered on a case-by-case basis.

The toxicokinetic and toxicodynamic properties of the test substance should be considered before appraising or proposing animal tests. Understanding these properties will enable appropriate protocols for the standard tests to be developed, especially with respect to tissue(s) to be investigated, the route of administration and the highest dose tested. If little is understood about the systemic availability of a test substance at this stage, toxicokinetic investigations or modelling may be necessary.

Certain substances may need special consideration, such as highly electrophilic substances that give positive results *in vitro*, particularly in the absence of metabolic activation. Although these substances may react with proteins and water *in vivo* and thus be rendered inactive towards many tissues, they may be able to express their mutagenic potential at the initial site of contact with the body. For such substances, test methods such as the comet assay or the gene mutation

assays using transgenic animals that can be applied to the respiratory tract, the upper gastrointestinal tract and skin may be appropriate. Specialised test methods may need to be applied in these circumstances, and these may not have recognised, internationally validated, test guidelines. The validity and utility of such tests and the selection of protocols should be assessed by appropriate experts or authorities.

1.8.3.1 Negative *in vitro* genotoxicity testing

In general, substances that are negative in the full set of *in vitro* tests are considered non-genotoxic, as only a very limited number of such substances have been found to be genotoxic *in vivo*. Most of these are pharmaceuticals designed to affect pathways of cellular regulation, including cell cycle regulation, and this evidence is judged insufficient to justify routine *in vivo* testing. The metabolic profile of a substance may however indicate that the standard *in vitro* tests are not able to detect a potential genotoxic effect and a further *in vitro* or *in vivo* test may be needed to ensure mutagenicity potential is adequately explored (e.g. use of an alternative to rat liver S9 mix, a reducing system, a metabolically active cell line, or genetically engineered cell lines might be judged appropriate).

1.8.3.2 Equivocal *in vitro* genotoxicity testing

In some cases, the results of the *in vitro* studies will not fulfil all the criteria for a clearly positive or clearly negative response defined in the corresponding OECD TGs. In those cases, expert judgement may allow judging the results as positive or negative without further investigation. For instance, a statistically significant increase compared to the concurrent negative control, associated with a dose-response relationship, could still be considered biologically relevant and concluded as positive even if the increased values remain within the negative historical control data distribution, in particular if there are doubts about the quality of the historical control data. Alternatively, re-examination of the test results, new or additional scoring of stored samples or slides from the test, or performance of a repeat experiment, under possibly modified experimental conditions, could also be useful to clarify the results and reach a conclusion.

If the results of a standard *in vitro* study remain equivocal, i.e. equally likely to be positive or negative, supporting data could be generated. For instance, further information on the mode of action, e.g. from mechanistic *in vitro* assays, may help assess the gene mutation and/or chromosomal aberration potential of the substance, based on a WoE approach, and decide on the need for *in vivo* follow-up testing.

1.8.3.3 Follow-up to positive *in vitro* genotoxicity testing

The nature of the *in vitro* response (gene mutation, structural or numerical chromosome aberration) must be considered when selecting the follow-up *in vivo* study or deciding on the need to combine *in vivo* studies to investigate specific endpoints and fulfil the information requirements. When scientifically justified, investigation of different endpoints and sampling of more than one tissue in the same study is also encouraged whenever possible, as this would provide a more comprehensive overview of the genotoxic potential of a substance and limit the number of animals used. When combining test methods, care should be taken not to impair the validity of the results from each individual test. Further recommendations and references for combining or integrating different test methods can be found in the respective OECD TGs and *OECD 238*.

The appropriate follow-up to *in vitro* positive results in genotoxicity testing is provided in section 1.6 of *ECHA Guidance Vol III Part A*. Special considerations are provided below.

For substances showing evidence of ***in vitro* clastogenicity**, both the *in vivo* micronucleus test and *in vivo* chromosomal aberration test are appropriate follow-up tests, provided that bone marrow exposure to the substance or its metabolites occurred. An *in vivo* comet assay may also be appropriate even if this test is an indicator assay detecting putative DNA lesions and not chromosome aberrations per se, as it can detect substances causing structural chromosome aberrations *in vivo*. However, only the *in vivo* micronucleus test is able to detect both clastogens and aneugens. Therefore, if a positive result for chromosome aberrations was obtained *in vitro* but aneugenicity was not investigated, **the rodent micronucleus test would be appropriate to address clastogenic and aneugenic potentials *in vivo*.**

In case of positive results in the *in vivo* micronucleus test and if the clastogen/aneugen mode of action has not been investigated in the *in vitro* micronucleus test, one of the centromere labelling or hybridisation procedures described in OECD TG 474 must be used (e.g. FISH with pancentromeric DNA probes or primed in situ labelling with pancentromere-specific primers) to determine whether the increase in the number of micronuclei is the result of clastogenic events (resulting in chromosome fragments contained in micronuclei) and/or aneugenic events (micronuclei contain whole chromosomes). Supporting information on the mode of action, e.g. from *in vitro* mechanistic studies, may also help clarify the mode of action of the substance.

Moreover, since the *in vivo* micronucleus test only investigates effects in the bone marrow, **combination with the *in vivo* comet assay is appropriate** to assess effects in both distant organs, such as the liver, and at sites of contact, such as the glandular stomach and the duodenum (oral administration) or the lung (inhalation). Investigating several genotoxic endpoints and different tissues in a combined study is necessary to reduce the uncertainties of not testing all organs and to generate complementary information that provides a comprehensive overview of the genotoxic potential.

For substances inducing **aneugenic effects but no clastogenic effects *in vitro***, as demonstrated in an *in vitro* micronucleus test, the *in vivo* micronucleus test is the only appropriate follow-up test.

For substances inducing **gene mutations**, the TGR assays are the most appropriate and usually preferred tests to follow up a positive *in vitro* gene mutation result and detect substances that induce gene mutations *in vivo*. With respect to the 3Rs principle and taking into account that a positive *in vivo* gene mutation result in somatic cells triggers *in vivo* gene mutation germ cell testing, male germ cells must always be collected, if possible, when a TGR study is performed. According to OECD TG 488, the 28-day administration period and sampling 28 days after the final treatment allows testing of mutations in somatic tissues and tubule germ cells from the same animals.

The Pig-a assay is another appropriate *in vivo* gene mutation assay in somatic cells to follow up on positive *in vitro* gene mutation results, provided that bone marrow exposure to the substance or its metabolites occurs. One advantage of the assay is the use of blood samples, which facilitates combination with other genotoxicity test methods and integration into repeated dose toxicity studies. However, the applicability of the OECD TG 470 is currently limited to rodent bone marrow erythroid cells. Therefore, bone marrow exposure to the substance or its metabolites is required, and the assay cannot be used to measure mutations in other organs such as the liver, the sites of contact tissues or the germ cells.

The *in vivo* comet assay can also detect substances inducing gene mutations, even if it is not a gene mutation assay but an indicator assay measuring DNA damage. This test can be used to analyse both sites of contact and distant organs, although the protocol described in the current OECD TG 489 is not applicable to mature germ cells. In comparison with the Comet assay, the TGR can be a more appropriate *in vivo* test to follow up gene mutations in vitro (except for local

genotoxicity). Furthermore, the TGR is given more weight in CLH than the Comet Assay (indicator test) and, in particular for weak mutagens, assay concordance of TGR and Comet Assay remains unclarified. In addition, the TGR can in some cases be more sensitive because the exposure duration of 28 days facilitates detection of accumulated mutations.

However, in case the comet assay is proposed for somatic cell investigation, male gonadal cells can be collected in the same study and slides prepared for later analysis. Since gonads contain a mixture of somatic and germ cells, positive results in male gonadal cells are not necessarily reflective of germ cell damage, but they indicate that the substance and/or its metabolites have reached the gonad and induced a genotoxic effect in this compartment.

The TGR and comet assays offer greater flexibility than the Pig-a assay, most notably with regard to the possibility of selecting a range of tissues for study on the basis of what is known of the toxicokinetics and toxicodynamics of the substance. The comet assay is an indicator assay detecting DNA lesions, while the TGR and Pig-a assays measure gene mutations, i.e. permanent transmissible changes in the DNA. Therefore, in cases where the gene mutation properties of a substance need to be specifically investigated, the TGR or Pig-a assay may be required.

The rat liver UDS test has a long history of use but is no longer considered appropriate to generate new information under BPR. The sensitivity of the UDS test has been questioned and its lower predictive value towards rodent carcinogens and/or *in vivo* genotoxicants has been confirmed in comparison with the TGR assay (*EFSA Clarification on genotoxicity*). Existing UDS studies can be submitted as supportive information when the liver is a target organ since the UDS is restricted to the detection of primary DNA repair in liver cells. The assay is of limited use as it is only an indicator of DNA repair indirectly showing DNA lesions and can only detect some types of DNA damage. The detected DNA repair patches depend on the DNA repair pathway involved and the proficiency of the cell type investigated, and not all gene mutagens are positive in the UDS test.

A positive result in the UDS assay can indicate exposure of the liver DNA and induction of DNA damage but it is not sufficient information to conclude on the induction of gene mutations. A negative result in a UDS assay is not a proof that a substance does not induce gene mutation. The test is no longer considered appropriate to generate new information under BPR and the above limitations should be considered for existing UDS data.

In case of positive results in any of the somatic tissues tested in the TGR, Pig-a or the comet assay, analysis of germ cell samples will be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

For substances inducing **both chromosome aberrations and gene mutations *in vitro***, the combination of the *in vivo* micronucleus test and the *in vivo* comet assay in a single study is the most appropriate follow-up option. The combined study, together with the results of *in vitro* mutagenicity studies, can be used to make definitive conclusions about the *in vivo* mutagenicity potential of the substance in somatic cells and the underlying mechanisms. The combined study helps limit the number of tests performed and the number of animals used while investigating several (site of contact and distant) tissues and addressing structural and numerical chromosomal aberrations as well as gene mutations.

For substances inducing **gene mutations or chromosomal aberrations *in vitro*, but are not systemically available**, or that are short-lived or reactive, an alternative strategy must be considered involving studies to focus on tissues at sites of contact, such as the glandular stomach and the duodenum (oral administration) or the lung (inhalation). Expert judgement should be used on a case-by-case basis to decide which tests are the most appropriate. The main options are the *in vivo* comet assay and the TGR assay. The route of exposure should be selected to allow the best possible assessment of the hazard to humans. For insoluble substances, the

possibility of release of active molecules in the gastrointestinal tract may indicate that a test involving the oral route of administration is particularly appropriate.

Non-standard studies supported by published literature may sometimes be more appropriate and informative than established assays. Guidance from an appropriate expert or authority should be sought before undertaking novel studies. Additional data that support or clarify the mechanism of action may justify a decision not to test further.

Evidence for *in vivo* DNA adduct formation in somatic cells, together with positive results from *in vitro* mutagenicity tests, are sufficient to conclude that a substance is an *in vivo* somatic cell mutagen. In such cases, positive results from *in vitro* mutagenicity tests may not trigger further *in vivo* somatic tissue testing. The possibility for effects in germ cells would need further investigation.

1.8.3.4 Test combination and integration and limitation of test animal use

Noting the 3Rs principles, the combination of *in vivo* genotoxicity studies or integration of *in vivo* genotoxicity studies into repeated dose toxicity studies is strongly encouraged, whenever possible and when scientifically justified. All the above-mentioned *in vivo* tests in somatic cells are in principle amenable to such integration, although sufficient experience is not yet available for all the tests. The maximum tolerated dose in a combined study using a (sub)chronic treatment may be significantly lower than the maximum tolerated dose following the acute administration currently recommended for some of the *in vivo* genotoxicity test methods. Therefore, combination with a (sub)chronic toxicity study can lead to a substantial reduction in systemic exposure to the substance and/or its metabolites compared to the *in vivo* genotoxicity test performed on its own. The impact of such a reduction on the relevance of negative *in vivo* genotoxicity results should be assessed.

It is possible for two or more endpoints to be combined into a single *in vivo* study, saving resources and numbers of animals used. For instance, as described in OECD TGs 489 and 474, the comet assay and the *in vivo* micronucleus test can be combined into a single acute study, although some modification of treatment and sampling times is needed. These same endpoints can be integrated into repeated dose (e.g. 28-day) toxicity studies (*EFSA Opinion on genotoxicity testing strategies*). The Pig-a assay can also be integrated into repeated-dose toxicity studies and different protocols exist for combining it with the *in vivo* micronucleus test and/or comet assay (see Annex 2 of OECD TG 470).

To ensure that the number of animals used in somatic cell genotoxicity tests is kept to a minimum, both males and females should not be used automatically. In general, the response of genotoxicity tests is similar between male and female animals (*OECD 238*). Therefore, in accordance with standard test guidelines, testing in one sex only is possible when the available data do not demonstrate relevant sex-specific differences, such as differences in systemic toxicity, target organ toxicity, metabolism or bioavailability. Some specific investigations can also encourage the use of one sex: for instance, if germ cell effects are to be analysed in a TGR assay, only males will be used because it is not possible to collect sufficient numbers of female germ cells to conduct the TGR assay.

As indicated in the *OECD 238* and in most of the *in vivo* test guidelines for genotoxicity testing themselves, concurrent positive and negative control animals should normally be used in every test to confirm the reliability of the method and validity of the results. However, if the test laboratory has demonstrated proficiency in the conduct of the test and has established a historical control database for the tissues of interest, it should be considered:

- whether to use concurrent positive control animals. As described in the guidelines of most of the above *in vivo* tests, the use of a concurrent positive control group may be replaced by appropriately stored samples from previous positive control animals, from the same species and strain, and with similar age as those treated with the test substance (frozen tissues or DNA samples for the TGR assays, fixed and unstained slides or cell suspension samples used as scoring controls for the *in vivo* micronucleus test, fixed and unstained slides for the chromosomal aberration test, or blood samples used as flow cytometry standards for the Pig-a assay. When concurrent positive control animals are not included in each study, laboratories should still occasionally perform additional tests with mutagen-treated animals to assure continued proficiency in detecting increases in mutant frequency. It should be noted that, according to OECD TG 489 and *OECD 238*, concurrent positive controls are always necessary when conducting the *in vivo* comet assay, since there is insufficient experience with the stability of alkali labile DNA sites in storage, no agreed tissue freezing and thawing methodology, and no standard method to assess whether a potentially altered response due to storage may affect the sensitivity of the test.
- whether a concurrent positive control group and a concurrent negative control group are to be used for all time points when multiple sampling times are used (e.g. for both the early and late time points in the *in vivo* micronucleus assay, or when single treatment with multiple sampling is used in the *in vivo* comet assay).

1.8.3.5 Evidence of target tissue exposure

The choice of any of the aforementioned *in vivo* assays can be justified only if it can be demonstrated that the tissues studied in the assay are exposed to the test substance and/or its metabolites.

A positive *in vivo* genotoxicity or mutagenicity test result demonstrates target tissue exposure, while for a negative result, evidence of target tissue exposure will be required to conclude that the substance is not genotoxic or mutagenic in the target tissue (exceptions would be intravenous administration or site of contact tissues). For instance, the *in vivo* micronucleus test (OECD TG 474), *in vivo* chromosomal aberration test (OECD TG 475) and Pig-a gene mutation assay (OECD TG 470) investigate cells of erythropoietic origin sampled from the bone marrow and/or peripheral blood and the corresponding test guidelines require demonstration of bone marrow exposure to conclude on a negative result.

Different pieces of evidence of target tissue exposure can be obtained from the *in vivo* genotoxicity or mutagenicity study itself or from an independent study using the same route and same species:

- Treatment-related effects or signs of toxicity in the target tissue (e.g. depression of the immature to mature erythrocyte ratio in the *in vivo* micronucleus test, depression of the mitotic index in the *in vivo* chromosomal aberration test, depression of the fraction of mutant reticulocytes among the total number of mutant erythrocytes in the Pig-a assay, histopathological changes in the *in vivo* comet assay).
- Measurements in the plasma or blood of the test substance and/or its metabolites.
- Toxicokinetic measurements of the substance and/or its metabolites in the target tissue.
- Systemic effects or signs of systemic toxicity, e.g. clinical signs.

Further guidance on how to demonstrate bone marrow exposure is available in *EFSA Clarification on genotoxicity* and in *OECD 238* section 4.2.2 "Proof of exposure (bioavailability)".

1.8.3.6 Substances that give negative results in an *in vivo* test for genotoxic effects in somatic cells

If the testing strategy described above has been followed and the first *in vivo* test is negative, the need for a further *in vivo* somatic cell test should be considered. A second *in vivo* test should then be proposed only if it is required to conclude on the genotoxic potential of the substance under investigation, i.e., if the *in vitro* data show the substance to have potential to induce both gene mutations and chromosome aberrations and the first *in vivo* test has not addressed both concerns comprehensively. In this regard, on a case-by-case basis, attention should be paid to the quality and relevance of all the available toxicological data, including the adequacy of target tissue exposure.

For a substance giving negative results in adequately conducted, appropriate *in vivo* tests, it will normally be possible to conclude that the substance is not an *in vivo* mutagen.

1.8.3.7 Substances that give positive results in an *in vivo* test for genotoxic effects in somatic cells

Substances that have given positive results in cytogenetic tests both *in vitro* and *in vivo* must be studied further to establish whether they specifically act as aneugens, and therefore whether thresholds for their genotoxic activity can be identified, if this has not been established adequately already. This should be done using *in vitro* methods and will support risk evaluation. Confirmation of the type of chromosomal aberration induced is also important to decide on appropriate follow-up testing.

Further investigations may be required for substances giving positive results in the *in vivo* genotoxicity tests in somatic cells. These may include an additional *in vivo* germ cell genotoxicity study to address any remaining concern.

No further information on germ cell mutagenicity is required for substances known to cause germ cell mutagenicity (i.e. meeting the CLP criteria for classification as germ cell mutagen category 1A or 1B) or known to be genotoxic carcinogens (i.e. meeting the CLP criteria for classification as category 1A, 1B or 2 for germ cell mutagenicity and category 1A or 1B for carcinogenicity). The first step is therefore to assess all available data to determine whether there is sufficient information to conclude that the substance poses a hazard as germ cell mutagen or genotoxic carcinogen. If this is the case, no further testing is justified.

Although the hazard class for mutagenicity primarily refers to germ cells, data showing the induction of genotoxic effects at site of contact tissues by substances for which no indication of sufficient systemic availability or presence in germ cells has been presented are also relevant and considered for classification. For such substances, at least one positive *in vivo* genotoxicity test in somatic cells like an *in vivo* comet assay may lead to classification in Category 2 germ cell mutagens and to the labelling as 'suspected of causing genetic defects'.

No germ cell study should be conducted if there is clear evidence that neither the substance nor its metabolites will reach the germ cells. Expert judgement is needed to evaluate the toxicokinetic and toxicodynamic properties of the test substance.

If specific germ cell testing is to be undertaken, expert judgement should be used to select the most appropriate test strategy. The *in vivo* germ cell study must address the concerns identified in somatic cells, i.e. the gene mutation concern, the chromosomal aberration concern, or both.

Guidelines are available for investigating chromosomal aberrations in rodent spermatogonial cells (OECD TG 483) and for the rodent dominant lethal test (OECD TG 478). Dominant lethal mutations are believed to be primarily due to structural or numerical chromosome aberrations. However, the rodent dominant lethal test is no longer considered appropriate to generate new information under BPR. Currently, there is no standard test method to detect numerical chromosomal aberrations in germ cells.

The TGR assays (OECD TG 488) are the only standard test methods detecting gene mutations in germ cells. Alternatively, other methods can be used if deemed appropriate by expert judgement.

The *in vivo* comet assay (OECD TG 489) is currently not recommended for mature germ cell testing, but positive results in male gonadal cells indicate that the substance and/or its metabolites have reached the gonad and can cause mutations in germ cells. This type of supporting evidence, in combination with positive results from an *in vivo* somatic cell mutagenicity test, may potentially be sufficient to warrant classification of the substance in category 1B for germ cell mutagenicity.

To date, there is no single standard test method or agreed combined study capable of detecting both chromosomal aberrations and gene mutations in germ cells in the same animals. When both concerns are raised by the *in vivo* somatic cell test results, it has to be decided case by case which test methods to use.

In principle, it is the potential for effects that can be transmitted to the progeny that should be investigated, but tests historically used to investigate transmitted effects (i.e. the heritable translocation test and the specific locus test) use a very large number of animals. They are rarely used nowadays and are not considered appropriate to generate new information under BPR.

To minimise animal use, it is recommended to include samples from both relevant somatic tissues and germ cell tissues (e.g. testes) in *in vivo* mutagenicity studies: the somatic cell samples can be investigated first and, if they are positive, germ cell tissues can then also be analysed. The possibility to combine reproductive toxicity testing with *in vivo* mutagenicity testing could be considered.

1.8.3.8 Remaining uncertainty

Reliable data can be generated from well-designed and conducted studies *in vitro* and *in vivo*. However, due to the lack of human data available and the inherent degree of uncertainty in testing, a certain level of uncertainty remains when extrapolating these testing data to the effect in humans.

1.8.4 Concluding on suitability for Classification and Labelling

In concluding on classification and labelling, all the available information needs to be considered. The *CLP Guidance* should be followed. If the data available is not sufficient, additional testing may be required as described in the *ECHA Guidance Vol III Part A*.

1.8.5 Concluding on suitability for risk assessment

Considerations on dose (concentration) response shapes and mode of action of mutagenic substances in test systems

If a substance is demonstrated to be e.g. an exclusive aneugen, it is assumed that its genotoxic properties are thresholded, in contrast to a substance having (also) clastogenic properties. The *EFSA aneugenicity Guidance* provides guidance on the risk assessment of aneugenic substance.

Considerations on the dose (concentration) response relationship and on possible mechanisms of action are important components of risk assessment. The default assumption for genotoxic substances is that they have a linear dose (concentration) response relationship. However, this assumption has been challenged by experimental evidence showing that both direct and indirect acting genotoxins can possess non-linear or thresholded dose (concentration) response curves.

Examples of non-DNA reactive mechanisms that may be demonstrated to lead to genotoxicity *via* non-linear or thresholded dose (concentration) response relationships include inhibition of DNA synthesis, alterations in DNA repair, overloading of defence mechanisms (antioxidants or metal homeostatic controls), interaction with microtubule assembly leading to aneuploidy, topoisomerase inhibition, metabolic overload and physiological perturbations (*e.g.* induction of erythropoiesis).

Some publications have also demonstrated the existence of non-linear or thresholded dose (concentration) response relationships for some DNA reactive genotoxic substances like alkylating substances. The underlying mechanisms seem linked to DNA repair capacity (Guérard et al., 2015).

Assessment of the significance of genotoxic responses mediated by such mechanisms would include an assessment of whether the underlying mechanism can be induced at substance concentrations expected to occur under relevant *in vivo* conditions.

In general, several concentrations/doses are tested in genotoxicity assays. At least three experimental concentrations/doses have to be tested as recommended in the OECD test guidelines for genotoxicity. Determination of experimental dose (concentration) dependent response is important to assess the genotoxic potential of a substance and may be used as indicated below. It should be recognised that not all of these considerations may be applicable to *in vivo* data.

- *OECD 238* lists the relevant criteria to be fulfilled for a result to be considered as a clear positive: (i) the increase in genotoxic response is concentration or dose related, (ii) at least one of the data points exhibits a statistically significant increase compared to the concurrent negative control, and (iii) the statistically significant result is outside the distribution of the historical negative control data (*e.g.* 95% confidence interval). In practice, the criterion for a dose (concentration) related increase in genotoxicity will be most helpful for *in vitro* tests, but care is needed to check for cytotoxicity or cell cycle delay which may cause deviations from a dose (concentration) response related effect in some experimental systems.
- Genotoxicity tests are not designed to support derivation of no effect levels. However, on certain occasions, the LOAEL may be a helpful tool in risk assessment. This is true specifically for genotoxic effects caused by non-DNA reactive thresholded mechanisms like aneugenicity. Further, it can give an indication of the mutagenic potency of the substance in the test at issue. Modified studies, with additional dose or concentration points and improved statistical power may be useful. The BMD approach presents several

advantages over the NOAEL/LOAEL approach and can be used as an alternative strategy for dose (concentration) response assessment (see also Section 2.3.2.1.3).

- Unusual shapes of dose (concentration) response curves may contribute to the identification of specific mechanisms of genotoxicity. For example, unusual shapes may be induced by oxidizing substances, or extremely steep increases can suggest an indirect mode of action or a metabolic switching which could be confirmed by further investigation.

Considerations on genetic risks associated with human exposure to mutagenic substances

There are no officially adopted methods for estimating health risks associated with (low) exposures of humans to mutagens. Most (if not all) tests used today are developed and applied to identify the mutagenic hazard *per se*. In regulatory practice, the assessment of human health risks for mutagenic substances that are also carcinogenic is considered covered by assessing and regulating the carcinogenic risks of these substances. The reason for this is that mutagenic events underlie these carcinogenic effects. Therefore, mutagenicity data is not used in deriving PoDs for risk assessment. See also Section 2.4.1 for guidance on assessing non-threshold carcinogens.

A different approach might be considered for mutagens with a thresholded effect, such as aneugens or those interfering with DNA repair enzymes[24].

1.9 Carcinogenicity

Table 20: Guidance to be considered together with the current guidance

Guidance	Section
ECHA Guidance Vol III Part A	
CLP Guidance	Part 3: Health Hazards, Section 3.6 CARCINOGENICITY
OECD GD 116	

1.9.1 Definition

Chemicals are considered carcinogenic if they induce cancer or increase its incidence. Substances which have induced benign and malignant tumours in well performed experimental studies on animals are considered also to be presumed or suspected human carcinogens unless there is strong evidence that the mechanism of tumour formation is not relevant for humans (see Section 3.6.1 of the *CLP Guidance*). Carcinogenic chemicals can increase the tumour incidence and/or malignancy or shorten the time to tumour occurrence. Benign tumours that are considered to have the potential to progress to malignant tumours are generally considered along with malignant tumours. Chemicals can induce cancer by any route of exposure, but carcinogenic potential and potency may depend on the conditions of exposure, such as route, level, pattern and duration of exposure. Carcinogens may be identified from epidemiological studies, from animal experiments and/or other appropriate means that may include (Q)SAR analyses and/or

[24] ECHA (2018) Committee for risk assessment RAC: Opinion on scientific evaluation of occupational exposure limits for benzene. https://echa.europa.eu/documents/10162/13641/benzene_opinion_en.pdf/4fec9aac-9ed5-2aae-7b70-5226705358c7

extrapolation from structurally similar substances (read-across). The determination of the carcinogenic potential of a chemical is based on a WoE approach (see Section 1.2.2.5). Classification criteria are given in the CLP Regulation.

Carcinogenesis involves the transition of normal cells to cancer cells via a sequence of stages that entail both genetic alterations (mutations) and non-genetic events. Non-genetic events are defined as those alterations/processes that are mediated by mechanisms that do not affect the primary sequence of DNA and yet increase the incidence of tumours or decrease the latency time for the appearance of tumours. For example altered growth and death rates, (de)differentiation of the altered or target cells and modulation of the expression of specific genes associated with the expression of neoplastic potential (e.g. tumour suppressor genes or angiogenesis factors) are recognised to play an important role in the process of carcinogenesis and can be modulated by a chemical agent in the absence of genetic change to increase the incidence of cancer.

Carcinogenic chemicals have conventionally been divided into two categories according to the presumed mode of action: genotoxic or non-genotoxic.

Genotoxic modes of action involve genetic alterations caused by the chemical interacting directly with DNA to result in a change in the primary sequence of DNA. A chemical can also cause carcinogenic effects via:

- genetic alterations indirectly following interaction with other cellular processes, or
- non-genotoxic modes of action.

Indirect genetic alterations may be induced via:

- epigenetic changes (e.g. DNA methylation), i.e. effects that do not involve alterations in genes but may influence gene expression,
- induction of oxidative stress,
- exceedance of the compensatory capacity of physiological conditions,
- disturbance of homeostatic controls,
- alterations in DNA repair,
- allelic loss (e.g. aneuploidy).

Non-genotoxic modes of action include:

- epigenetic changes (e.g. DNA methylation), i.e. effects that do not involve alterations in genes but may influence gene expression,
- chronic cytotoxicity with subsequent regenerative cell proliferation (e.g. induction of urinary bladder tumours in rats due to persistent irritation/inflammation, tissue erosion and regenerative hyperplasia of the urothelium following the formation of bladder stones),
- activation of specific receptors (e.g. PPARα, which is associated with liver tumours in rodents; or tumours induced by various hormonal mechanisms),
- immune modulation, e.g. broad immunosuppression,
- hormonal perturbation.
- altered cell-cell communication,

Note that in the above, epigenetic alterations are included under both non-genotoxic and indirect mechanisms, and a clear differentiation between the two may not always be possible.

Key hallmarks for non-genotoxic carcinogens have been identified, as well as test methods that address the mechanisms of hallmarks of cancer or endpoints connected to them. For more information see (Jacobs *et al.*, 2020).

The objective of investigating the carcinogenicity of chemicals is to identify potential human carcinogens, their modes of action, and their potency.

With respect to carcinogenic potential and potency, the most relevant source of information is human epidemiology studies (e.g. cohort, case control studies). In the absence of human data, animal carcinogenicity tests are used to identify carcinogens. The results of these studies have to be extrapolated to humans, both in qualitative and quantitative terms. This introduces uncertainty with regard to potency and relevance to humans, due to species specific factors such as differences in chemical metabolism and TK, and inherent difficulties in extrapolating from the high doses used in animal bioassays to those normally experienced by humans.

Once a chemical has been identified as a carcinogen, there is a need to elucidate the underlying mode of action, i.e. whether the chemical is genotoxic directly (DNA reactive, e.g. causes chromosome breakage or loss) or indirectly (i.e. causes disturbance e.g. in cell division or DNA damage response). In risk assessment a distinction is made between different types of carcinogens.

For genotoxic carcinogens exhibiting direct interaction with DNA, it is not generally possible to infer the position of the threshold from the NOEL on a dose-response curve, even though a biological threshold below which cancer is not induced may exist.

For non-genotoxic carcinogens, no-effect thresholds are assumed to exist and to be identifiable if appropriately designed studies of the dose-response for critical non-genotoxic effects are conducted. No-effect thresholds may also be present for certain carcinogens that cause genetic alterations via indirect effects on DNA following interaction with other cellular processes (e.g. carcinogenic risk would manifest only after chemically induced alterations of cellular processes had exceeded the compensatory capacity of physiological or homeostatic controls). However, in the latter situation the scientific evidence needed to convincingly underpin this indirect mode of genotoxic action may be more difficult to achieve. Human studies are generally not available for making a distinction between the modes of action, and a conclusion on this depends on the outcome of mutagenicity/genotoxicity testing and other mechanistic studies. Animal studies (e.g. the carcinogenicity study, repeated dose studies, and experimental studies with initiation-promotion protocols) may also inform on the underlying mode of carcinogenic action.

The cancer hazard and mode of action may also depend on exposure conditions such as the route of exposure. A pulmonary carcinogen, for example, can cause lung tumours following chronic inhalation exposure, but there may be no cancer hazard with dermal exposure. Therefore, all relevant effect data and information on human exposure conditions are evaluated in a WoE approach to provide the basis for regulatory decisions.

1.9.2 Data to be used in the effects assessment

1.9.2.1 Non-human data for carcinogenicity

1.9.2.1.1. Non-testing data for carcinogenicity

Although significant challenges remain, non-testing techniques exist for elucidating mechanistic, toxicokinetic or toxicodynamic factors important in understanding carcinogenicity. These include

evaluation of structural similarities and analogues (i.e. read-across and grouping) and (Q)SAR models. Such information may assist in priority setting, hazard identification, elucidation of the mode of action, potency estimation and deciding on testing strategies based on a WoE evaluation.

Genotoxicity is an important mechanism for carcinogenesis and is often decisive for the choice of risk assessment methodology.

Models predicting test results for genotoxic endpoints for closely related structures are known as local or congeneric (Q)SARs. Congeneric models are less common for carcinogenicity than for mutagenicity.

For non-genotoxic carcinogenicity, a large number of different mechanisms may be involved. Although many potentially useful models exist, their applicability depends on the proposed mechanism and chemical class.

Several global models exist which attempt to predict the carcinogenic hazard of diverse non-congeneric groups of substances. These models may also assist in screening, priority-setting, deciding on testing strategies and/or the assessment of hazard or risk based on WoE.

1.9.2.1.2. Testing data on carcinogenicity

(a) *In vitro* data

A variety of *in vitro* data may be available that must be evaluated within the context of the overall toxicological effects of a substance under evaluation. Where standard protocols do not exist, studies are conducted in accordance with expert judgement using protocols tailored to the specific substance, target tissue and cell type or animal species under evaluation. *In vitro* tests can currently not be used as stand-alone methods for the identification of carcinogenic properties of substances. They can however be used in identifying specific carcinogenicity MoAs.

Genotoxicity studies: the ability of substances to induce mutations or genotoxicity can be indicative of carcinogenic potential. Correlation between carcinogenicity and mutagenicity/genotoxicity is weaker for *in vitro* studies than for appropriately designed *in vivo* studies.

***In vitro* cell transformation assays** assess the ability of chemicals to induce changes in the morphological and growth properties of cultured mammalian cells that are presumed to be similar to phenotypic changes that accompany the development of neoplastic or pre-neoplastic lesions *in vivo*. These assays are restricted to the detection of effects of chemicals at the cellular level and will not be sensitive to carcinogenic activity mediated by effects exerted at the level of intact tissues or organisms.

Mechanistic studies:

- Cell proliferation: sustained cell proliferation can facilitate the growth of neoplastic/pre-neoplastic cells and create conditions favouring spontaneous changes that promote neoplastic development.
- Altered intercellular gap junction communication: exchange of growth suppressive or other small regulatory molecules between normal and neoplastic/pre-neoplastic cells through gap junctions is suspected to suppress phenotypic expression of neoplastic potential. Disruption of gap junction function may attenuate the suppression of neoplastic potential by normal cells.

- Hormone or other receptor binding: a number of agents may act through binding to hormone receptors or sites for regulatory substances that modulate the growth of cells and/or control the expression of genes that facilitate the growth of neoplastic cells. These interactions are diverse and generally very specific.
- Immunosuppressive activity: neoplastic cells frequently have antigenic properties that permit their detection and elimination by normal immune system function. Suppression of normal immune function can reduce the effectiveness of immune surveillance and permit the growth of neoplastic cells induced by exogenous factors or spontaneous changes.
- Ability to inhibit or induce apoptosis: apoptosis constitutes a sequence of molecular events that results in the death of cells, most often by the release of specific enzymes that result in the degradation of DNA in the cell nucleus. Apoptosis is integral to the control of cell growth and differentiation in many tissues. Induction of apoptosis can eliminate cells that might otherwise suppress the growth of neoplastic cells; inhibition of apoptosis can permit pre-neoplastic/neoplastic cells to escape regulatory controls that might otherwise result in their elimination.
- Ability to stimulate angiogenesis or the secretion of angiogenesis factors: the growth of pre-neoplastic/neoplastic cells in solid tumours will be constrained in the absence of vascularisation to support the nutritional requirements of tumour growth. Secretion of angiogenesis factors stimulates the vascularisation of solid tumour tissue and enables continued tumour growth.

In vitro data can only give preliminary information about the carcinogenic potential of a substance and possible underlying modes of action. For example, *in vitro* genotoxicity studies may provide information whether the substance is likely to be genotoxic *in vivo*, and thus a potential carcinogen, and on the potential threshold or non-threshold mode of action underlying carcinogenicity.

In vitro cell transformation results can help in concluding in a WoE evaluation whether a chemical has carcinogenic potential. Such results do not inform of the underlying modes of action since they are restricted to the detection of effects at the level of single cell and may be produced by mechanistically distinct processes.

Studies can also be conducted to evaluate the ability of substances to influence processes facilitating carcinogenesis. Such studies need to be designed and assessed on a case-by-case basis.

Overall, there are significant uncertainties in extrapolating *in vitro* data to an *in vivo* situation. *In vitro* data may however provide insights into the nature of the *in vivo* studies that might be conducted to define carcinogenic potential and/or mechanisms.

(b) Animal data

Animal data may provide direct or indirect information for assessing the carcinogenic potential of a substance to humans.

Carcinogenicity studies (conventional long-term/life-time studies) are typically conducted using rats and mice, but information may be available also from studies in guinea pig, Syrian hamster, mini-pig, dog and primates. Exposures to test substances may be via oral, inhalation or dermal exposure routes. The exposure route may be decided on the basis of foreseen routes of exposure relevant to humans or based on information such as epidemiology studies or repeated dose toxicity studies in animals.

Short- and medium-term bioassay data (e.g., mouse skin tumour, rat liver foci model, neonatal mouse model): multiple assays permit the detection and quantitation of putative pre-neoplastic changes in specific tissues. The induction of such pre-neoplastic foci may be indicative of carcinogenic potential. Such studies may be applicable on a case-by-case basis for obtaining supplemental mechanistic and dose-response information that may be useful for risk assessment.

Genetically engineered (transgenic) rodent models: transgenic animals can be more susceptible to carcinogenesis, increasing the sensitivity of the study and/or decrease the latency with which spontaneous or induced tumours are observed. The genetic changes in a given strain of engineered animals can increase sensitivity to carcinogenesis in a broad range of tissues or can be specific to the changes requisite for neoplastic development in one or only a limited number of tissues. While conventional bioassays are used for hazard identification and potency estimation, studies using genetically engineered animals are informative on potential hazard and possible mode of action, but less on carcinogenic potency as they are considered to be highly sensitive to tumour induction.

Genotoxicity studies *in vivo*: the ability of substances to induce mutations or genotoxicity can be indicative of carcinogenic potential.

Repeated dose toxicity tests can identify tissues that may be specific targets for toxicity and subsequent carcinogenic effects. Particularly significant would be pre-neoplastic changes (e.g. hyperplasia or metaplasia) suspected to be precede tumour development.

Studies on the induction of sustained cell proliferation: substances can induce sustained cell proliferation via compensatory processes that continuously regenerate tissues damaged by toxicity. Some substances can also be tissue-specific mitogens, stimulating cell proliferation in the absence of overt toxic effects. Mitogenic effects are often associated with the action of tumour promoters. Both regenerative cell proliferation and mitogenic effects can be necessary, but not sufficient, for tumour development but have sufficiently different mechanistic basis that care should be exercised in assessing which is occurring.

Studies on immunosuppressive activity: suppression of normal immune surveillance functions can interfere with immune system functions that serve to identify and eliminate neoplastic cells.

Studies on TK can identify tissues or treatment routes that might be the targets for toxicity and can deliver data on exposure and metabolism in specific organs. Linkages to subsequent carcinogenicity may or may not exist, but such data can serve to focus carcinogenesis studies on specific tissue types or animal species.

Other studies on mechanisms/modes of action, e.g. toxicogenomics, proteomics, metabonomics and metabolomics: carcinogenesis is associated with multiple changes in gene expression, transcriptional regulation, protein synthesis and other metabolic changes.

In vivo data can give direct information about the carcinogenic potential of a substance, possible underlying modes of action, and potency.

Knowledge of the historic tumour incidence for the strain of animal used is important, and laboratory specific data are preferable. Attention to the study design is essential because of the requirement for statistical analyses. The quality, integrity and thoroughness of the reported data from carcinogenicity studies are essential to the subsequent analysis and evaluation of studies. If the available study report does not include all the information required by the test guideline, expert judgment is required to assess the reliability and acceptability of the study.

The final design of a carcinogenicity bioassay may deviate from OECD guidelines if expert judgement and experience in the testing of analogous substances supports the modification of protocols. Carcinogenicity data may sometimes be available also in species other than those specified in test guidelines.

Data may be available from non-conventional carcinogenicity studies, such as short- and medium-term carcinogenicity assays with neonatal or transgenic animals. While such animal model systems may help in detection of carcinogens in a shorter period of time and using fewer animals, their sensitivity and specificity has to be further ensured. See also the ICH harmonised Guideline (2022)[25].

Study findings may not clearly demonstrate a carcinogenic potential, even when standard guidelines have been followed. For example, there may only be an increase in the incidence of benign tumours or of tumours that have a high background incidence in control animals. Expert judgment is required, and detailed and substantiated rationale should be given if such positive findings are dismissed as not relevant.

Repeated dose toxicity studies may provide helpful additional information to the WoE to determine whether a substance has the potential to induce cancer, and for potential underlying modes of action. For example, the induction of hyperplasia (through cytotoxicity and regenerative cell proliferation, mitogenicity or interference with cellular control mechanisms) and/or the induction of pre-neoplastic lesions may contribute to the WoE. Toxicity studies may also provide evidence of immunosuppressive activity, a condition favouring tumour development under chronic exposure.

TK data may reveal the generation of metabolites with structural alerts. It may also give important information as to the potency and relevance of carcinogenicity and related data collected in one species and its extrapolation to another, based on differences in absorption, distribution, metabolism and or excretion of the substance. Species specific differences may be demonstrated in experimental studies or by toxicokinetic modelling.

Positive carcinogenic findings on animals require careful evaluation and this should be done with other toxicological data (e.g. *in vitro* and *in vivo* genotoxicity studies, TK data, mechanistic studies, (Q)SARs) and the exposure conditions including route of exposure. Such comparisons may provide evidence for specific mechanisms of action that may then be evaluated for relevance for humans.

A substance may exhibit limited genotoxicity *in vivo* but the relevance of this property to carcinogenicity is uncertain if genotoxicity is not observed in tissues that are the targets of carcinogenesis, or if genotoxicity is observed via routes not relevant to exposure conditions (e.g. intravenous injection) but not when the substance is administered via routes of administration known to induce cancer. In such instances, the apparent genotoxic properties of the substance may not be related to the mechanisms believed to underlie tumour induction. For example, oral administration of some inorganic metal compounds will induce renal tumours via a mechanism believed to involve organ specific toxicity and forced cell proliferation. Although genotoxic responses can be induced in non-target tissues for carcinogenesis via intravenous injection, there is only limited evidence to suggest that this renal carcinogenesis entails a genotoxic mechanism.

In general, tumours induced by a genotoxic mechanism (known or presumed) are, in the absence of further information, considered to be of relevance to humans even when observed in tissues

[25] https://database.ich.org/sites/default/files/S1B-R1_FinalGuideline_2022_0719.pdf

with no direct human equivalent. Tumours shown to be induced by a non-genotoxic mechanism are, in principle, also considered relevant to humans but there is a recognition that some non-genotoxic modes of action do not occur in humans. This includes, for example, some specific types of rodent kidney, thyroid, urinary bladder, forestomach and glandular stomach tumours induced by rodent-specific modes of action.

The information available for substances identified as carcinogenic based on testing and/or non-testing data should be further evaluated to identify underlying modes of action and potency and to subsequently allow for a proper quantitative risk assessment.

For the use of historical control data, see Section 1.8.3.4, and EFSA supporting publication on HCD (Coja et al., 2022).

1.9.2.2 Human data for carcinogenicity

The most definitive epidemiological studies on chemical carcinogenesis are generally cohort studies of occupationally exposed populations, and less frequently the general population. Cohort studies evaluate groups of initially healthy individuals with known exposure to a given substance and follow the development of cancer incidence or mortality over time. With adequate information regarding exposure of individuals, dose dependent relationships with cancer incidence or mortality in the overall cohort can be established. Case control studies retrospectively investigate individuals who develop a certain type of cancer and compare their chemical exposure to that of individuals who did not develop disease. Case control studies can be nested within cohort studies and can help increase the precision with which cancer can be associated with a substance.

Besides the identification of carcinogens, epidemiological studies may provide information on actual exposure in workplaces and/or the environment and the associated dose-response for cancer induction.

Although instrumental in the identification of known human carcinogens, epidemiology studies are often limited in their sensitivity by a number of technical factors. The extent and quality of information is often limited on exposure history or other determinants of health status within a cohort. Given the long latency between exposure to a carcinogen and the onset of clinical disease, robust estimates of carcinogenic potency are difficult to generate.

Occupational and environmentally exposed cohorts often have co-exposures to carcinogenic substances and there may be other confounding factors that have not been documented or are incompletely documented. This can be particularly problematic in the study of industry sectors (e.g. base metal production) known to entail co-exposures to known carcinogens (e.g. arsenic) present as trace contaminants in the raw materials being processed. Retrospective hygiene and exposure analyses for such sectors are often capable of estimating exposure to the principal materials being produced, but data documenting critical co-exposures to trace contaminants may not be available. Increased cancer risk may be observed in such settings, but the source of the increased risk can be difficult to determine. Finally, a variety of lifestyle confounders (smoking, drinking, dietary patterns and ethnicity) influence the incidence of cancer but are often inadequately documented. Thus, modest increases in cancer at tissue sites known to be impacted by confounders (e.g. lung and stomach) can be difficult to interpret.

Epidemiological data may potentially be used for hazard identification, exposure estimation, dose-response analysis, and risk assessment. Expert judgement should be used to evaluate the degree of reliability for each study on the carcinogenic potential of a substance. Particular attention should be given to exposure data and to the choice of the control population. The

presence or absence of concurrent exposures to other substances and the methods used for assessing the relevant dose levels should be explicitly documented. A series of studies revealing similar excesses of the same tumour type, even if not statistically significant, may suggest a positive association, and a meta-analysis may be used to increase the sensitivity.

Interpretation of epidemiology studies must include an assessment of the adequacy of exposure, the size of the study cohort relative to the expected frequency of tumours at tissue sites of special concern and whether basic elements of study design are appropriate (e.g. a mortality study will have limited sensitivity if the cancer induced has a high rate of successful treatment). Such factors can limit the sensitivity of a study – unequivocal demonstration that a substance is not a human carcinogen is difficult and requires detailed and exact measurements of exposure, appropriate cohort size, adequate intensity and duration of exposure, sufficient follow-up time and sound procedures for detection and diagnosis of cancers of potential concern. Conversely, excess cancer risk in a given study can also be difficult to interpret if relevant co-exposures and confounders have not been adequately documented.

Once identified as a carcinogenic substance on the basis of human data, well-performed epidemiology studies may be valuable for providing information on the relative sensitivity of humans as compared to animals.

1.9.3 Remaining uncertainty

Adequate human data for evaluating the carcinogenic properties of a chemical are most often not available, and alternative approaches have to be used.

Test guidelines for identifying genotoxic carcinogens are available and adequately cover this property. Animal carcinogens acting by a genotoxic mode of action may reasonably be regarded as human carcinogens unless there is convincing evidence that the mechanisms by which mutagenicity and carcinogenicity are induced in animals are not relevant to humans. There is however uncertainty on the carcinogenic potency in animals and humans.

Conventional carcinogenicity protocols in animals have been found to be insensitive for some well-established human carcinogenic substances (e.g. asbestos and arsenic compounds). These substances can be shown to be carcinogenic when the test conditions are modified, thus illustrating the possibility that a chemical could pose a carcinogenic hazard in humans but be missed in conventional animal studies.

1.9.4 Concluding on suitability for Classification and Labelling

In concluding on classification and labelling, all the available information needs to be considered. The *CLP Guidance* should be followed. If the data available is not sufficient, additional testing may be required as described in the *ECHA Guidance Vol III Part A*.

1.9.5 Concluding on suitability for risk assessment

Where a chemical is identified as a carcinogen, dose-response assessment is an essential further step to characterise carcinogenic risks for certain exposure conditions or scenarios. A critical element in this assessment is the identification of the mode of action underlying the observed tumour formation and whether this induction of tumours takes place via a genotoxic mechanism.

It is generally assumed that, in the absence of data to the contrary, an effect-threshold cannot be identified for genotoxic carcinogens exhibiting direct interaction with DNA, and it is thus not

possible to define a no-effect level for carcinogenicity. However, in certain cases a threshold for carcinogenicity may be identified by demonstrating that an increase in tumours did not occur at exposures below those associated with local chronic cytotoxicity and regenerative hyperplasia. For certain genotoxic carcinogens causing genetic alterations, a practical threshold may exist for the underlying genotoxic effect. For example, this has been shown to be the case for aneugens, or for chemicals that cause indirect effects on DNA that are secondary to another effect such as oxidative stress that overwhelms natural antioxidant defence mechanisms.

Non-genotoxic carcinogens exert their effects through mechanisms that do not involve direct DNA reactivity. It is generally assumed that these modes of actions are associated with threshold doses, and it may be possible to define no-effect levels for the underlying toxic effects of concern. There are numerous modes of action involved in non-genotoxic carcinogenicity. For example, chronic cytotoxicity with subsequent regenerative cell proliferation is a mode of action by which tumour development can be induced. The induction of urinary bladder tumours in rats, for example, may be due to persistent irritation/inflammation/erosion and regenerative hyperplasia of the urothelium following the formation of bladder stones which eventually results in tumour formation. Specific cellular effects, such as inhibition of intercellular communication may facilitate the clonal growth of neoplastic/pre-neoplastic cells.

The identification of the mode of action of a carcinogen is based on a combination of results in genotoxicity tests *in vitro* and *in vivo* and observations in animal experiments, e.g. site and type of tumour and parallel observations from pathological and microscopic analysis. If the mode of action of tumour formation is identified as having a threshold, a PoD should be derived for concluding the risk assessment.

1.10 Reproductive toxicity

1.10.1 Definition

The BPR requires that active substances are assessed for reproductive toxicity (information requirement 8.10, BPR Annex II). Reproductive toxicity includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring. Adverse effects on or via lactation are also included under reproductive toxicity, but for classification purposes such effects are treated separately so that a specific hazard warning about this effect can be provided for lactating mothers.

In more specific terms, each of these three differentiations is characterised by multiple diverse endpoints, which relate to impairment of male and female reproductive functions or capacity (fertility) and the induction of harmful effects on the progeny (developmental toxicity, including developmental neurotoxicity and developmental immunotoxicity).

- *Adverse effects on sexual function and fertility:* Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.
- *Adverse effects on development of the offspring:* Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification

under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency.

- *Effects on or via lactation*: It is recognised that for many substances there is no information on the potential to cause adverse effects on the offspring via lactation. However, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies.

As the assessment of reproductive toxicity is part of the core data set, several tests are prescribed in Annex II of the BPR that specifically address reproductive toxicity. In short, the objectives of these tests are to:

- have adequate information to conclude whether classification and labelling for adverse effects on sexual function and fertility and on development is warranted or can be with sufficient confidence excluded (e.g. by ensuring that sufficiently high dose levels have been tested);
- have sufficient information for the purpose of risk assessment;
- obtain information relevant for the assessment of endocrine disrupting properties.

While reproductive toxicity studies are part of core information requirements for biocidal active substances, these studies can be waived for substances that already meet the criteria for classification as germ cell mutagen (category 2, 1A or 1B) and carcinogen (category 1A or 1B)[26], as the results of reproductive toxicity testing are unlikely to have added value for risk assessment. This is because the risk characterisation for such substances will be based on the assumption that a threshold exposure level for adverse health effects cannot be identified, which will normally lead to a recommendation for the most stringent risk management measures. Therefore, reproductive testing will not normally be required for germ cell mutagens and carcinogens (category 1A or 1B), unless there are case-specific reasons suggesting that the information gained from testing will be needed for the risk characterisation. As a consequence, toxic properties on reproduction cannot be excluded for germ cell mutagens and carcinogens that have not been tested for reproductive toxicity. Notwithstanding these provisions, studies on reproductive toxicity may still be needed to conclude on endocrine disrupting properties (see section 1.11).

Table 21: Guidance to be considered together with the current guidance

Guidance	Section
ECHA Guidance Vol III Part A	1.10 Reproductive toxicity
CLP Guidance	3.7 Reproductive toxicity

[26] While the BPR states this as "genotoxic carcinogen, i.e. germ cell mutagen (category 2, 1A or 1B) and carcinogen (category 1A or 1B)", genotoxic carcinogens is not a recognized category under CLP.

1.10.2 Data to be used for the hazard and risk assessment

This section provides information on the evaluation of the available data. Both non-human (nonanimal approaches and *in vivo* animal studies) and human data are considered.

1.10.2.1 Non-animal data

1.10.2.1.1 Physico-chemical properties

It may be possible to infer from the physico-chemical characteristics of a substance whether it is likely to be absorbed following exposure by a particular route and, furthermore, whether it (or an active metabolite) is likely to pass the placental, blood-brain or blood-testes barriers, or be secreted in milk. Information on the physico-chemical properties may contribute to a WoE assessment.

1.10.2.1.2 Chemical grouping or read-across and (Q)SAR models

There are a large number of potential targets/mechanisms associated with reproductive toxicity which, on the basis of current knowledge, cannot normally be adequately covered by a battery of (Q)SAR models. (Q)SAR approaches are currently not well suited for reproductive toxicity and no firm recommendations can be made concerning their routine use in a testing strategy. A particular challenge for this endpoint is the complexity and amount of information needed from various functions and parameters to evaluate the effects on reproduction. Not all necessary aspects can be covered by a (Q)SAR prediction. Another limitation of (Q)SAR modelling is that dose-response information (e.g. NOAEL) required for risk assessment is not provided. A negative result from current (Q)SAR models cannot be interpreted as demonstrating the absence of a reproductive hazard without other evidence to support this.

1.10.2.1.3 *In vitro* data and Adverse Outcome Pathways

The design of alternatives to *in vivo* testing for reproductive toxicity is especially challenging in view of the complexity of the reproductive process and large number of potential targets/mechanisms associated with this broad area of toxicity. In addition, many *in vitro* approaches do not include elements of maternal-foetal crosstalk and biotransformation which may differ depending on the organ and the estimation of the PoD values for risk assessment may be challenging. Furthermore, *in vitro* approaches often lack information if they correctly predict the *in vivo* outcome. Due to these limitations, the assessment of reproductive toxicity can currently not rely on *in vitro* methods alone. However, *in vitro* assays as well as non-mammalian tests can contribute to the overall WoE assessment as supporting evidence. In all cases of this nature, expert judgement must be used to assess the adequacy of the data as inadequate data shall not be used as a primary support for classification or risk assessment (see CLP Annex I, 3.7.2.5.4).

In vitro assays may provide mechanistic information on key events in adverse outcome pathways (AOPs) that are expected to precede reproductive toxicity adverse outcomes. Some assays are designed to assess the ability of a chemical to interact with the endocrine system, e.g. bind and activate or block the androgen receptor (AR) or the estrogen receptor (ER). These include cell-free or whole cell binding assays, cell proliferation assays and transcription assays. The following

adopted *in vitro* EU test methods[27], OECD test guidelines and US EPA guideline cover modes of action relevant for reproductive toxicity:

- OECD Test Guideline 455: Performance-Based Test Guideline for Stably Transfected Transactivation *in vitro* Assays to Detect Estrogen Receptor Agonists and Antagonists (EU B.66)
- OECD Test Guideline 493: Performance-Based Test Guideline for Human Recombinant Estrogen Receptor (hrER) *in vitro* Assays to Detect Chemicals with ER Binding Affinity (2015) (B.70)
- OECD Test Guideline 458: Stably Transfected Human Androgen Receptor Transcriptional Activation Assay for Detection of Androgenic Agonist and Antagonist Activity of Chemicals
- OECD Test Guideline 456: H295R Steroidogenesis Assay (EU B.57)
- OPPTS 890.1200 Aromatase Assay (Human Recombinant)

Several other assays, or combinations thereof, have been proposed to predict (specific aspects of) developmental (neuro)toxicity[28], but up to date none of the tests in the battery have validated OECD test methods, there are several uncertainties as regards their predictive capacity and applicability domain and the DNT *in vitro* battery has not been accepted as a stand-alone replacement of the DNT *in vivo* OECD tests methods for the regulatory use. However, this is an area of active research and it is recommended to consider the latest status of alternative methods from the ECVAM website (https://joint-research-centre.ec.europa.eu/eu-reference-laboratory-alternatives-animal-testing-eurl-ecvam_en), as well as internationally agreed testing methods by OECD (https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788).

Validated and non-validated *in vitro* tests, provided the applicability domain is appropriate, could be used with other data in a WoE assessment to gather the information required to support hazard identification. *In vitro* techniques can be used in mechanistic investigations, which can also provide support for regulatory decisions. Also, *in vitro* tests can be used as supporting evidence when assessing the toxicological properties by read-across from analogous substance(s) or within a substance grouping approach, providing the applicability domain is appropriate. Positive and negative *in vitro* test results can be of value in a read-across assessment.

As mentioned above, a key issue when assessing reproductive toxicity is the complexity of the reproductive process and the large number of potential targets/mechanisms. Current developments on adverse outcome pathways (AOPs) may help in connecting mechanistic information (including molecular initiating event) to an adverse outcome and support other available data. While for the assessment of reproductive toxicity it is not required to know the mechanism of the reproductive adverse outcome, this is different from the assessment of endocrine disruption. In assessing endocrine disruption, there is an additional requirement for an assessment of the biologically plausible link between endocrine activity and adversity. While negative results from *in vitro* tests, (Q)SAR predictions and/or *in chemico* assays do not provide enough confidence for regulatory decision making to demonstrate absence of a reproductive

[27] COMMISSION REGULATION (EU) 2023/464 of 3 March 2023 amending, for the purpose of its adaptation to technical progress, the Annex to Regulation (EC) No 440/2008 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

[28] See e.g. Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In-Vitro Testing Battery (https://one.oecd.org/document/ENV/CBC/MONO(2023)13/en/pdf)

hazard[29], they may provide valuable support for read-across justification and contribute to a WoE assessment.

1.10.2.2 Animal data

Relevant animal data may be available from a wide variety of studies, which give different amounts and types of information (depending for example on exposure duration, parameters measured, statistical power, etc.) on the potential reproductive toxicity of a substance. Such information may include but is not limited to:

In vivo studies providing information on reproductive toxicity:

- Extended one-generation reproductive toxicity study (EU B.56, OECD TG 443);
- Two-generation reproductive toxicity study (EU B.35, OECD TG 416);[30]
- Prenatal developmental toxicity study (EU B.31, OECD TG 414);
- Developmental Neurotoxicity Study (EU B.53, OECD TG 426).
- One-generation reproductive toxicity study (EU B.34, OECD TG 415).
- A reproduction/developmental toxicity screening test (EU B.63, OECD TG 421);
- Combined repeated dose toxicity study with the reproductive/developmental toxicity screening test (EU B.64, OECD TG 422).

Repeated dose toxicity studies which may include parameters relevant for reproductive toxicity (sexual function and fertility):

- 28- and 90-day repeated-dose toxicity studies (EU B.7, OECD TG 407; EU B.26, OECD TG 408[31]), where relevant parameters are included, for example semen analysis, oestrous cyclicity, organ weights of reproductive organs and accessory sex organs, and/or reproductive organ histopathology.

Short-term *in vivo* tests on endocrine disrupting modes of action in intact or non-intact animals, e.g.:

- Uterotrophic bioassay in rodents: a short-term screening test for estrogenic properties (EU B.54, OECD TG 440; *OECD GD 71*);
- Hershberger bioassay in rats: a short-term screening assay for (anti)androgenic properties (EU B.55, OECD TG 441 and *OECD GD 115*);
- Studies on juvenile/peripubertal animals;
- *US EPA Guidance for thyroid assays*

[29] BPR, Annex IV, 1.3: Qualitative or Quantitative structure-activity relationship ((Q)SAR). Results obtained from valid qualitative or quantitative structure-activity relationship models ((Q)SARs) may indicate the presence, but not the absence of a given dangerous property.
[30] An existing two-generation reproductive toxicity studies (EU B.35, OECD TG 416 adopted 2001 or later) can fulfil the standard information requirement regarding reproductive toxicity. If new studies are needed, an extended one-generation reproductive toxicity study is required (EU B.56, OECD TG 443).
[31] OECD TG 408 was revised in 2018 to include endocrine endpoints to combine with the existing sensitivity to reproductive effects, including the measurement of thyroxine (T4), triiodothyronine (T3), thyroid stimulating hormone (TSH) and thyroid gland weight.

Other studies which may provide supporting information, e.g.:

- Mechanistic studies (e.g. *in vitro* methods) that provide information on pathways relevant for reproductive toxicity, e.g. endocrine endpoints or *in vitro* models aimed at developmental toxicity testing like the mouse Embryonic Stemcell Test (EST);
- Reproductive and/or developmental effects in other non-mammalian species such as fish (e.g. Fish Sexual Development Test (OECD TG 234) or the Test No. 240: Medaka Extended One Generation Reproduction Test (MEOGRT) (OECD TG 240) and amphibians (Amphibian Metamorphoses Assay (OECD TG 231) or Larval Amphibian Growth and Development Assay (EU C.53, OECD TG 241).

1.10.2.3 *In vivo* reproductive toxicity tests

1.10.2.3.1 Prenatal development toxicity study

The prenatal developmental toxicity study (EU B.31, OECD TG 414) provides a focused evaluation of potential effects on prenatal development, although only effects that are induced and manifested after implantation and before birth can be detected. Detailed information on external, skeletal and visceral malformations and variations and other developmental effects such as post-implantation losses and effects on foetal weights are provided. Caesarean section allows precise evaluation of the number of foetuses affected.

For a comprehensive assessment of prenatal developmental toxicity, information from two species, one non-rodent (preferably rabbit) and one rodent (preferably rat) is assessed. In case one (or both) of the default species were deemed not suitable species (regarding the human relevance) for prenatal developmental toxicity testing, an adequate justification should have been provided. Results from prenatal developmental toxicity studies are considered relevant to humans unless it is conclusively demonstrated that the clearly identified mechanism or mode of action has no relevance for humans or when the toxicokinetic differences are so marked that it is certain that the hazardous property will not be expressed in humans and a substance which produces an adverse effect on reproduction in experimental animals should not be classified. The DevTox database (https://devtox.org/index_en.php) can help identify the observed structural abnormalities in PNDT studies as malformations or variations.

A prenatal developmental toxicity study (EU B.31, OECD TG 414) does not provide information on postnatal development or male sexual function and fertility, and it provides no or limited information on female sexual function and fertility as the treatment period of dams normally starts on gestation day 6 and the dams and foetuses are terminated at the end of gestation period. However, if exposure started already on gestation day 0, effects on preimplantation loss could indicate adverse effects on female fertility. It is noted however, that even if the treatment started already on gestation day 0, the exposure period prior to the day of implantation would be very short as compared to OECD TG 443 and 416, and thus lack of such effects in OECD TG 414 would not demonstrate the lack of such toxic properties for the substance. Effects on maintenance of pregnancy in terms of reduced gestation length may potentially be identified as well.

1.10.2.3.2 Extended one-generation reproductive toxicity study

The test method of the extended one-generation reproductive toxicity study (EOGRTS, EU B.56, OECD TG 443) describes a flexible modular study design with several investigational options allowing each jurisdiction to decide on the study design required for the respective regulatory context. The study design for BPR is described in detail in *ECHA Guidance Vol III Part A*.

Additional guidance and recommendations for the EOGRTS are provided by ECHA[32] as well as *OECD GD 151*.

The extended one-generation reproductive toxicity study allows evaluation of the effects of the test substance on the sexual function and fertility of the adult males and females and pre- and postnatal developmental toxicity as the exposure period and investigations of developmental parameters continue until the end of adolescence. The interaction between maternal animals and their offspring (nursing behaviour, ability to suckle) is investigated during lactation until weaning, amongst other investigations. The BPR standard information requirement includes Cohorts 1A and 1B for reproductive toxicity, including the extension of cohort 1B to produce the F2 generation thereby covering the complete reproductive cycle. Hence, the EOGRT study also provides information on the sexual function and fertility of the offspring (F1 generation), addressing the potential effects after exposure of the most sensitive life stages (i.e. in utero and early postnatal period). The extension also provides information on developmental toxicity of the second filial generation and provides key information or the assessment of endocrine disruption.

1.10.2.3.3 Two-generation reproductive toxicity study

The two-generation reproductive toxicity study (OECD TG 416, EU B.35) is a general test which allows evaluation of the effects on sexual function and fertility and development of the test substance on the complete reproductive cycle. The investigated parameters relevant for the assessment of sexual function and fertility include alterations to the female and male reproductive system, oestrous cycle length and normality, sperm parameters, sexual behaviour fertility (including reduced number of implantation site) and parturition in P and F1 generations, and sexual maturation in F1 generation (measured by the day of vaginal opening in females and preputial separation in males). Investigations of developmental toxicity of the conceptus include pre- and post-natal effects in offspring such as post-implantation losses, number and sex of pups, stillbirths, live births, the presence of gross anomalies, physical or behavioural abnormalities, altered growth and organ weights and functional deficiencies. It was the standard BPR information requirement until 15 April 2022 but is currently no longer part of the core data requirements. However, studies conducted in accordance with OECD TG 416 (adopted 2001 or later) are considered appropriate to address this information requirement also for the assessment of endocrine disruption if the study is available and was initiated before 15 April 2022.

1.10.2.3.4 Developmental neurotoxicity

Developmental neurotoxicity (DNT) is a separate information requirement under the BPR, which is usually specifically investigated in OECD TG 426 or in DNT cohorts 2A and 2B of EOGRTS with additional investigation for cognitive functions (see also *ECHA Guidance Vol III Part A*, section 1.10.3). All of these listed sources of information include tests for clinical observations, motor activity, motor and sensory function, cognitive functions (such as associative learning and memory) as well as neuropathological examination and brain weight measurement.

[32] ECHA (2023) Evaluating results from 55 extended one-generation reproductive toxicity studies under REACH: Final report of the EOGRTS review project.
https://echa.europa.eu/documents/10162/17228/final_report_eogrts_review_project_en.pdf/9d0b31f1-eff0-e9db-be8c-ac72d5e4b2e5?t=1679916891564

The OECD TG 426 standard set up includes the assessment of associative learning and memory, which is not included in the standard setup of DNT cohorts of EOGRTS. Testing for cognitive functions needs to be added if DNT is investigated via EOGRTS. Both developmental neurotoxicity studies (OECD TG 426, OECD TG 443 including DNT cohorts and additional investigation for cognitive functions) are designed to provide information on the potential functional and morphological hazards to the nervous system arising from exposure of the offspring during the nervous system developmental period. The offspring in an OECD TG 426 study are exposed when a substance is administered to the mothers daily as a minimum from the time of implantation (starting on gestation day [GD] 6) and throughout lactation (until postnatal day [PND] 21). Cohort 2B of an EOGRTS is terminated on PND 21 or 22 and therefore the offspring are exposed only in utero via their mother and during the lactation period. In cohort 2A of an EOGRTS, the offspring are exposed via the mother in utero, through lactation and directly at least after weaning until termination on ~PND 66-77. It is to be noted that when exposure occurs via feed, there is also some direct exposure of the offspring via feed during the lactation period when the pups start eating the same feed as their mothers at around PND 10.

In case of offspring exposure, lactational transfer and direct dosing need to be considered to ensure a continuous dosing period. As the nervous system continues to develop until around PND60 in rats, exposure that is finished at weaning (be it direct dosing or via lactation) does not cover all critical windows of neurodevelopment[33].

It is important to note that classification for developmental toxicity is not limited to effects induced during pregnancy or as the result of parental exposure. It also covers effects interfering with normal development that resulted from exposure of the developing offspring until sexual maturation. Any effects in the offspring resulting from such developmental exposure, manifested at any point in their life span, is taken into consideration. This includes effects investigated after sexual maturation in cohort 2A of EOGRTS (and in the offspring in OECD TG 426 if the exposure had continued after sexual maturation) which should be addressed and concluded under developmental toxicity. This is because in EOGRTS, cohort 2A is exposed in utero and postnatally until PND 66-77. In this scenario (or in any other study where the exposure has continued after the developmental period), it is not possible to know how much prenatal exposure and/or postnatal developmental exposure until sexual maturation and/or exposure after sexual maturation contributed to the manifestation of effects observed after sexual maturation[34].

In the assessment of developmental toxicity, including DNT, the severity and nature of the effect should be considered. Note that the CLP criteria for developmental toxicity do not discriminate between the reversible and irreversible effects for classification. Also reversible effects may at the time of their manifestation interfere with normal development of the organism in a toxicologically significant manner. If the behaviour of offspring in neurobehavioral tests is affected by other toxicity than neurotoxicity, that is also relevant for the assessment of developmental toxicity because developmental toxicity covers any effect which interferes with normal development of the conceptus.

Treatment related effects in a developmental neurotoxicity study are relevant to developmental toxicity classification and can be used as a PoD for the risk assessment. The effects are considered relevant to humans, unless it is conclusively demonstrated that the identified mechanisms or modes of action are not relevant for humans or when the toxicokinetic differences

[33] See Annex I of RAC/62/2022/05 (https://echa.europa.eu/documents/10162/17090/rac_clh_guidance_note_neurotoxicity_en.pdf/96717ed9-55d3-10e0-785b-093d07e267f3?t=1665034511575).
[34] RAC Guidance Note: Addressing developmental neurotoxicity and neurotoxicity under the current CLP hazard classes (https://echa.europa.eu/documents/10162/17090/rac_clh_guidance_note_neurotoxicity_en.pdf/96717ed9-55d3-10e0-785b-093d07e267f3?t=1665023711575)

are so marked that it is certain that the hazardous property will not manifest in humans or it can be clearly demonstrated that the effects are solely secondary non-specific effects (secondary non-specific consequence of maternal toxicity). Note that if the developmental neurotoxic effects are mediated via endocrine activity, they are relevant also for the assessment of endocrine disruption in addition to the assessment of developmental toxicity.

1.10.2.4 Relation between maternal toxicity and developmental toxicity

Developmental effects should be considered in relation to adverse effects occurring in the mothers, as developmental toxicity may be secondary non-specific consequence of maternal toxicity. Based on pragmatic observation, maternal toxicity may, depending on severity, influence development via non-specific secondary mechanisms, producing effects such as depressed foetal weight, retarded ossification, and possibly resorptions and certain malformations in some strains of certain species. However, the limited number of studies which have investigated the relationship between developmental effects and general maternal toxicity have failed to demonstrate a consistent, reproducible relationship across species. Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are solely secondary non-specific consequences of maternal toxicity. When a substance is so toxic that maternal death or severe inanition results, or the dams are prostrate and incapable of nursing the pups, it is reasonable to assume that developmental toxicity is produced solely as a secondary consequence of maternal toxicity and discount the developmental effects. It is to be noted that in rabbits, the body weight gain may not be useful indicator of maternal toxicity because of normal fluctuations in body weight during pregnancy. Maternal mortality greater than 10 % is considered excessive and the data for that dose level shall not normally be considered for further evaluation. Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity.

1.10.2.4.1 Effects on or via lactation

Effects on or via lactation may occur in several ways: substances may reach the milk and as a result lead to exposure of a breastfed child or the quality and quantity of the milk may be affected by maternal exposure to the substance. However, for many substances there might not be sufficient information on the potential to cause adverse effects on the offspring via lactation.

In general, the extended one generation study (OECD TG 443), or the two-generation study (OECD TG 416) alone may not provide sufficient information on the potential to cause adverse effects on or via lactation. Absorption, metabolism, distribution and excretion studies may indicate the likelihood that the substance is present in potentially toxic levels in breast milk. Human data, while rare, can also be used to assess a hazard to babies during lactation. Thus, to best assess effects on or via lactation, any relevant existing information on the substance under study, including physico-chemical, toxicokinetic and developmental toxic properties must be considered together. Cross-fostering may establish whether developmental toxicity to the offspring is caused by lactational exposure or via uterine exposure.

It should be born in mind that the newborn may be more sensitive than the adult. Not only because of specific developmental endpoints, but also in view of a possibly higher intake of the substance per body weight and the immaturity of detoxification pathways and physiological barriers. Moreover, some effects may become apparent only later in life. However, the mere presence of the substance in the milk would normally not be sufficient to support classification

for effects on or via lactation, unless supported by a strong justification for a concern to offspring via breast feeding. This can be based for example on a quantitative assessment of the transfer via the milk and comparing this level to the toxicity threshold in the pups.

The exposure route per se (i.e. whether it is prenatally via mother, during lactation via milk or direct exposure of developing offspring e.g. via feed or gavage or exposure via inhalation or dermal route) inducing the developmental toxic effects does not influence the classification for developmental toxicity but classification for developmental toxicity must be applied independently of the classification for effects on or via lactation if the CLP criteria for developmental toxicity are met.

1.10.2.4.2 Human data on reproductive toxicity

Epidemiological studies in the general population or in occupational cohorts may provide information on possible associations between exposure to a chemical and adverse effects on reproduction. Clinical data and case reports (e.g. biomonitoring after accidental substance release or case studies from intoxications) may also be available.

The quality and reliability of existing human data for hazard assessment should be critically reviewed. This includes a detailed evaluation of the study design (and deviations), strength and relevance of the effects, and exposure information. Possible confounding factors should be taken into account.

When evidence of a reproductive hazard has been derived from animal studies it is unlikely that the absence of evidence of this hazard in an exposed human population will negate the concerns raised by the animal model. This is because there will usually be methodological and statistical limitations to the human data. For example, statistical power calculations indicate that a prospective study with well-defined exposure during the first trimester with 300 pregnancies could identify only those developmental toxins that caused at least a 10-fold increase in the overall frequency of malformations; a study with around 1000 pregnancies could identify only those developmental toxins that caused at least a 2-fold increase (EMEA, 2006). Extensive, high quality and preferably prospective data are necessary to support a conclusion that there is no risk from exposure to the chemical.

1.10.3 Conclusions on reproductive toxicity

For the assessment of the hazard regarding reproductive toxicity, adverse effects on sexual function and fertility, adverse effects on development and effects on or via lactation should all be assessed and concluded separately and independently.

1.10.4 Concluding on suitability for Classification and Labelling

In concluding on classification and labelling, all the available information needs to be considered. The *CLP Guidance* should be followed. If the data available is not sufficient, additional testing may be required as described in the *ECHA Guidance Vol III Part A*.

Note also that CLP does not define a specific dose above which the production of an adverse effect is considered to be outside the criteria leading to classification for reproductive toxicity.

Most OECD Test Guidelines refer to 'limit test' which does not inform of a level above which effects would not be relevant for classification.

1.10.5 Concluding on suitability for risk assessment

In order to be suitable for risk assessment, appropriate PoDs (e.g. NOAEL/LOAEL) have to be established. All reproductive toxicity endpoints should be considered collectively, using a WoE approach to establish the most relevant endpoint and NOAEL to be used in risk assessment.

1.11 Endocrine disruption

Table 22: Guidance to be considered together with the current guidance

Guidance	Section
ECHA Guidance Vol III Part A	1.13.3 Endocrine disruption
ECHA/EFSA ED Guidance	
CLP Guidance	3.11. Endocrine disruption for human health
OECD GD 150	

1.11.1 Definition

The BPR requires that active substances are assessed for endocrine disruption (information requirement 8.13.3, BPR Annex II). While this requirement has always been in the BPR, the scientific ED criteria established in Regulation (EU) 2017/2100 were published in 2018[35], together with a specific *ECHA/EFSA ED guidance* on how to assess whether active substance would meet the ED criteria. These criteria for endocrine disrupting properties are specific in that they require the assessment of mode of action rather than an assessment of adversity alone. The criteria of an endocrine disruptor under the BPR[36] is based on the definition of WHO/IPCS (2002) of an endocrine disruptor:

> *A substance shall be considered as having endocrine-disrupting properties that may cause adverse effect in humans if, […] it is a substance that meets all of the following criteria, unless there is evidence demonstrating that the adverse effects identified are not relevant to humans:*
>
> *(a) it shows an adverse effect in an intact organism or its progeny, which is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population 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;*

[35] 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

[36] The same definition is used for Plant Protection Products, as established in Regulations (EU) 2018/605 and (EU) 2023/707.

(b) it has an endocrine mode of action, i.e. it alters the function(s) of the endocrine system;

(c) the adverse effect is a consequence of the endocrine mode of action.

The 'endocrine mode of action' as stated in point (b) should be interpreted as 'endocrine activity' while the term 'the adverse effect is a consequence of endocrine mode of action' in point (c) covers the link between the adverse effect and the endocrine activity identified in points a) and b). In the context of the assessment of endocrine disruption, the following definitions are used in line with *CLP Guidance*:

- '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 the mixture the potential to alter one or more functions of the endocrine system;
- '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;
- '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.

In 2023, endocrine disruption was introduced into CLP[37] as a separate hazard class with sub-categorisation[38] and a conclusion on the ED properties has to be included in the CLH dossier. ED Category 1 of the new CLP mirrors the scientific ED criteria specified in the BPR, and substances classified as ED Cat 1 are considered to meet the BPR ED criteria. The assessment under BPR requires a conclusion regarding the ED properties of the active substance, as well as the biocidal products, without sub-categorisation.

To conclude on the ED properties of the product, evaluating bodies have to determine whether a biocidal product has ED properties because of a non-active substance contained therein. Further guidance is available in the documents agreed at the CA meetings[39].

The identification of a substance as endocrine disruptor for human health indicates that a substance may cause an endocrine mediated adverse effect at any life stage. The nature of such effects, and sensitivity to them, may depend on the life stage investigated. Generally, the developing foetus, pups and peripubertal animals are considered more sensitive to endocrine modulation than adults.

The assessment and classification for endocrine disruption for human health is independent of the classification of the substance for other hazard classes, including endocrine disruption for environment. The data needed for the assessment will often come from the tests on reproductive toxicity and developmental toxicity[40] and carcinogenicity and other repeated dose studies. There are also several tests in the BPR information requirements, as specified in Annex II 8.13.3.1,

[37] For completeness, it is noted that under CLP the ED classification needs to be based on available data and the generation of any new data is not required for the purpose of CLP.
[38] COMMISSION DELEGATED REGULATION (EU) 2023/707 of 19 December 2022 amending Regulation (EC) No 1272/2008 as regards hazard classes and criteria for the classification, labelling and packaging of substances and mixtures.
[39] https://circabc.europa.eu/ui/group/e947a950-8032-4df9-a3f0-f61eefd3d81b/library/386abfea-55ce-4764-8a31-f9d4f6ceaf0a?p=1&n=10&sort=modified_DESC
[40] Although a core information requirement, in some cases reproductive and developmental toxicity studies might have been waived e.g. because the substance already meets other exclusion criteria. Notwithstanding these provisions, studies on reproductive toxicity may need to be conducted to obtain information on endocrine disrupting properties.

that specifically address certain aspects of endocrine disruption. Any additional available relevant information should be also included in the ED assessment. The objectives of these tests are to have sufficient information to conclude:

- whether adverse effects occur, and/or
- whether the substance shows endocrine activity.

It is important to acknowledge that the ED criteria do not differentiate between various modalities but cover all endocrine activities and their adverse outcomes. However, currently the detailed guidance is available for the assessment of endocrine disrupting modes of action that are caused either via estrogen (E), androgen (A), thyroid (T) and steroidogenic (S) (EATS) modalities. These EATS modalities are the pathways for which most knowledge is currently available and there is relatively good mechanistic understanding how substance-induced perturbations may lead to adverse effects via an endocrine activity. At present, only for the EATS modalities there are standardised *in vivo* (EATS) and *in vitro* (EAS) test guidelines (OECD TGs, EPA), where there is a broad scientific agreement on the interpretation of the effects observed on the investigated parameters.

For non-EATS modalities some considerations are available, and while assays are described in the scientific literature for non-EATS modalities, there are no endorsed test guidelines. Nevertheless, also non-EATS modalities are a valid basis to consider that a substance meets ED criteria, and several biocidal active substances have already been concluded to meet the ED criteria via a non-EATS modality[41].

1.11.2 Data to be used in the assessment of ED properties for human health

The assessment of endocrine disruption is independent of the assessment of other hazard classes, though part of the evidence is obtained from studies that also provide information on other toxic properties such as reproductive toxicity and carcinogenicity. Specific *ECHA/EFSA ED guidance* is available for the assessment of endocrine disruption in the context of the BPR and the PPPR, and additional guidance is available in *CLP Guidance*. Both guidance documents build in turn on the *OECD GD 150*. *OECD GD 150* provides guidance on the interpretation of effects measured in relevant OECD test guidelines, which may arise as a consequence of perturbations of the EATS modalities, and how these effects might be evaluated to support identification of endocrine disruptors. *OECD GD 150* also includes a description of the OECD conceptual framework, categorized the relevant assays into five levels:

- Level 1: Existing data and existing or new non-test information;
- Level 2: *in vitro* assays providing data about selected endocrine mechanism(s)/pathways(s);
- Level 3: *in vivo* assays providing data about selected endocrine mechanism(s)/pathway(s);
- Level 4: *in vivo* assays providing data on adverse effects on endocrine relevant endpoints;
- Level 5: *in vivo* assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism.

[41] https://echa.europa.eu/regulations/biocidal-products-regulation/approval-of-active-substances/bpc-opinions-on-active-substance-approval

Relevant data for the assessment of endocrine disrupting properties for human health is made on the basis of an assessment of the total WoE using expert judgment. This means that all available information that bears on the determination of endocrine disruption for human health 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 humans or animals;

(b) data from analogue substances using structure-activity relationships (SAR) by the means of read-across or grouping,

(c) any additional relevant and acceptable scientific data.

Relevant human data concerning repeated dose toxicity, carcinogenicity and reproductive toxicity may be available from case reports, epidemiological studies, medical surveillance and reporting schemes, and national poison centres. Such information may be relevant for the assessment of endocrine disruption.

Evaluation of the data on adversity

Data on adversity is considered applying the WoE determination and expert judgement considering both positive and negative results, the relevance of the study designs for the assessment of adverse effects, 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; the route of exposure, toxicokinetic and metabolism studies; the concept of the limit dose (concentration), and international guidelines on maximum recommended doses (concentrations) and for assessing confounding effects of excessive toxicity. From reproductive toxicity studies, all adverse effects on sexual function and fertility and development is assessed; see table 14 of *ECHA/EFSA ED Guidance*. Systemic toxicity from all studies, e.g. repeated dose toxicity studies, carcinogenicity studies and reproductive toxicity studies, is considered when any endocrine related organs are affected. This includes for example effects on reproductive organs, thyroid, adrenals, pituitary and nervous system.

For the EATS modalities, the *OECD GD 150* provides guidance on how to interpret parameters normally investigated in (eco)toxicity studies. The *OECD GD 150* differentiates between:

- '*EATS-mediated*' 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*) 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 human health are effects on uterine weight, disturbed estrous cyclicity, or increases in thyroid gland weight, or changes in histopathology of the follicular cells of the thyroid gland.
- '*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 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 are litter size and gestation length, or changes in spatial associative learning and memory, which alone cannot be considered to be endocrine mediated (e.g., without supportive

mechanistic evidence on endocrine activity and evidence of a biologically plausible link between the endocrine activity and the observed adverse effect(s)).

The parameters reported in *OECD GD 150* as relevant to support ED identification 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).

Guideline studies other than those listed in *OECD GD 150*, may also include apical endpoints that can be affected by an endocrine MoA, and therefore may provide relevant information. Furthermore, information on the broader (eco)toxicological profile of the substance may provide better understanding of potential indirect effects on the endocrine system.

The information used to assess a substance can be from standard studies or other scientific data, e.g. literature studies, Q(SAR) data and internationally recognised databases. For further details see *ECHA/EFSA ED Guidance* (section 4) and *CLP Guidance*.

Evaluation of the data on endocrine activity

In line with the *ECHA/EFSA ED guidance*, it is recommended to assess T parameters separately and EAS properties in combination. In each assessment, the types of evidence for endocrine activity can be separated into the following, as defined in *ECHA/EFSA ED Guidance*:

- ***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 hormone production). These parameters are measured in assays currently placed under OECD CF level 2.
- ***In vivo* mechanistic** – parameters measured *in vivo* that provide information on endocrine activity that are usually not considered adverse. This group applies mainly to parameters measured within assays placed at OECD CF level 3. In addition, changes in hormone levels are considered *in vivo* mechanistic even when they are measured in OECD CF level 4 and 5 assays. It should be noted that certain parameters within OECD CF level 3 *in vivo* assays when measured in an animal model (e.g. Hershberger assay OECD TG 441 or fish short-term reproduction assays OECD TG 229) may also provide additional information on adversity in certain circumstances and therefore should be treated as those parameters grouped as 'EATS-mediated' or 'sensitive to, but not diagnostic of EATS' (see below).

In vitro data

Numerous *in vitro* tests are available to investigate specific endocrine modalities, including the following OECD TGs. This includes, but is not limited to, the following tests that are indicated in BPR Annex II 8.13.3.1:

- Estrogen receptor transactivation assay (OECD TG 455);
- Androgen receptor transactivation assay, (OECD TG 458);
- H295R steroidogenesis assay (OECD TG 456);
- Aromatase assay (human recombinant) OPPTS 890.1200.

The currently validated *in vitro* systems consist of (a monolayer of) one cell type that focuses on a specific pathway. *In vitro* tests lack the complexity of an intact organism, and in particular, considerations of adsorption, distribution, metabolism, excretion (ADME) properties are not covered by current *in vitro* test guidelines. Therefore, when interpreting the results of *in vitro* tests, these limitations should be taken into consideration. In order to (partly) overcome these

limitations, several *in vitro* tests can be run utilising metabolising systems, potentially metabolising the parent compound into a substance/metabolite that is active, less active or inactive. Therefore, all mechanistic information should be considered together to reach a conclusion.

While most current *in vitro* assays focus on specific nuclear hormone receptors, not all ED effects are receptor mediated. In addition, only a limited number of receptors is usually investigated, and substances might be able to act via more than one mechanism. The available *in vitro* tests are not expected to detect all types of endocrine activity. Because of this, and because of the inherent limitations of *in vitro* systems highlighted above, conclusions on the endocrine activity of the substance can only be drawn in the context of what the *in vitro* assays can evaluate, and a negative *in vitro* result alone cannot be used to exclude possible endocrine disrupting activity on the endocrine modality under investigation. In addition, the applicability domain of *in vitro* tests must be considered.

The development and validation of specific *in vitro* assays is an area of active research, especially assays that investigate endpoints relevant for the assessment of endocrine disruption. Several ED relevant assays are included in the US EPA ToxCast programme, accessible via the CompTox Chemical Dashboard (https://comptox.epa.gov/dashboard/). Preset (EDSP) filters exist for assays relevant for E, A, T and S, and more information on the specific assays is provided in the ToxCast Assay Description Documentation (https://www.epa.gov/comptox-tools/exploring-toxcast-data). In Europe, EURL ECVAM maintains a tracking system (https://tsar.jrc.ec.europa.eu/) on alternative, non-animal methods on their way to regulatory implementation which provides an overview of the stages of different methods in terms of acceptance as a recognised test method for use in various sectors.

Special consideration of the ToxCast ER Bioactivity Model

The output data from the ToxCast ER Bioactivity Model, which builds on a number of *in vitro* assays, has equivalent predictive capacity as the 'Uterotrophic bioassay in rodents' (OECD TG 440, *OECD GD 71*) for substances with no or low metabolising potential; i.e., both methods can detect substances that are estrogen agonists and antagonists *in vivo*. ToxCast ER Bioactivity Model results can be used similarly to uterotrophic assay data on endocrine activity, however, if the substance has metabolising potential, additional data on metabolites or other endocrine activity data is needed to reach a conclusion. Since the ToxCast ER bioassay lacks metabolic capacity, *in vivo* data has higher weight if the prediction is in conflict with this. However, several adaptations to consider Phase I metabolism capability are under development and have been applied to over 700 ToxCast substances (Hopperstad et al., 2022). The applicability domain should be considered; see further information on use of ToxCast ER Bioactivity model in (Browne *et al.*, 2015) and (Browne *et al.*, 2017).

A similar model has been proposed for the androgen pathway as well (Judson *et al.*, 2020) and the model predictions are available via the CompTox dashboard. However, the AR model activity and the results from the Hershberger assay were not as concordant as for the ER model and the Uterotrophic assay. In part, this might be due to the less robust reference dataset for the Hershberger assay, as explained in (Kleinstreuer *et al.*, 2018).

In silico data

In silico predictions may be used as supporting information for endocrine modalities in a WoE approach. In particular, by providing information on the molecular initiating event (MIE), *in silico* predictions can be used to support the identification of endocrine modes of action. The different types of *in silico* prediction methods can be grouped as:

1. molecular modelling of receptor interactions,

2. (Q)SAR modelling of receptor-based activity,
3. profilers based on structural alerts and decision trees.

For further details, software tools and literature-derived (Q)SAR models for predicting endocrine activity or nuclear receptor binding, see *ECHA/EFSA ED Guidance* (Section 4.1) and Appendix D.

In vivo data

In vivo studies can also provide information on endocrine activity, as EATS-mediated adverse effects infer an underlying *in vivo* mechanism that should be used for the identification of the endocrine activity. While information on endocrine activity (e.g. change in hormone levels) can be obtained from the standard studies required to assess e.g. reproductive toxicity, the *OECD GD 150* also lists dedicated assays for providing *in vivo* mechanistic information, such as the Uterotrophic and Hershberger assays. For further details, see *ECHA/EFSA ED Guidance*. BPR Annex II recommends the following three assays to investigate endocrine activity *in vivo* in mammals:

- Uterotrophic bioassay in rodents (OECD TG 440);
- Hershberger bioassay in rats (OECD TG 441);
- Pubertal development and Thyroid Function in Intact Juvenile or Peripubertal Male Rats (OPPTS 890.1500).

Mode of action analysis and evaluation of biologically plausible link

A mode of action (MoA) can be described as a series of biological events, i.e., key events (KEs) that lead to a specific adverse effect. 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 limited to hormone-receptor interactions. The assessment should, when possible, include consideration of the modified Bradford Hill criteria: essentiality, dose/incidence and temporal concordance, specificity, consistency, analogy. When data are available, in particular dose/incidence and temporal concordance are valuable to support or disprove the plausibility of the key event relationships and should always be assessed. Additional guidance on performing the mode of action analysis is provided in the *ECHA/EFSA ED guidance* and *CLP Guidance*.

The International Programme on Chemical Safety (IPCS) Mode of Action and human relevancy framework[42], see e.g. (M. E. Meek *et al.*, 2014a) and (M. E. Meek *et al.*, 2014b) provides a methodology for analysing and transparently laying out the evidence for the MoA of a substance, linking adverse effect and endocrine activity. The OECD Adverse Outcome Pathway (AOP) activity (*OECD GD 260*, *OECD 184*) also provides a similar structured framework and WoE methodology.

In the WoE considerations in the MoA framework, both biological plausibility and empirical support are weighted, however, biological plausibility is the most influential consideration. Biological plausibility does not need to be demonstrated with substance specific data. Existing scientific knowledge can be used, e.g., textbooks and peer reviewed scientific literature. AOPs can be helpful to establish biological plausibility, but they are not a prerequisite. Several adverse outcome pathways related to endocrine disruption have been established and endorsed (see e.g., OECD Series on AOPs or EFSA PPPR Panel 2023), and there is continuous development of additional AOPs in various stages in the AOPwiki (aopwiki.org). It should be noted that the presence of an AOP in the AOPwiki does not necessarily indicate its relevance or reliability.

[42] https://www.who.int/publications/i/item/9789241563499

Depending on the stage of development of the AOP in AOPwiki ("Under Development", "Under Review", "ESCA approved" and "WPHA/WNT Endorsed"), the amount of data needed to support biological plausibility may vary considerably. The validity of an AOP should be considered using expert judgement.

Special considerations on assessment of thyroid modality

Special consideration needs to be given to the assessment of the thyroid modality. As addressed in the *ECHA/EFSA ED guidance*, evidence for the assessment of this modality will mostly come from (older) OECD CF level 4 and 5 studies where investigations are limited to thyroid parameters investigated in those studies (e.g. OECD TGs 407, 408, 409, 416, 443 and 451-3). Most of the available evidence will concern thyroid weight and thyroid histopathology, without information on concomitant changes in thyroid hormone levels, especially in the absence of studies that could provide thyroid-relevant mechanistic information. While several assays are available that target specific relevant key events in thyroid disruption signalling (see *OECD 207*), and validation efforts are ongoing (*OECD 403*) there are currently no validated level 2 assays available for the investigation of thyroid-related activity in the OECD conceptual framework.

The evaluation of potential thyroid disruption may therefore be hampered by the limited parameters tested in the available toxicity studies. For example, repeated dose toxicity studies may not investigate the potential MIEs or adverse outcomes manifested as e.g. developmental neurotoxicity or cardiovascular toxicity.

As thyroid hormones are essential for normal human brain development, both prenatally and postnatally, even small changes in foetal thyroid hormone levels (e.g. due to decrease of maternal TH levels) may result in adverse outcomes, in particular related to developmental neurotoxicity. In children, disruption of thyroid function in the mother during pregnancy and in the first years of the child's life can lead to neurodevelopmental impairments including low IQ scores (Päkkilä *et al.*, 2015; Korevaar, Tiemeier and Peeters, 2018), cognitive and neurobehavioral defects (Henrichs *et al.*, 2010), and hearing loss (Crofton, 2004). In adults, THs are responsible e.g., for maintenance of cellular metabolism and cardiovascular function (Mullur, Liu and Brent, 2014; Yamakawa *et al.*, 2021).

US EPA has published a test guideline specifically aimed at investigating thyroid disruption in peripheral blood of dams and offspring, the Comparative Thyroid Assay (CTA). In this assay, altered TH levels in blood are an indication of endocrine activity. As disruption of thyroid homeostasis is the initial, critical effect that may lead to adverse effects on the developing nervous system, the CTA may provide information for classification on thyroid mediated adversity instead of a rat DNT study (OECD TG 426). If a CTA is available which provides evidence that the HPT axis is not altered in the foetus or offspring, then this result should be considered in the overall WoE for adversity.

Additional guidance on the assessment of the thyroid modality is provided in the *CLP Guidance*.

Specific considerations regarding adverse effects on (developmental) neurotoxicity and immunotoxicity

Adverse effects on the (developing) nervous system can be elicited by various mechanisms, including endocrine activity. The endocrine system also works closely with the immune system, influencing and modulating the immune system throughout all life stages. (Developmental) neurotoxic and immunotoxic effects therefore have to be considered as adverse effects relevant for the assessment of endocrine disruption when there is evidence that they are mediated by endocrine activity and there is evidence of a biologically plausible link between the endocrine activity and the adverse (D)NT or (D)IT effect. In the absence of evidence for endocrine activity, DNT and DIT are considered in the assessment of reproductive toxicity.

1.11.3 Remaining uncertainty on endocrine disruption

In the assessment of endocrine disruption, there can be an increased level of uncertainty due to:

- inconsistent results within a study or among studies (e.g. positive and negative; pointing towards different directions)
- low quality of study/studies (e.g. low reliability, issues with study design such a dose level setting)

Studies performed in the past were not designed to detect endpoints specifically for endocrine disruption, nor to provide mechanistic information for the adverse effects. The endpoints included in these older studies can suffer from low specificity and sensitivity if not performed correctly (e.g. TH measurements).

The methods for detecting endocrine activity are limited and only focus on a subset of potential mechanisms of endocrine system interference, especially when considering the number of assays that have undergone OECD validation.

Due to the complexity of the TH system, and the current lack of validated *in vitro* tests, some additional uncertainty exists for the assessment of the thyroid modality. It is e.g. possible that only hormone (T3/T4) levels or TSH are altered, not both, and it can still lead to a severe adverse effect via endocrine MoA. Therefore, changes in TH levels and related adverse effects must be carefully assessed and considered for classification.

1.11.4 Conclusions on endocrine disruption

It is important to ensure that the assessment results in a clear conclusion on the endocrine disrupting properties of an active substance. In this assessment, all endocrine relevant endpoints for an endocrine pathway should be considered collectively, using a WoE approach. Substance can potentially induce endocrine disruption by any route of exposure (e.g. when inhaled, ingested, applied to the skin or injected), but endocrine disruption potential and potency may depend on the conditions of exposure (e.g. route, level, pattern, and duration of exposure; age at the time of exposure).

The quality and consistency of the data should be given appropriate weight. Both positive and negative results should be assembled together in a single WoE determination. There can be no firm rules to conducting a WoE assessment, as this involves expert judgment and because the combination and reliability of information available for a particular substance is normally unique. The WoE assessment should consider all toxicity endpoints together, not considering endocrine relevant endpoints in isolation but focusing on a pattern of (endocrine) effects. Residual uncertainties have to analysed and a level of confidence for the conclusion assigned. See also section 1.2.2.5 for more information on WoE.

As the BPR requires a conclusion on the ED properties of an active substance (and biocidal product), there should be sufficient information in the dossier to conclude on the presence or absence of particular endocrine disrupting mode(s) of action. If there is any information suggesting that the active substance may have endocrine disrupting properties, or if there is incomplete information on key parameters relevant for concluding on endocrine disruption, then additional information or specific studies shall be required to elucidate: (1) the mode or the mechanism of action; and/or (2) potentially relevant adverse effects in humans or animals.

In addition to concluding on the ED properties of the active substance, a conclusion is also needed regarding the biocidal product. This requires an assessment of whether the active substance and/or the non-active substance(s) should be considered to have ED properties based on the scientific criteria in the BPR, and/or REACH and/or CLP[43].

1.11.5 Concluding on suitability for Classification and Labelling

In concluding on classification and labelling, all the available information needs to be considered. The *CLP Guidance* should be followed. If the data available is not sufficient, additional testing may be required as described in the *ECHA Guidance Vol III Part A*.

In 2023, a separate hazard class for endocrine disruption was introduced under CLP, with two categories. The allocation to Category 1 or 2 depends on the strength of the available evidence for endocrine activity and adversity and the biological plausibility of a link between them:

- *Category 1: Known or presumed endocrine disruptors for human health*
- *Category 2: Suspected endocrine disruptors for human health*

While CLP makes a distinction between known/presumed (Cat 1) and suspected (Cat 2), the BPR does not make a similar distinction. Given that Cat 2 refers to the cases where the evidence is insufficient to classify as Cat 1, and the CLP criteria for Cat 1 are similar to the ED criteria from the BPR, it is considered that only substances classified as Cat 1 meet the exclusions criteria by meeting the ED criteria as formulated in the BPR. However, contrary to CLP, the BPR requires sufficient information to be available for concluding on the ED properties of the biocidal active substance.

1.11.6 Concluding on suitability for risk assessment

The regulatory consequences of ED properties have been set in the BPR. As a general rule, endocrine disruptors are not approved on the basis of hazard, without a risk assessment or consideration of exposure. Derogations may apply on a case-by-case basis.

A consolidated approach for the risk assessment of ED substances is currently not available, but more experience is needed before advancing in developing a risk assessment approach. In any such approach, the existence of a threshold is a key factor. While for many ED effects a threshold might exist, its identification is currently hindered by lack of data and uncertainties. On a case-by-case basis, and when toxicological and mechanistic data allow a conclusion, it might be possible to establish a reference value (level with no unacceptable effects) considering a WoE approach and appropriate selection of uncertainty factors.

1.12 Phototoxicity

Several classes of chemicals, even when not toxic by themselves, may become reactive under exposure to environmental light, inducing toxic effects known as phototoxicity. Chemicals can

[43] According to CA-Sept24-Doc.5.5, CLP Regulation is applicable to biocidal active substances and biocidal products (mixtures), including the new hazard classes for endocrine disruption introduced by Delegated Regulation (EU) No 2023/707. It is noted that the criteria established through Delegated Regulation 2017/2100 correspond the endocrine disruptors category 1 under the CLP Regulation. Other documents may become available from the CA meetings at: https://circabc.europa.eu/ui/group/e947a950-8032-4df9-a3f0-f61eefd3d81b/library/386abfea-55ce-4764-8a31-f9d4f6ceaf0a?p=1&n=10&sort=modified_DESC.

be photoreactive following systemic exposure and distribution to the skin, or after topical exposure/application.

The parameters that trigger phototoxicity testing are described in Section 1.13.1 of *ECHA Guidance Vol III Part A*. Briefly, there is possible concern of phototoxicity if the active substance absorbs light within the range of natural sunlight (290-700 nm) and is liable to reach the eyes or light-exposed areas of skin, either by direct contact or through systemic distribution.

1.12.1 Definitions

Phototoxicity is a toxic response elicited by topical or systemic exposure to photoreactive chemicals after the exposure of the body to environmental light (see definitions in OECD TG 495).

There are three types of phototoxic reactions:

- **Photoirritation** is a skin response to a photoreactive chemical elicited by topical or systemic exposure to photoreactive chemicals after the exposure of the body to environmental light.
- **Photoallergy** (or photosensitisation) is an immune-mediated reaction in which light may cause a structural change in a chemical, so that it acts as a hapten, possibly by binding to proteins in the skin.
- **Photogenotoxicity** is a genotoxic response after exposure to a chemical either directly by photoexcitation of DNA or indirectly by excitation of photoreactive chemicals.

Some chemicals can cause all three types of reactions.

1.12.2 Test methods and tiered assessment

Although two different photoirritation testing tools have been developed and validated (see Table 23), there are currently no validated test methods to evaluate photosensitive or photogenotoxic potential of chemicals (*OECD GD 397*). Therefore, the only type of phototoxic reaction that can be evaluated with validated methods is photoirritation. Moreover, only *in vitro* methods for the assessment of photoirritation are validated; there are no validated *in vivo* methods.

For the phototoxic hazard categorization of the biocidal active substance requiring phototoxicity testing, a stepwise tiered approach can be used.

Table 23: Test methods and tiered approach for the hazard assessment of phototoxicity

	Test method	Endpoint investigated Principle and properties of test method	Tiered test requirement Recommended strategy
Experimental evaluation of phototoxicity	OECD TG 432 *In vitro* 3T3 NRU Phototoxicity Test (3T3 NRU PT)	**Photo-cytotoxicity** investigated by the relative reduction in viability of cells exposed to the chemical in the presence versus absence of light. The sensitivity of the 3T3 NRU-PT is high and if a compound is negative in this assay, it would have a very low probability of being phototoxic in humans. However, a positive result in the 3T3 NRU-PT should not be regarded as indicative of a likely clinical phototoxic risk, but rather a flag for follow-up assessment (*ICH Photosafety Guidance*).	**Tier 1** To be requested as first step for assessment the biocidal active substances that require phototoxicity testing ➔ If negative, no further testing needed. Conclude on photoirritation potential based on the result. ➔ If positive proceed to Tier 2
	OECD TG 498 *In vitro* Phototoxicity - Reconstructed Human Epidermis Phototoxicity test method (RhE PT)	**Photo-cytotoxicity** investigated by the relative reduction in viability in RhE tissues exposed to the chemical in the presence versus absence of a noncytotoxic dose of simulated sunlight Appropriate test for Tier 2 according to SCCS 2021[44]: *As a second tier, the biological effects can be further evaluated on a reconstructed human skin model with some barrier properties.*	**Tier 2** ➔ If negative, no further testing needed. Conclude on photoirritation potential based on the result. ➔ If positive proceed to Tier 3
Toxicokinetic characterisation	ADME data OECD TG 427/428 (*in vivo* / *in vitro* skin absorption)	ADME data to assess the distribution and retention in the light-exposed tissues (e.g. skin, eyes). Dermal absorption to determine the penetration of the substance through the skin into the systemic compartment.	**Tier 3** If distribution, retention and dermal absorption of the substance are low, concern for phototoxic potential is deemed low (OECD IATA, 2024)**.**

If the outcome from OECD TG 432 is positive, the test chemical is subjected to the RhE PT as a follow-up testing. No further testing would be needed if the chemical exhibits no significant phototoxic effects in 3T3 NRU PT or RhE PT. In case positive predictions are made at this step, further assessment on the skin and eye distribution of the test chemical may be needed for risk assessment.

[44] SCCS/1628/21, Scientific Committee on Consumer Safety, SCCS NOTES OF GUIDANCE FOR THE TESTING OF COSMETIC INGREDIENTS AND THEIR SAFETY (March 2021): https://ec.europa.eu/health/sites/default/files/scientific_committees/consumer_safety/docs/sccs_o_250.pdf

If a test chemical is positive in the *in vitro* phototoxicity testing systems, the *in vivo* phototoxic risk might not be high if the chemical has low distribution and/or accumulation at the light exposed tissues such as skin and eyes. Therefore, toxicokinetic testing can be applied to the tested chemicals with "positive" prediction. Since the nominal dose vs. intake may differ, careful consideration on experimental conditions and chemical suitability should be made to avoid false negative predictions.

Further guidance on the assessment of phototoxicity is found in the *OECD GD 397*.

2 Effect assessment – hazard characterisation

2.1 Introduction

Hazard characterisation is performed using all the information available to derive systemic reference values that are protective for all systemic effects and external reference values where possible. The reference values should be protective for the whole population. For some effects it is not possible to derive reference values, and consequently a qualitative or semi-quantitative approach is needed (see Section 4.4).

For the derivation of acceptable exposure levels (AELs) and external reference doses (ADI, ARfD, AEC), all available hazard information regarding systemic toxicity and local effects is evaluated and, where possible, dose descriptors (NOAEL, LOAEL, NOAEC, LOAEC, BMD) are established for setting the PoD.

- A systemic effect is normally observed distant from the site of first contact when the substance or its metabolites becomes systemically available after having passed through a physiological barrier (skin or mucous membrane of the gastrointestinal tract or the respiratory tract).
- A local effect is observed at the site of first contact and is caused irrespective of a substance becoming systemically available.

Toxic effects on surface epithelia may also reflect indirect effects as a consequence of systemic toxicity or secondary to systemic distribution of the substance or its metabolite(s).

2.2 Identification of critical effects

In the first step of hazard assessment, the whole data package should be evaluated for assessment of the most relevant critical (i.e. the most sensitive) effects considering the biological plausibility of the dose-effect relationship, its consistency over the whole data package, the severity and reversibility of the effect, the mode of action if possible, and relevance for humans.

Appropriate studies should then be identified from which the relevant PoDs for each of the relevant exposure time frames can be used to establish AEL values (see Section 2.3.1). Indications of route specific sensitivity and dose-response relationship are taken into account when considering the relevant critical NOAELs.

Furthermore, the data package should be evaluated with respect to local effects at the port of entry, e.g. lesions in the airways in inhalation studies or on the skin in dermal studies. If the data package allows, external reference values could be derived as explained in Sections 2.3.7 and 2.3.8.

Before deriving reference values, it is important to determine whether the substance exerts its effects by a non-threshold mode of action (e.g. non-threshold mutagens or non-threshold carcinogens) or whether it is possible to derive a threshold. If the substance exerts its effects by a threshold mode of action, PoDs are set for the most critical effect(s) for deriving reference values.

If the substance exerts its effects entirely or partly by a non-threshold mode of action (e.g. for mutagenicity, carcinogenicity) or it is not possible to derive a threshold, a reference value cannot be derived and for these effects semi-quantitative or qualitative approach has to be followed for hazard and risk characterisation.

The decision on a threshold or non-threshold mode of action may not always be easy to make. It is possible that a biological threshold may be postulated (e.g. sensitisation, endocrine disruption), but the data do not allow identifying it. In case of uncertainty, assuming a non-threshold mode of action is the prudent choice.

For risks related to exposure to carcinogens, mutagens or reprotoxic substances at work, Directive 2004/37/EC (amended by Directive 2022/431[45] to include also reprotoxic substances) requires that occupational exposures are avoided/minimised as far as technically feasible. The Directive clarifies that safe levels can be identified for most reprotoxic substances, and the specific requirements of the Directive apply only to non-threshold reprotoxic substances.

2.2.1 Hazard information underlying the derivation of AELs/AECs

2.2.1.1 Toxicokinetics and dermal absorption

Data on toxicokinetics (TK) will provide information on the fate of the active substance in the human body. Sufficient information on absorption via the routes of exposure used in animal studies should be available for setting systemic reference values or to address species specific mechanisms if relevant. This is especially important when systemic reference values are derived from dermal studies.

Studies on dermal absorption can contribute significantly to the risk characterisation of biocides, noting that dermal exposure is often a major route of exposure. Guidance on TK is provided in Section 1.3 as well as within the *ECHA Guidance Vol III Part A*.

For further guidance on dermal absorption, see also *EFSA Guidance on dermal absorption*, *OECD GD 28* and *OECD 156*.

2.2.1.2 Acute toxicity

For acute toxicity, quantitative risk characterisation is performed. While acute toxicity may not be characterised by a NOAEL/LOAEL (or NOAEC/LOAEC), these can be used if available from sub-acute toxicity studies. LD_{50} or LC_{50} values are based on lethality and are not suitable for risk characterisation. For the derivation of acute AELs, information on acute effects from relevant studies (see section 2.3.1) can be used. Dermal exposure is normally compared to data from a repeated dose study.

Information relevant for acute toxicity may also be available from human case reports, such as poisoning incidents. The use of such information for risk characterisation will depend on expert judgement on the reliability and relevance of the reported information. Possible shortcomings include the unavailability of a no-effect level or a dose-response relationship.

[45] Directive (EU) 2022/431 of the European Parliament and of the Council, amending Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens at work

2.2.1.3 Irritation and corrosivity

Irritation and corrosivity are particularly significant for non-professional use since one must assume that no PPE is worn during application of products. Dermal contact can be significant depending on the formulation type and method of application for the product.

Quantitative risk characterisation is not appropriate, but a semi-quantitative or qualitative risk characterisation may be carried out (see Section 4.4).

2.2.1.4 Sensitisation

A qualitative risk characterisation is performed for sensitisation, as the proposed quantitative methodologies for dermal sensitisation are neither harmonised nor considered sufficiently protective, thus needing further scientific clarification (see Section 4.4).

2.2.1.5 Repeated dose effects

Repeated dose effects in the 28-day study, 90-day study, and long-term toxicity study are of concern whenever exposure occurs repeatedly, and especially if the effects are irreversible or only partially reversible. Most effects can be assessed in a quantitative risk characterisation; for possible non-threshold effects refer to the specific sections on genotoxicity, carcinogenicity and endocrine disruption.

2.2.1.6 Genotoxicity

Data from genotoxicity studies do not allow deriving a reference dose since a non-threshold mode of action is usually assumed for genotoxic substances, and the study setup is normally not adequate for assessing a threshold. A qualitative risk characterisation is performed. For genotoxic carcinogens, a semi-quantitative or qualitative assessment may be performed (see Section 4.4).

In some cases there may however be robust evidence of an indirect mode of action and a quantitative approach may be possible based on expert judgment[46]. Examples of non-DNA reactive mechanisms that may be demonstrated to lead to genotoxicity via non-linear or threshold dose/concentration response relationships are provided in Section 1.8.5.

2.2.1.7 Carcinogenicity

If a threshold mode of action is identified, a PoD should be derived using data from carcinogenicity studies. If carcinogenicity has a non-threshold mode of action (e.g. genotoxic carcinogens), a semi-quantitative or qualitative approach (see Section 4.4) should then be followed.

[46] https://echa.europa.eu/documents/10162/13579/jtf_opinion_task_2_en.pdf

2.2.1.8 Toxicity to reproduction and development

Effects on the reproductive system are often threshold-based allowing quantitative risk characterisation. Effects on the development of offspring can also be due to a genotoxic mechanism; in this case qualitative risk characterisation would be appropriate. The relevant effects can also occur on developmental neurotoxicity (DNT) or developmental immunotoxicity (DIT).

If AELs are based on severe reproductive effects, the need for an additional AF should be considered based on the severity of effects, their relationship to toxicity observed in the dams, and comparing the effect level with effects seen in other animals. For further guidance on setting an additional AF, see Section 2.3.4.

It should be taken into account that where the general public may be exposed, they are unprotected and may not be aware of exposure. This implies the need of stringency in setting the AF.

Fertility and developmental effects are relevant in considering repeated exposure, but effects on fertility have been reported already following short-term exposure. Developmental effects can occur following short-term exposure if this coincides with the critical formative stages of embryonic and foetal development.

2.2.1.9 Endocrine disruption

Effects seen in animal studies that are relevant for endocrine disruption are systemic, and as such they can be used in setting of NOAELs. However, where a substance is concluded to be an endocrine disruptor, no consolidated approach is available for the risk assessment. A critical aspect is the existence of a threshold that can in principle be seen in an animal experiment, while setting a reference value covering endocrine disruption might not be possible. Please see also 1.11.6.

2.2.1.10 Other toxicity endpoints

In addition to the above-mentioned effects, other effects such as immunotoxicity and neurotoxicity are relevant for professional and non-professional users. Secondary exposure can also be significant, including for children, especially if the use of the biocidal product leaves residues that are not removed.

2.3 Threshold effects

2.3.1 Relevant time frames in AEL derivation

A comparison of the relevant critical dose descriptors (NOAEL, NOAEC, LOAEL, LOAEC, BMD) for different time frames provides useful information on the influence of exposure duration on the severity and spectrum of toxicity. Assessing the entire data package can elucidate the time-dependency of toxicity, supporting an adequate assessment in varying time frames of exposure.

Three AELs are derived for different durations: acute AEL, medium-term AEL and long-term AEL, considering all available information on the time-dependency of toxicity.

For acute AEL, PoDs should optimally be derived from acute studies with single exposure, designed to establish a dose-response relationship including NOAELs. Relevant information on acute effects may however be available from subacute, subchronic and chronic studies, where particular weight should be given to observations at the beginning of the studies.

The proposed time frames to be considered for setting the different reference values are given in Table 24.

Table 24: Time frames relevant for setting and applying an AELs

AEL	Relevant toxicity studies	Relevant time frame of human exposure
Acute AEL	Single dose studies designed to determine PoDs* or repeated dose studies with acute effects, e.g. - acute neurotoxicity - 28-day/90-day studies - developmental toxicity - *In vivo* genotoxicity studies (acute systemic effects, i.e. clinical signs)	≤ 24h
Medium-term AEL	Repeated-dose studies, e.g. - 28-day/90-day studies - 90-d neurotoxicity - 12-month dog - developmental toxicity - 2-generation study	> 24 h – 3 months (max. 6 months)
Long-term AEL	Chronic or repeated dose studies, e.g. - 18-month/24-month chronic/carcinogenicity - 2-generation study - EOGRTS - developmental toxicity - 12-month dog	> 6 months (min. 3 months)

* Data from LD_{50} studies can be considered supportive if appropriate acute effects were investigated

In selecting the PoD for any time frame, the most relevant and most critical effects for the corresponding time frame should be considered, regardless of the study where they were identified. Table 24 provides guidance on deciding the relevant studies, but in addition:

- For acute effects, the PoD can also come from a repeated dose study when the critical effect is observed at the beginning of dosing and is relevant for single exposure, or when the critical acute effects were not adequately evaluated in a single dose study.
- For medium-term effects, long-term studies could be considered if there are indications that effects only become evident in chronic toxicity studies while they might be initiated earlier (sub-acute/sub-chronic). The indicated duration of up to 3 months can be extended up to 6 months based on the available dataset, and considering the toxicokinetic

properties of the active substance. For example, slow elimination could lead to prolonged internal exposure even after cessation of exposure. The reversibility of the repeated-dose and chronic effects have to be considered.

- For long-term effects, studies of shorter duration can be considered if the PoD is lower than the one based on a chronic toxicity study.

When valid developmental studies are available, all relevant critical effects should be evaluated together with observations from other studies. If the PoD from a valid developmental toxicity study is lower than in other studies and this cannot be explained by dose intervals, this PoD should be used in deriving the relevant AEL value that is protective to the whole population, including pregnant women. It should however be noted that developmental studies are often the only studies to use gavage dosing, which can give rise to effects related to Cmax. These may include clinical signs that may not be relevant to dermal exposures where Cmax is generally lower.

2.3.2 Dose-response assessment

The quantitative extrapolation of hazard from the animal experiment to humans is based on the most relevant endpoints, whereby a set of relevant points of departure (PoD) is established to cover the different exposure time frames and routes.

2.3.2.1 Identification of points of departure for systemic effects

2.3.2.1.1 Point of departure of an individual study

It is generally considered that many adverse health effects are not expressed until the substance (or metabolite) reaches a threshold concentration in the relevant organ. Reaching this threshold depends on the level and route of exposure of the organism to the substance, and this may vary considerably for different routes of exposure and for different species. The differences may result from toxicokinetics and toxicodynamics and/or mechanisms of action. The observed (no) effect level in a toxicity test is influenced by the sensitivity of the test system and dose spacing and is a surrogate for a "true" no adverse effect level.

The sensitivity and setup of a study may limit the reliability of the NOAEL. Such limitations may be related to the toxicological endpoint, the potency of the substance, the exposure period and frequency, dose spacing, the variability within the species, the number of dose groups and the number of animals per dose group. If a reliable NOAEL cannot be derived, at least a LOAEL should be identified if the study is overall considered reliable.

2.3.2.1.2 Selecting the points of departure

Considering the adequacy of data and the effects seen, the study in the most sensitive and relevant species resulting in the lowest dose descriptor (e.g. NOAELs, NOAECs, LOAELs, LOAECs, BMDs) is selected for establishing the PoD for reference value derivation.

If there are several studies addressing the same effects, normally the lowest relevant value should be used in reference value derivation. However, when the dose spacing in comparable studies results in different PoDs, it may be appropriate to consider these studies together, providing that both the study design and endpoints addressed are comparable. Regarding the similarity of the study design, one must consider the dosing regime, the duration and route of

exposure and the species/strain of animal. An 'overall NOAEL' should be the highest value identified in the available studies that provides a reasonable margin (≥2) over the lowest LOAEL, also considering the shape of the dose–response curve.

When valid developmental studies are available, all relevant critical effects should be evaluated together with observations from other studies. If the dose descriptor from a valid developmental toxicity study is lower than in other studies and this cannot be explained by dose spacing, this dose descriptor should be used in deriving the relevant AEL value that is protective to the whole population, including pregnant women. It should however be noted that developmental studies are often the only studies to use gavage dosing, which can give rise to effects related to Cmax. These may include clinical signs that may not be relevant to exposure via skin or via food, where Cmax is generally lower. Where duly justified, and based on expert judgment, it could be decided not to consider such effects in reference value derivation.

As a general rule, if several relevant PoDs are available, the one that would result in the lowest AEL for a given time frame should be chosen. The lowest PoD may not always result in the lowest AEL value as this also depends on the AFs used in deriving the reference value.

Using NOAEL as the PoD causes some uncertainty as it relies on information regarding one dose level rather than the full information on dose-response. Optimally, the shape of the dose-response curve should be taken into account and, in principle, the steeper the dose-response curve, the more reliable the NOAEL/LOAEL is.

Unless a threshold mechanism of action is clearly demonstrated, it is generally assumed that thresholds cannot be identified in relation to:

- mutagenicity or genotoxicity, (see section 1.8.5 for exceptions and further guidance),
- genotoxic carcinogenicity (for further guidance, see Appendix R.8-6 of *REACH Guidance R.8*),
- endocrine disruption.

For some of these effects, it may be possible to show dose-response relationship under experimental conditions, without being able to use this information in deriving reference values.

2.3.2.1.3 Benchmark dose (BMD)

Where the data allows, the BMD methodology should be preferred instead of using NOAEL/LOAEL as dose descriptor. This involves fitting a mathematical equation to the experimental dose-response data points and using all the plausible fit equations to select a BMD. The BMD is the dose that results in a predetermined level of adverse response, i.e. the critical effect size or benchmark response. The lower confidence limit (BMD_L) is normally taken as the PoD for determining reference values. The ratio of BMD_L and BMD_u provides a measure of uncertainty of the BMD and the experimental data.

The BMD is derived using all experimental data and reflects the dose-response pattern better than NOAEL/LOAEL. It is independent of predefined dose levels and dose spacing, resulting in a more consistent PoD that reflects more accurately the true potency of the substance, and provides a quantification of the uncertainties in the dose-response data.

For further guidance and information on the benefits of using the BMD, see *EFSA BMD Guidance*.

2.3.2.2. Identification of PoD for local effects

Irritant, corrosive and sensitising effects are normally driven more by concentration than a (systemic) dose, and the PoD for these properties is normally set as a concentration where possible. For guidance on this, see Section 4.4.2.

2.3.3. Modification of PoD

In some situations, it may be necessary to modify the PoD. Such situations can result from:

1. Difference in bioavailability between experimental animals and humans at the relevant level of exposure and via the same route of exposure;
2. Absence of a dose descriptor for a human exposure route;
3. Differences in human and experimental exposure duration and conditions;
4. Differences in respiratory volumes between experimental animals and humans, considering the activity level of both animals and humans.

Modification of a PoD may not be appropriate when human exposure is evaluated based on biological monitoring data, as the calculation of AEL/AEC values can be straightforward if studies in animals or humans are available that relate the effect directly or indirectly to the biomonitoring metric.

Further Guidance and worked examples on modification of PoD is provided in the *REACH Guidance R.8*, Section R. 8.4.2.

2.3.4. Assessment factors for systemic effects

Reference values such as AELs are derived by applying assessment factors (AF) to the (modified) PoDs. This accounts for extrapolation from animal toxicity data to the exposed human population.

The rationale for the choice of the AFs should always be explained in detail.

Default values

In the absence of sufficient chemical-specific data, a default AF of 100 is applied to the relevant NOAEL, resulting in the reference value for the corresponding time frame. The basis for this approach is a 10-fold factor for interspecies variation (toxicokinetic factor 4, toxicodynamic factor 2.5) and a 10-fold factor for intraspecies variation (toxicokinetic factor 3.16, toxicodynamic factor 3.16).

Chemical-specific values

A chemical-specific AF can be introduced to replace a default AF if specific information is available on all these factors:

1. Interspecies differences in toxicokinetics
2. Interspecies differences in toxicodynamics
3. Human variability in toxicokinetics
4. Human variability in toxicodynamics

Human data

Scientifically valid human data can be used to reduce the level of uncertainty in comparison to extrapolation from animal models. Such data may include biomonitoring studies, epidemiological data and medical poisoning records and historical human volunteer studies. Human volunteer studies should not be performed for the purposes of the BPR.

Human volunteer studies that have been performed for the purpose of regulatory frameworks other than the BPR should include clear statements that they were performed in accordance with internationally accepted ethical standards. If human data are used to derive a reference value, the interspecies AF can be omitted.

Additional assessment factors

In addition to uncertainties in interspecies differences and intraspecies variability, additional AFs should be considered for the following elements:

1. Nature and severity of the effect

   If the severity of the critical effect at the LOAEL is of particular significance, an additional AF between 2 and 10 can be considered even when a NOAEL was identified in the relevant study. For example, an additional assessment factor is applied for anticoagulant rodenticides (see Section 2.3.6.1).

2. A human subpopulation having greater susceptibility to the active substance

   If particularly vulnerable subpopulations may be exposed, an additional AF can be considered.

3. Difference in frequency or pattern of exposure in the study providing the NOAEL and the estimated human exposure (e.g. 6 h in the animal study and 8 or 24 h for humans)

4. Duration extrapolation should be handled on a case-by-case basis, ensuring that the best available data is used to derive reference values. The possibility for duration extrapolation cannot be used to justify waiving. Default values for duration extrapolation:

   - Subchronic to chronic: AF of 2
   - Subacute to subchronic: AF of 3
   - Subacute to chronic should normally not be necessary, but in exceptional cases an AF of 6 could be used. This could be e.g. if the chronic data is of insufficient quality to derive reference values, but it can nevertheless be concluded that chronic exposure does not result in more severe effects.

5. LOAEL to NOAEL extrapolation

   If the AEL is based on a LOAEL and not a NOAEL, an additional AF has to be considered. The value of this factor should be set based on the slope of the dose-response curve and the magnitude of the effect at the LOAEL. The use of LOAELs to set AELs is generally discouraged but can be acceptable where the effects at the LOAEL are of moderate magnitude and severity, noting that this will make use of existing animal data, potentially reducing the need for additional animal studies.

6. Slope of the dose-response curve

7. Overall quality of the toxicity data package

In each case, expert judgment is required, and it is necessary to consider the whole data package and avoid excessive AFs. The extent of overall uncertainty should be considered and reflected in the overall AF, especially when the default AF of 100 is exceeded.

Data waiving would normally not result in requiring an additional AF. It is however possible that waiving is justified although this will result in information loss that is not possible to cover by other studies. In such cases, an additional AF may be justified.

Allometric scaling

In the DNEL methodology under REACH, interspecies differences are assessed according to the allometric scaling principle (species differences in caloric demand) in combination with an additional default factor of 2.5 to account for remaining uncertainties.

Allometric scaling can be used when the toxic effect is essentially determined by the area under the (plasma) concentration curve over time, as opposed to the peak plasma concentration or another pharmacokinetic variable. Allometric scaling should not be applied, or should be adjusted, if 1) there are indications of significant interspecies differences in the bioavailability of the substance, 2) its clearance is known not to scale approximately with the body weight to the power of 0.75, 3) the kinetics cannot be assumed as dose-proportional over the dose range considered, or 4) if the animal species is especially susceptible or unsusceptible to the effects in question.

The following values are used for different species:

- Rat: 4 × 2.5 = 10
- Dog: 1.4 × 2.5 = 3.5
- Mouse: 7 × 2.5 = 17.5

These values could also be used for biocides, generally as a refinement step in derivation of reference values. Where applied, the *REACH Guidance R.8* (Chapter R.8.4.3.1) should be used.

Physiologically based kinetic (PBK) modelling

When considered of sufficient reliability, data from PBK modelling can be used to refine the AFs. PBK models provide a documentable, scientifically defensible means of bridging the gap between animal bioassays and human risk estimates.

Further information on characterisation, validation and reporting of PBK models is available in *OECD GD 331*. *REACH Guidance R.8* provides further information on the refinement of AFs by using PBK modelling.

2.3.5. Assessment factors for local effects

For local effects at the port of entry (skin, eye, respiratory tract and GI tract) it is sometimes justified to assume that either toxicokinetics or toxicodynamics (or both) do not contribute significantly to interspecies differences. This could be the case for example in direct/pH-driven chemical action on tissue/cell membranes. Based on sound scientific reasoning, the default 10-fold interspecies factor might then be reduced depending on the mode of action.

For local acute effects on the respiratory tract, it is prudent to assume that humans would be more sensitive than animals unless there is data to inform on this uncertainty. This is because there could be significant quantitative differences in deposition, airflow patterns, clearance rates

and protective mechanisms between humans and animals. The default interspecies dynamic factor of 2.5 should be applied.

When local reference values are set based on animal studies and there is no information of effects in humans at similar dose/concentration levels, the intraspecies AF should normally be 10. When setting the intraspecies AF based on human data, the dynamic factor of 3.16 should normally not be changed. The kinetic factor 3.16 cannot be excluded if the study population is small and no sensitive populations are studied. It is nevertheless possible to set an intraspecies AF lower than 10 (e.g. 3.16) even when dynamic and kinetic differences cannot be excluded, taking into account factors such as mode of action (e.g. pH-related irritancy at the first site of contact and no local metabolism involved) and low severity of the effects at LOAEC.

2.3.6. Derivation of systemic AELs

Depending on use patterns of biocidal products, humans will be exposed either as professional or non-professional users or due to secondary exposure, for example after application of biocidal products for domestic use. Risk assessment has to consider specific effects on sensitive sub-populations where appropriate, such as infants, children, the elderly, or women of childbearing age.

Systemic AELs are established as general health-based reference values for the human population as a whole, including sensitive sub-populations. These AELs are normally derived independently of the route of exposure, representing the internal (absorbed) dose available for systemic distribution from any route of exposure and are expressed as internal levels (mg/kg bw/day).

As the AEL should cover the whole population, the same AEL is valid for professionals and non-professionals. However, in exceptional cases where information is available on age specific kinetic differences, different AELs could be set for professionals and non-professionals. As an example, a lower AF was applied for the toxicokinetic component of intraspecies variability, where variability was shown to be minimal in all age groups below 75 years.

AELs should be established for acute, medium-term, and long-term exposure based on the full toxicological data package available. The values can be interpreted to cover up to daily exposure of the general human population (or a specific sub-population) likely to be without an appreciable risk of adverse effects during the specified time frame. The AELs should be established for each duration even if the toxicological data package does not indicate e.g. any acute hazard. In such a case, the acute AEL may be the same as the medium-term AEL value.

The majority of toxicity studies are oral studies, while the risk assessment in most cases focuses on the dermal and the inhalation exposure routes. To avoid the need for animal testing via different routes of exposure, systemic AELs are normally set on the basis of the available (mostly oral) studies by converting the external NOAEL to an internal NOAEL using the (oral) absorption value (e.g. for 30% oral absorption the AEL is: 0.3 × NOAEL / AF). If systemic AELs are derived from dermal or inhalation studies, information on absorption via the relevant route is used. Route specific information can reduce the uncertainties in risk characterisation associated with route-to-route extrapolation.

If there are local effects at the port of entry, or indications of route specific differences in toxicity that are not due to differences in absorption, route specific reference values may be considered.

All reference values that are derived from e.g. NOAEL/NOAEC values by applying AFs should be rounded to a single significant figure if the impact of rounding is less than 10%, and to two

significant figures if the impact of rounding to one significant figure exceeds 10%[47]. Rounding should happen as late as possible in the assessment process.

See also Section 4.3.2 for the use of a surrogate AEL in the context of combined exposure to multiple substances.

2.3.6.1 Specific situations

Anticoagulant rodenticides

For anticoagulant rodenticides, long-term studies are mostly not available, and acute studies are not suitable for setting AELs due to bioaccumulation, combined with the additive effects of anticoagulants. In terms of exposure and study duration, teratogenicity studies have been more relevant for AEL setting, and for this purpose the developmental study in the most sensitive species should be used.

Due to the specific nature of effects of anticoagulant rodenticides, an AF of 3 for duration extrapolation to chronic scenarios has been applied, and an additional AF of 3 has been used for all anticoagulant rodenticides due to the severity of the effect.

Pyrethroids

When appropriate data exists for dermal and inhalation routes, this data should be used to derive route specific systemic AELs, rather than using oral data and route-to-route extrapolation. Extrapolation would be problematic due to extensive hepatic first pass metabolism. This approach requires that 1) appropriate route specific data is available, and 2) large first pass metabolism is demonstrated or likely.

2.3.7. Derivation of external reference values for route specific effects

For active substances or biocidal products that produce local effects on the skin or the respiratory tract independently of systemic toxicity, a (systemic) AEL might not appropriately cover the actual (external) exposure. For some active substances it may be appropriate to assess both systemic effects and local effects quantitatively, and in such cases an AEC value can also be derived in addition to AEL values.

A route specific reference value is also needed if available data are showing that toxicity via a specific route (e.g. inhalation) is critically different from what is expected by absorption data in combination with oral studies. An external reference value could then be considered for the route in question.

For inhalation, an external reference value (AEC) should then be derived as local concentration in mg/m^3 air for the quantitative or semi-quantitative assessment where appropriate.

For dermal effects, a NOAEC should be set where the information is sufficient, without deriving an AEC value. In setting a NOAEC for an active substance, one should avoid deriving a value conflicting with an established specific concentration limit (SCL) under CLP. However,

[47] This is in accordance with Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured Data; EFSA Journal 2012;10(3):2579; https://www.efsa.europa.eu/en/efsajournal/pub/2579.

information that was not available when setting the SCL should be considered. For dermal effects, it is preferable not to set a defined limit for acceptable exposure. Local dermal effects seen in the studies and/or expected to take place in humans should however be described, providing a NOAEC/LOAEC that would usually be expressed as a percentage concentration. The usefulness of the information on dermal effects from animal studies may also be limited because the study setup would not necessarily reflect the human exposure situation. Nevertheless, where adequate information is available regarding cumulative dermal effects and this information is considered relevant for humans, an AEC could be derived.

For oral effects, setting a reference value would normally not be relevant but case-by-case consideration is needed. In some cases, a NOAEC could be set, without deriving an AEC. An oral AEC could be considered in exceptional cases.

However, as indicated above, for irritation/corrosion and sensitisation the derivation of PoD is difficult and, in most cases, a qualitative risk assessment will be performed (see Section 4.4.2).

2.3.8. Derivation of external reference values for exposure via food

In this section, exposure via food refers to all dietary exposure, including drinking water.

An external reference value for dietary exposure is needed when residues in food or feed are expected to arise from the use pattern of a biocidal product.

ADI and ARfD should always be derived if appropriate information is available and the substance exerts adverse systemic effects or local effects via the oral route. These reference values might not be derived 1) if not scientifically justified (e.g. highly reactive substances where no residues are expected), 2) if the effects have entirely or partly a non-threshold mode of action, or 3) it is currently not possible to derive a threshold.

- **ADI** (acceptable daily intake) is an estimate of the amount of a substance in food or drinking water that can be consumed over a lifetime without presenting an appreciable risk to health (WHO, 1987[48]). The ADI is expressed in mg/kg bw/day.
- **ARfD** (acute reference dose) is an estimate of the amount of a substance in food or drinking water that can be ingested over a short period of time, usually during one meal or one day, without appreciable health risk to the consumer (JMPR, 2002[49]). The ARfD is expressed in mg/kg bw.

The setting of the ADI and ARfD should follow:

- the WHO Guidance on Dose-Response Assessment of Health-Based Guidance Values (2020[50]),
- the principles for ADI and ARfD setting in plant protection products,
- the current guidance in selecting the critical PoDs and appropriate AFs.

JMPR has given detailed consideration to the use of particular toxicological endpoints that are most relevant to establishing ARfDs (reviewed by (Solecki *et al.*, 2005)), with a focus on

[48] WHO 1987, International Programme on Chemical Safety, Principles for the safety assessment of food additives and contaminants in food. Environmental Health Criteria 70
[49] JMPR 2002, Pesticide residues in food –Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues
[50] WHO/IPCS, 2020, Principles and methods for the risk assessment of chemicals in food, Chapter 5: Dose-Response Assessment and Derivation of Health-Based Guidance Values

interpreting effects that have been problematic when deciding whether an effect is relevant to acute exposure. More recently, JECFA has published guidance on the establishment of ARfDs for residues of veterinary drugs, covering toxicological, pharmacological and microbiological effects (*JECFA ARfD guidance*). These can be used as supportive guidance in ARfD setting for biocides.

What to consider in setting ADI and ARfD

Already existing ADI and ARfD values from other European frameworks (e.g. food and feed additives, veterinary medicinal products, plant protection products) should be taken into consideration whenever possible. Conflict of scientific opinion should be avoided[51]. Deviations from reference values already identified by other regulatory bodies may be possible on a case-by-case basis if different information or new methodology is available and a robust justification is provided.

For ADI derivation, long-term oral studies are normally used because in these studies the test substance is normally incorporated in the diet and administered for the majority of the lifetime on a daily basis, reflecting the ADI concept. Unless justified otherwise, it is recommended to consider deriving ADI and $AEL_{long\text{-}term}$ on the basis of the same (long-term) NOAEL.

For ARfD derivation, short-term studies are most suitable. If the critical effect has not been adequately evaluated in a single dose study, a repeated dose toxicity study should be used. Normally, all indications of acute toxicity observed in repeated dose studies should be considered as potentially relevant, in particular effects observed at the beginning of repeated dose studies. This also applies to developmental effects, which typically result from exposure during sensitive periods. Unless justified otherwise, it is recommended to consider deriving ARfD and AEL_{acute} on the basis of the same (acute) NOAEL. The effects that are not relevant for residue intake should be disregarded, considering the administration route in the animal study.

Gavage administration may result in marked differences in kinetics following the bolus administration of a high dose, compared to more frequent intakes of small amounts through the diet. Local gastrointestinal effects might not be relevant if they can be shown to be due to gavage administration, and dietary administration does not produce the same effects. For example, if diarrhoea and vomiting in dogs are due to local effects and high active substance concentrations following specific dosing methods such as capsule administration or gavage, then these effects should not be considered in setting the ADI/ARfD.

If an active substance administered via food/diet exerts local toxicological effects on the gastrointestinal tract, these may be considered relevant for ARfD derivation. Exceptionally these could also be used for ADI derivation if the local effect may lead to long-term effects (e.g. persistent low grade local irritation leading to inflammation over time). For such direct effects, a reduction of the AF may be considered. While the principles in Annex II of the plant protection products Regulation 1107/2009 require applying at least the default AF of 100 for both ADI and ARfD derivation, a deviation may be justifiable when sufficient information is available. For ARfD derivation, a reduction of the AF for human toxicokinetic differences may be justified if it can be assumed that the concentration rather than the total intake determines the effects.

The rabbit is known to be sensitive to gastrointestinal disturbances due to a disruption in the balance of the caecal microflora. Some biocidal substances disturb the balance of the rabbit intestinal/caecal microflora leading to malnutrition and subsequent maternal toxicity, while

[51] See also Article 95 of REACH Regulation and ECHA Management Board Decision 18/2013.

humans might be exposed to higher doses without similar concern. For such substances, the information from a prenatal developmental toxicity study might not be relevant for humans.

2.3.9. Deriving reference levels (AELs) when a community/national OEL is available

When an EU IOEL exists, under conditions described in the *REACH Guidance R.8* (Appendix R 8-13), the basis for the IOEL can be considered in deriving a reference value for active substances, applying the methodology described in this guidance. Other Occupational exposure limits can be considered as additional information.

2.4. No threshold identified

When no reliable PoD can be set for a given endpoint, a fully quantitative approach may not be possible. This usually applies for irritation/corrosion, sensitisation, mutagenicity/ carcinogenicity and endocrine disruption (see Section 2.2.1.9).

For local effects, additional guidance for qualitative and/or semi-quantitative risk characterisation is provided in Section 4.4.2 of this guidance.

For mutagens and carcinogens where no threshold can be identified, a semi-quantitative approach can be considered (see Section 2.4.1).

For endocrine disruption, there are currently no qualitative or semi-quantitative approaches.

2.4.1 Non-threshold carcinogens

As a general rule, a risk for the general public from secondary exposure to a non-threshold carcinogenic biocidal substance is unacceptable. See also reference to Directive 2004/37/EC in Section 2.2.

A qualitative risk assessment should always be performed, and this should lead to identification of strict risk management measures to be used.

If the information allows, a semi-quantitative risk assessment can be performed to inform on the residual exposure levels; it then needs to be concluded whether these are tolerable/acceptable or should be further reduced. This assessment can be performed according to the methodologies described in *REACH Guidance R.8*, always considering the human relevance of the mode of action.

- The 'linearised' approach concerns lifetime cancer risk and is based on the assumption of a linear dose-response for the carcinogenic effect, assuming a supra- or sublinear dose-response when appropriate. A relevant dose-descriptor is selected and, if necessary, modified to adjust for the differences in human and animal exposure routes, conditions etc. The DMEL is derived for a specified cancer risk level, and for each relevant exposure pattern, by a linear high to low dose extrapolation. The specified risk level of very low concern has to be decided on a policy level: based on experience in applying cancer risk values within and outside the EU, levels of 10^{-5} and 10^{-6} have been considered as indicative tolerable lifetime cancer risk levels when deriving reference values for workers and the general population, respectively. These values represent an increase of lifetime cancer risk in 1 per 100 000 exposed individuals (10^{-5}) or 1 per 1 000 000 exposed individuals (10^{-6}).

- In the 'Large Assessment Factor' approach, the dose-descriptor is selected and modified to adjust for the differences in human and animal exposure routes, conditions etc. AFs are applied to derive a DMEL for each relevant exposure pattern. The AFs include the ones used for threshold effect assessments, and additional AFs for the nature of the carcinogenic process and to account for the reference point not being a NOAEL. An intraspecies AF of 10 should be used for biocides instead of 5 that is used for workers in REACH. The resulting overall AF is generally much higher than for threshold effects.

In most cases, a similar DMEL is reached when applying either of the approaches above. The values can be used in judging the significance of the residual exposure remaining after introducing the strict risk management measures, and can guide in further targeting the risk management measures. Exposure levels below the DMEL are considered to represent an appropriately low risk of effects (cancer).

A narrative description of the overall quality of the data has to be provided, giving special attention to reliability of the exposure assessment and representativeness of actual exposure situations.

The assessment based on the REACH guidance cited above should be done on a case-by-case basis, considering all biocide-specific guidance as well, noting e.g. the need to correct external reference values (such as DMEL) for absorption. Expert judgment will play a considerable role in the assessment. Conclusions on the cancer risk should be indicated in a clear and transparent manner, with special consideration of risk management measures.

If a DMEL cannot be derived due to the absence of cancer data, the possibility of read-across should be considered to derive a DMEL. Alternatively, the TTC concept may be used (see Section 4.2.4).

3. Exposure assessment

3.1. Introduction

The BPR requires a risk assessment of biocidal products before these can be placed on the market. The estimation of human exposure is a fundamental element of the risk assessment and requires quantification of the levels of exposure for both users of the biocidal product and others who may be exposed following its use.

Biocidal products are authorised for the proposed use(s) and there is no legal basis for determining and assessing worst case conditions for a potential misuse. Misuse should thus not be considered in the exposure assessment of biocidal products.

In most cases experimental exposure data are not available and the exposure assessments are based on dedicated exposure models if possible. Otherwise, an exposure study shall be carried out by the applicant to indicate appropriate safety for humans during use if authorisation is still to be granted.

This guidance presents a tiered approach for conducting exposure assessment with refinement options to be chosen using higher tier methodologies when needed. This can be the case when risk is identified for specific exposure scenarios and refinement needs to be considered.

This section outlines the principles of exposure assessment for the assessment of exposure from biocidal products.

For the actual estimation of exposure, additional technical guidance on types of generic models, calculations and default parameters is provided in *BHHEM*.

Note that there are several references in this section to *BHHEM* for detailed information on the methodology and the reader is advised to read this section in conjunction with *BHHEM*.

3.2. General principles of exposure assessment

3.2.1. Introduction

The fundamental concept underlying the approach for human exposure assessment is the need to establish the full range of human exposure situations that could occur from the use of a biocidal product and to consider all routes of exposure. The exposure assessment therefore requires:

- Information on the product type / formulation that will be the source of exposure;
- identification of the exposed population (industrial, professional, non-professional, general public);
- identification of exposure scenarios / patterns of use for each population including routes of exposure;
- calculation & quantification of potential chemical intake

Understanding the source of exposure is the first step in preparing the exposure assessment. Identification of the product type(s) where the active substance is contained is needed to enable mapping of the patterns of use with specific product type(s) and/or formulations and the corresponding exposure via different routes of each exposed population.

3.2.2. Patterns of use / exposure scenario

For exposure assessment, the different types of potential users as well as the exposure of individuals via secondary (indirect, unintentional exposure) pathways of exposure need to be considered. As a first step, a list of potential uses and releases enables identification of the populations/individuals that are likely to be exposed directly or indirectly to the biocidal product.

Regarding the potential exposed population from the use of biocidal products, these can be divided into four categories:

- Industrial users;
- Professional users;
- Non-professional users (consumers);
- General public (adults, infants, and children).

3.2.2.1. Industrial and professional users

The terms 'industrial users' and 'professional users' are used to indicate the area where a task is performed and the intended use: within or outside industrial settings.

Both industrial and professional users come into contact with the biocidal product as a consequence of their professional life. In general the professional user is subject to EU and national worker protection legislation, such as the EU Chemical Agents Directive (Directive 98/24/EC on the protection of the health and safety of workers from the risks related to chemical agents at work) and has residual risk controlled through control measures and the use of PPE.

There are also trained professional users, who will have expert knowledge and skill in handling hazardous biocidal products and their pattern of use will show greater frequency and/or duration of use, leading to greater quantities of product used. These users are considered to be in possession of the required knowledge, skills and competencies to be able to consider the risks to themselves and other non-target species. For example, when using rodenticides, they would be expected to follow integrated pest management before deciding that use of a rodenticide is necessary to control an infestation. They are also expected to observe more complex instructions for use and RMMs in the product authorisation than non-trained professional users. A justification/explanation should be added to the assessment why a trained professional user is considered instead of a professional user as a user category (e.g. national legislation requirements)

A use of a biocidal product shall be limited to trained professional users in e.g. the following cases:

- the required task or RMMs would exceed the standard education of the typical professional user,
- fumigation with gases classified as Acute Tox. 1, 2 or 3,
- self-contained breathing apparatus (SCBA) was assigned for the use,
- biocidal products classified as 1A or 1B for carcinogenicity (Carc.), germ cell mutagenicity (Muta.) or reproductive toxicity (Repr.)

See also Example 5 in Section 4.4.2.3.3 concerning professionals trained for the task vs. professionals not trained for the task.

3.2.2.2. Non-professional users

The non-professional user (consumer) is a member of the general public who may be exposed to biocides by using a consumer product. The consumer is unlikely to take informed measures to control exposure and may not follow exactly the instructions for using the biocidal product. In general, the non-professional pattern of use can be expected to have a lower frequency and/or duration of use.

The consumer exposure assessment should address the intended uses of the product. However, since consumers include diverse user groups (e.g. adults, elderly, children), assumptions applied for professionals cannot necessarily be taken into account in each case. Therefore, the exposure assessment should include conservative assumptions. For example, some consumers may experience higher exposures compared to others based on physical ability, e.g. to spillages during mixing and loading processes based on non-routine use or equipment not being available (e.g. funnel).

Consumers will not normally use PPE unless it is very strongly recommended by the manufacturer and/or provided with the product. Only typical clothing should normally be assumed when carrying out consumer exposure assessments.

3.2.2.3. General public

The general public (adults, infants, children) are the individuals that are likely to be inadvertently exposed to the biocidal active substance directly or indirectly via the environment and/or other routes of exposure without using the biocidal product themselves. This would cover both residents living in areas treated with biocides and bystanders that are adjacent to an area treated with a biocide. Longer exposure may take place to residents, while for bystanders, acute exposure would normally be assumed.

3.2.3. Primary (direct) and secondary (indirect) exposure scenarios

3.2.3.1. Principles

For each of the identified populations that are likely to be exposed to the biocidal product, the type of expected exposure needs to be defined. The type of exposure expected for each of the identified populations should be characterised as primary (direct) or secondary (indirect).

Primary exposure (see Section 3.3) occurs to the individual who actively uses the biocidal products. The user may be a professional at work or a non-professional. Professional users differ from non-professional users in a number of aspects and a distinction between the two is necessary in exposure assessments.

Secondary exposure (see Section 3.4) may occur during or after the actual use or application of the biocidal product. For professional users it is useful to make a distinction between *intentional* and *incidental* secondary exposure scenarios. An intentional secondary exposure scenario is any secondary exposure incurred during a worker's regular employment duties, for example, a carpenter exposed to wood dust impregnated with a biocide. In most instances the professional users' flowchart will provide the most suitable approach for these scenarios. Incidental secondary exposure relates to any exposure not necessarily incurred during employment but resulting from the professional use of a biocide. Home laundering of contaminated work clothes is a typical example of incidental secondary and non-professional exposure. In most instances these exposure scenarios are best assessed using the methodology

for non-professional uses (consumers) as a realistic worst case with refinement options if needed.

The user of a product may be subject to both primary and secondary exposure, whereas the "non-user" (general public) will only experience secondary exposure. Primary exposures are generally higher than secondary exposures, while some specific subgroups of the population may experience higher secondary exposures because of their specific behaviour, e.g. children crawling on a treated carpet.

3.2.3.2. Routes of exposure

For both primary (direct) and secondary (indirect) exposure scenarios, human exposure can occur through any or all of the following exposure routes:

- inhalation route;
- dermal contact (dermal route);
- ingestion (oral route);
- eye contact (ocular route).

The likelihood of the biocides entering the body by the three major routes should be determined: inhalation, absorption through the skin, or ingestion. Although not a major route of exposure, the potential for local effects resulting from exposure of the eyes will also need to be considered, particularly when handling irritant/corrosive substances. If exposure via one or more of the pathways does not occur, no further assessment is needed for that route of exposure. Exposure assessment should be performed for each relevant route of exposure.

Once all the exposure assessments for all relevant routes have been explored, the systemic (internal) dose from these is calculated so that the single internal exposure value is compared with the corresponding AEL for quantitative risk characterisation.

3.2.3.2.1 Inhalation exposure

In some cases, inhalation exposure can be the predominant route of exposure, e.g. when using volatile material (vapour pressure >0.01 Pa at 20°C) or from a process generating aerosols in an enclosed space. Inhalation exposure is usually derived from the airborne concentration in the breathing zone of the exposed individual. It may refer to the active substance or to the product in use and is expressed as mg/m^3 as a time weighted average concentration over time. The potential inhalation exposure can be reduced by technical measures such as local exhaust ventilation, or by using RPE. The resulting actual exposure takes the effectiveness of these risk management measures into account.

3.2.3.2.2 Dermal exposure

Exposure to the skin is usually a significant aspect of human exposure to biocides and can be subdivided into potential or actual dermal exposure.

Potential dermal exposure is the amount that deposits on the clothes or gloves and on exposed skin. The most common metric measurement for biocides is the amount of biocidal product that deposits per unit time[52] (mg/min) or task (mg/cycle);

Actual dermal exposure is an estimate of the amount of contamination that reaches the skin. It is dependent on the effectiveness of clothing and PPE and is often expressed as weight of biocidal product on skin (mg on skin). Actual dermal exposure arises through:

- direct deposition on exposed skin;
- permeation through clothing, penetration of clothing around fastenings, openings and along seams;
- incidentally through contact with surfaces, and when putting on and taking off contaminated clothing or PPE.

For the assessment of professional exposure, it is estimated that the calculated external dose (mg/min × duration of exposure, resulting in mg per person) will stay on the skin for the whole shift or even longer. Similar assumptions should be made for non-professionals, meaning that for daily exposure, the skin contamination remains for that day, unless thorough cleaning of the skin can be assured.

3.2.3.2.3 Ingestion exposure

This is the amount entering the mouth, excluding what is inhaled. There are no standard methods for quantifying exposure by ingestion, but it can be inferred from biological monitoring studies. It is expressed as mg per event or mg/day. It is usually assumed that ingestion exposure in workplaces does not occur when good hygiene is assumed. This may not be true in all cases, especially when there is a regular contact between the contaminated skin and the mouth region. At present there are no established ways to estimate oral exposure to humans, apart from biomonitoring where oral, dermal and inhalation exposure are integrated.

3.2.3.2.4 Systemic exposure

The estimates of exposure described above cover the three major routes outlined above and relate to external exposure. To estimate systemic exposure, two approaches can be taken.

The first is to calculate the systemic body burden from these values. This conversion is based on the selection and use of a variety of physiological default values (e.g. body weight and breathing rate) for specific situations.

The second approach (reverse reference scenario, section 3.2.5.4) is to use route specific external exposure data and compare that to limit values for each relevant route of uptake. This approach could be preferred if the underlying data for the first approach is deemed unreliable, and in particular if there is reliable information on the route of exposure under assessment.

Guidance and default values regarding dermal absorption and physiological factors are given and referred to in Section 1.3.3.3, as well as in the *ECHA Guidance Vol III Part A*. In addition, the

[52] For liquids mg/min is often used interchangeably with ul/min for water-based formulations with a density close to 1. For liquids more generally, expressing dermal exposure in ul/min and using a weight/volume concentration of active substance will avoid the need to correct for density.

"Default Human Factor Values for Human Health Exposure Assessment" within the *BHHEM* should also be consulted.

The most appropriate way of assessing total systemic exposure is by human biomonitoring (HBM). HBM can be an important tool for assessing exposure to biocides and their health risks providing information on cumulative, aggregated and internal exposure accounting for different exposure sources and routes. However, applying HBM data in exposure and risk assessment is limited by guidance available e.g. for interpreting the biomarker levels concerning adverse health effects and for linking internal exposure levels to external intake/exposure (Santonen *et al.*, 2023). Exposure assessment is usually based on the intended use of a biocidal product containing the active substance (and SoCs).

3.2.4 Tiered approach in human exposure assessment

If measured exposure data is available and is representative, covering all the tasks in the scenario and is accompanied with contextual information, such data should be used as the first step in a tiered approach to human exposure assessment. Sometimes this is considered as part of the tier 1 assessment. Each measurement must include separate sampling and preparation. It is not sufficient to add a sample extract several times to a measuring device (e.g. GC, HPLC). In addition, all essential underlying parameters (e.g. temperature, room size, air exchange) must be specified.

Where there is a lack of pre-existing measured exposure data, a tiered approach to model exposure using mathematical exposure models needs to be planned and conducted. It is useful to initially conduct an exposure assessment based on realistic worst-case assumptions and to use default values when model calculations are applied. If the outcome of the risk assessment based on worst-case exposure assumptions is that the use of a biocidal product does not present unacceptable risk, no further refinement of the exposure estimate is required. If unacceptable risk is identified, the assessment must be refined using additional data and/or reasoned arguments based on expert judgement to allow a more informed decision.

This Tiered approach is a logical stepwise part of risk assessment, using the available information in reducing unnecessary requirements for human exposure surveys or studies. The three Tiers described below illustrate how this iterative process might progress. The tiering scheme should be read together with Section 3.3 regarding refinement options for exposure assessment.

The tiering from low to higher tiers can include options regarding exposure controls, including PPE for professional users, or higher tier methodology such as mathematical models and probabilistic approaches versus deterministic ones used in lower tiers.

Tier 1

This screening Tier should be kept simple. The assessor should select the top end value from a single exposure study, the recommended indicative value from an empirical (database) model, or a worst-case estimate from a mathematical exposure model. Tier 1 estimates should be based on realistic worst-case frequency and duration of use and must not take account of exposure reduction measures such as LEV or mechanical ventilation, or PPE, unless these measures have already been included in the measured data used for exposure assessment.

Dermal absorption data, either default values according to *EFSA Guidance on dermal absorption*, or application-specific measured data, are already considered in Tier 1 exposure assessments.

If the Tier 1 assessment shows unacceptable risk, a refined exposure estimate is required if possible.

Tier 2

The second Tier is more complex and requires further specific data and/or reasoned arguments to produce a more refined exposure assessment. The exposure studies/models are used in the same way as in Tier 1 but specific data on frequency and duration of use, transfer factors and the effects of exposure reduction measures may be used to modify the exposure assessment. The use of PPE by non-professional can normally not be assumed. The options for exposure reduction measures and appropriate defaults are discussed in Section 3.3. Information on quantitative assessment of these measures is included in the *BHHEM*.

If Tier 2 shows unacceptable risk, a third iteration will be required if possible.

Tier 3

The most detailed level of risk assessment may involve further refinements in the exposure modelling or commissioning of surveys or studies with the actual product or with a surrogate. The surveys must be representative, cover all the key tasks within the scenario and provide detailed information on patterns of use.

If Tier 3 shows unacceptable risk and no further refinement is possible, safe use cannot be demonstrated.

3.2.5 Exposure estimation - types of exposure data and approaches

Although substance specific measured data are preferred over modelled data if available, it may contain considerable uncertainty due to temporal and spatial variations as well as deficiencies in the quality and quantity of the available measured data. It is therefore advisable to compare measured data with modelled exposure estimates. This will require a critical analysis of the results and reasoned arguments to explain the similarities or differences between the two estimates. The ultimate choice of exposure estimates should be made based on the robustness/representativeness of the measured and modelled data for the situation and conditions under consideration.

3.2.5.1 Product specific exposure data

In general, product and use specific measured exposure data are preferred for the assessment of biocidal products. However, in some cases other data sets or approaches may have been established or agreed for the assessment, for example those mentioned in the *BHHEM*.

Measured exposure data for the specific product and information describing this data may be available from workplace exposure assessments or dedicated monitoring surveys. The data should be accompanied by sufficient information to place the exposures in context with respect to the pattern of use and control. All data will require careful evaluation and should have been collected following good occupational hygiene practice, preferably applying standardised procedures particularly with respect to sampling strategy, measurement methods and analytical techniques.

3.2.5.2 Generic exposure data

Generic exposure data describes measured exposure data obtained from similar operations utilising similar biocidal products. The data are collected from worker exposure studies or, in the case of consumers, from simulation studies using analogous products. These data are used to develop simple (generic) database exposure models for particular product types and specific use scenarios.

Generic exposure modelling is a useful tool because of its ability to predict the likely levels of occupational exposure of users of biocides and to estimate the effect of changes in conditions of use on exposure. Where representative measured exposure data is not available that would cover all the tasks in the scenario and be accompanied with contextual information, modelling is the initial basis for exposure assessment. Generic exposure models may also be used instead of, or together with exposure data if there is significant uncertainty associated with the quality of this data.

Generic exposure data can also be used to develop more complex computer-based data models.

3.2.5.3 Mathematical models

In the absence of product specific and/or generic exposure data for a particular use scenario, mathematical exposure models can be used. As in the case of generic exposure models, mathematical exposure models may also be used instead of, or together with exposure data for the specific product and generic models if there is significant uncertainty associated with the exposure estimates derived from the first two approaches. Further details of mathematical exposure modelling are provided in Appendix 3-4.

3.2.5.4 Reverse reference scenarios

In the absence of product specific or generic exposure data or suitable mathematical models, a reverse reference scenario can be used to determine the acceptable exposure level. A reverse reference scenario can be used to determine an estimate of the maximum amount of exposure that might be acceptable and its likelihood of occurrence as a realistic worst case. Using the relevant AEL, it is possible to calculate the amount of product that would lead to that dose by a specific route. This calculated amount can be compared with the amount of exposure that is considered realistic. It is also possible that in specific cases AELs are not available (e.g. for SoCs) and it is necessary to use other reference values. An example on using a reverse reference scenario is provided in Appendix 3-3.

3.2.5.5 Suitability of exposure data sources

Any representative and robust data source that describes relevant exposures can be used in the exposure assessment, when the contextual information is available.

Single values must be drawn from the distributions to estimate exposures where no directly relevant data exist. Distributions of human exposure data are commonly accepted as being approximately log-normal.

Exposure estimates for a single scenario can be estimated by a percentile from the data distribution. However, if this is repeated several times, simple addition of percentile values can

show gross deviations in the final estimate, especially with high or low percentiles. This applies to:

- summing the data for several daily treatment cycles;
- summing the data for the inhalation and dermal exposure routes;
- adding the phase of use estimates;
- combining primary and secondary exposure;
- aggregate exposure from all sources of the chemical.

The elements regarding uncertainty in exposure estimates when combining tasks need to be considered in higher tier methodologies (see section 3.3.2) if risk has been identified in a Tier 1 or Tier 2 (see section 3.2.4 for Tiers in Exposure Assessment).

An alternative to extracting values from data distributions is to use the entire data distribution in a probabilistic assessment. This is of particular importance for estimating combined exposure.

3.3. Primary (direct) exposure assessment

This section presents a summary of the main components from the pattern of use that are needed in the different types of exposure scenarios.

The essentials of exposure assessment for primary (direct) exposure are:

- Product composition & physico-chemical properties (physical state, concentration, vapour pressure of the active substance);
- Type of user: who will use the product;
- Duration and frequency of use, for each stage of use (see Section 3.3.1);
- Method of application or task: where and how the product will be used (see Section 3.3.2);
- Expected exposure controls (see Section 3.3.3.1);
- Refinement of exposure assessment if risk is not acceptable (see Section 3.3.3).

Product specific data is used as the first option. In the absence of such data, the next option is the use of default parameters (generic exposure data) or specific models available for the exposure scenario under consideration.

If an acceptable risk is identified, no further refinement is needed. If unacceptable risk is identified, refinement of exposure should be performed. This can be conducted taking into account:

- refinement of parameters (defaults) used in the exposure assessment, with appropriate justification,
- application of exposure control measures: for industrial/professional users this can include PPE but not for non-professional users (with rare exceptions),
- generation of product specific data,
- uncertainty assessment of the various steps of the exposure assessment performed.

Information on the pattern of use can be gathered through surveys or generic data on similar products. Specific information on patterns of use for many biocidal product types is limited, and such information may need to be generated to facilitate the assessment.

The most relevant data requirements for primary (direct) exposure assessment are listed in Table 25.

Table 25: Overview of requirements for primary (direct) exposure assessment

	Data Requirements	Priority	Comment
Product	Physical properties	Essential	Liquid / solid / particle size, aerosol, volatility
	Package details	Essential	Volume, material, closure, bulk delivery
	Formulation details	Essential	Active substance and co-formulants, in situ generation, ready-to-use product, concentrate
	Site inventory	Desirable	Amount, delivery frequency
	Storage information	Desirable	
Purpose of product	Where used	Essential	Location / system treated
	Description of tasks	Essential	How used, application rates
	Equipment used	Essential	Pressures, volumes
Use environment	Containment	Essential	Barriers to exposure, ventilation
	Pattern of control	Essential	Full containment, LEV, segregation, dilution ventilation
	Use pattern	Essential	Closed system, within a matrix, non-dispersive, wide dispersive
	Room parameter	Desirable	Room volume, room size, ventilation
Mixing and loading phase	Task	Essential	Description
	Frequency of task	Essential	Events per day
	Duration of task	Essential	Event duration
	Quantity used per task	Desirable	
	Dilution rate	Essential	
Application phase	Task	Essential	Description, continuous / intermittent / event
	Frequency of task	Essential	Events per day
	Duration of task	Essential	Event duration
	Quantity used	Essential	Not always relevant
	Area / volume treated	Essential	Not always relevant
	Timing	Desirable	Season/ weather conditions
	Task	Essential	Description

Post-application phase	Frequency of task	Essential	Events per day
	Duration of task	Essential	Event duration
Disposal	Task description	Desirable	e.g. strip old coatings, collect dead vermin
Primary exposure	Use sector	Essential	
	Mode of exposure	Essential	Inhalation / dermal / oral, by task
	Proximity to exposure source	Desirable	Distance e.g. arm's length
	Operators per task	Desirable	
Some data may be better expressed as ranges and likely values, rather than as single values. If ranges are given, the worst-case assumption should be used for scenario calculations.			

3.3.1. Duration and frequency of use

The frequency and duration of a task are major determinants of exposure. The frequency of a task is critical in deciding whether the exposure is chronic or acute. It should be expressed as events per day and on how many days per year the user is exposed. Duration of exposure should be expressed as minutes or hours per day.

In some cases, there may be variability in the pattern of use across the EU (e.g. different user groups; professional user versus non-professional user/consumer) based on e.g. regional or climatic differences.

The relevance of a claimed pattern of use must be considered especially in product authorisation. Justification is needed where the pattern of use does not follow a harmonised approach.

3.3.2. Method of application or task

Primary exposure concerns industrial users, professionals and non-professionals (consumers) who use and apply a biocidal product. The overall exposure scenario will consist of a series of tasks that can be allocated to three distinct phases of use:

1. **Mixing & loading** includes the tasks involved in delivery and handling of bulk ready-for-use and concentrate products, dilution of concentrates and the introduction of product to the application apparatus/system.
2. **Application** involves all uses of biocidal products, including application by hand or hand-held tools, dipping, spraying, foaming, handling treated articles, and in machining. This can lead to the exposure of people who are present during the product application (secondary exposure).
3. **Post-application** includes exposure taking place when cleaning and maintaining equipment and tools. Secondary exposure is included in the post-application phase.

The contribution to each route of exposure may vary considerably between these phases depending on the biocidal product and method of application, given that mixing and loading can reflect exposure to a concentrate, application to a diluted product, post-application to vapour or dried residue and removal to waste material. Exposure data often relates to full-shift sampling and therefore includes all three phases of use. However, it is important to ensure that each phase of use has been accounted for in the exposure assessment.

3.3.3. Refinement of exposure estimates

3.3.3.1. Exposure controls

Exposure can be prevented by a variety of means, including elimination, substitution and modification of a process or substance to reduce emission or release.

For biocides, preventing exposure may not be reasonably practicable and it must be controlled. Further details regarding refinement of exposure estimates are included in Appendix 3-1.

Non-professionals and the residential environment

Whilst non-professional users may wear overalls, gardening or kitchen gloves, or even a dust mask, such usage cannot be assured and must not be assumed in exposure estimation. An exception is anti-foulant products for which the use of gloves can be assumed in the exposure assessment. Other exceptions might be possible in line with CA meeting agreements[53]. For inhalation exposure, no exposure reduction should be assumed.

3.3.3.2. Higher tier methodologies

Higher tier methodologies usually include more elaborate exposure assessment using probabilistic approaches and/or more complex mathematical models. Also, as part of refinement of the exposure estimate, uncertainty analysis is an option to allow understanding of the validity of the data that will be used.

Further guidance for dealing with remaining uncertainty in exposure assessment and characterisation of human exposure models is available via the WHO/IPCS harmonisation work and can be further consulted for the exposure assessment of biocidal products:

- *WHO/IPCS Guidance on uncertainty and data quality in exposure assessment*
- *WHO/IPCS Guidance on principles for human exposure models*

3.4. Secondary exposure scenarios

Three main categories of potential sources of secondary (indirect) exposure are:

- environmental sources from the point of view of areas treated with biocidal products (e.g. a room fumigated with a biocidal product, swimming pool treated with disinfectants),
- treated articles,
- dietary exposure sources covering potential exposure via consumption of food where residues of biocidal products may be present.

[53] Documents finalised at the CA meetings: https://circabc.europa.eu/ui/group/e947a950-8032-4df9-a3f0-f61eefd3d81b/library/386abfea-55ce-4764-8a31-f9d4f6ceaf0a?p=1&n=10&sort=modified_DESC. Note in particular CA-Dec22-Doc.5.5 on sensitisers (https://circabc.europa.eu/ui/group/e947a950-8032-4df9-a3f0-f61eefd3d81b/library/c2e2aafb-8d51-456f-9dd9-c40312c37ba8/details) and CA-March23-Doc.5.7 on antifoulings (https://circabc.europa.eu/ui/group/e947a950-8032-4df9-a3f0-f61eefd3d81b/library/7cb8642e-876e-4aa0-889d-533ceb059667/details).

If risk is not identified when comparing the exposure assessment estimate to the corresponding hazard threshold, no further refinement is needed. If risk is identified, refinement of exposure should be performed in line with Section 3.3.3. This can take into account refinement of parameters used in the exposure assessment with appropriate justification, generation of product specific data including measured data, or uncertainty assessment of the various steps of the exposure assessment.

3.4.1. Use at home

Exposure is assessed for residents or bystanders who are present during or following the use of a biocidal product at home. The post application phase is particularly important for secondary exposure assessment in a domestic area or application by non-professionals because:

- residues may remain in the treated area;
- prolonged contact is possible because people live there;
- children, the elderly and other sensitive subgroups are present.

A task-based approach does not apply to post application phase. Instead, a scenario-based approach is used, including the following post-application scenarios:

1. Children playing on the floor where biocides have been applied. Dermal exposure takes place due to contact with contaminated surfaces such as floors and walls. Oral contact may take place via hand-mouth transfer and toy-mouth transfer.
2. People present in the house after application, being exposed to residues in air and on surfaces.

The exposed population is anyone in the environment who may:

- inhale residual aerosols following use of sprays, during or immediately after application;
- inhale vaporised biocide following any type of application;
- have dermal contact to recently applied or dried biocide;
- ingest dislodged deposits (by infants or inadvertently by adults, for example during eating, drinking or smoking).

Post application exposure of children is often the most critical type of exposure to a biocidal substance. Children are a sensitive group (higher ventilation in relation to body weight) playing at ground level where the concentration of residues may be higher, and the duration of contact may be prolonged, often days or weeks (compared to shorter exposure time during application).

For frequency and duration of exposure, accurate scenario data should be used if available. When such information is not available or is not considered reliable, default values should be used.

For possible secondary exposure scenarios, see *BHHEM*. Additional information on secondary scenarios is available in *REACH Guidance R.15*.

3.4.2. Dietary exposure and human exposure via environment

Indirect exposure of humans via food and/or the environment may occur by consumption of food and drinking water, inhalation of air and ingestion of soil. Human exposure via the environmental compartments is assessed by estimating the total daily intake of a substance based on the predicted environmental concentrations for (surface) water, groundwater, soil and air.

In addition, for dietary exposure in three specific use areas, estimation of risk needs to be addressed for specific product types. For use scenarios from product types not listed below, dietary exposure may be less likely but must still be considered on a case-by-case basis.

Estimating dietary risk from transfer of biocidal active substances into foods (non-professional uses) (see *ECHA Guidance Vol III Part D*) is relevant for:

- PT 4 (Food and Feed area disinfectants);
- PT 5 (Drinking water disinfectants);
- PT 6 (Preservatives for product during storage);
- PT 18 (Insecticides, acaricides & products to control arthropods);
- PT 19 (repellents and attractants).

Estimating Transfer of Biocidal Active Substances into Foods – Professional Uses (see *ECHA Guidance Vol III Part D*) is relevant for:

- PT 3 (Veterinary hygiene products);
- PT 4 (Food and Feed area disinfectants);
- PT 5 (drinking water disinfectants);
- PT 8 (Wood preservatives);
- PT 12 (Slimicides);
- PT 14 (Rodenticides);
- PT 18 (Insecticides, acaricides & products to control arthropods);
- PT 19 (Repellents & attractants).

Estimating Livestock Exposure to Biocidal Active Substances (see *ECHA Guidance Vol III Part D*) is relevant for:

- PT 3 (Veterinary hygiene products);
- PT 4 (Food and Feed area disinfectants);
- PT 5 (Drinking water disinfectants);
- PT 8 (Wood preservatives);
- PT 12 (Slimicides);
- PT 18 (Insecticides, acaricides & products to control arthropods);
- PT 19 (Repellents & attractants);
- PT 21 (Antifouling products).

3.4.3. Treated articles

Articles treated with or incorporating biocidal products can lead to consumer and environmental exposure as well as exposure of professional users if there is any release. In some uses, exposure may be most significant from treated articles during service life (e.g. PT 7, 8, 9, 10). Specifically, articles consisting of polymers can be used in a large range of consumer applications, which makes the exposure situation very complex and may result in the need to assess the aggregated exposure from the use of different articles.

Direct contact with materials treated with biocidal products may result in transfer to the skin if the biocidal product is dislodgeable, i.e. can be removed from the surface.

The possibility of transfer via the oral route should also be considered. This can be relevant due to e.g. mouthing by infants or children or leaking/leaching from treated articles.

3.5. Combined scenarios & combined exposure assessment

A combined scenario should cover a complete working day under realistic worst-case conditions for each user type: industrial, professional, non-professional.

The estimated combined exposure for a worker is added up from the exposure arising from the individual tasks through the different phases of use. The inhalation, dermal and oral exposure estimates per scenario are added together to provide a total systemic dose. The total estimates for different scenarios may be combined to provide a total exposure estimate for each user type (industrial, professional, non-professional).

For instance, for industrial or professional users the tasks may include scenarios for handling concentrated material (mixing and loading), spraying a formulation and handling a wet object post-application. Appropriate selection from available data distributions should allow a realistic estimate of daily exposure from the combination of the scenarios which takes into account the time exposed.

It is important to recognize that simple addition of precautionary estimates can lead to gross errors, and it should be considered if it is relevant and realistic to combine primary and secondary exposure estimates.

Aggregate exposure to a specific substance includes primary and secondary exposure and exposure to the same chemical in different products and matrices including treated articles. This applies to both dossiers where only one PT is assessed and those where more than one PT is assessed.

The relevance of combining secondary exposure from residential uses should also be considered, such as non-professional dietary exposure in combination with other non-professional or secondary exposure. This is particularly relevant for secondary exposure via treated articles.

It might not be feasible to aggregate the personal daily exposure to a chemical substance through all sources as some sources may not fall within the framework of the BPR.

3.6. Assessment of data quality

3.6.1. Criteria for quality assessment of exposure data

The criteria to judge the quality of exposure surveys and study reports are set out below. It is imperative that all data generated adhere to appropriately designed protocols and carefully conducted studies.

3.6.2. Acceptability

Scientifically sound and well-documented state-of-the-art data are given preference over default assumptions. The conduct and reporting of studies must be in compliance with the most recent test protocols and requirements.

Documentation is adequate when studies have been carried out in compliance with Good Laboratory Practice and defined in terms of all the following components:

1. Detailed protocol, which bridges the study conduct and the conclusions that may be reached;
2. The study should be carried out with adequate and validated equipment by committed and qualified scientific and technical staff, described in terms of organisation, personnel, and resources;
3. Statement on the study model which bridges the actual observed data and the general application, be it deterministic, empirical or statistical;
4. Fully described study design, containing all forms of data handling (sampling, chemical and statistical analysis);
5. Quality assurance procedure, including external audits;
6. Statement of overall uncertainty, indicating the errors due to variables in the study and possible bias;
7. All documents relevant to the study should be retained, the report indicating the absolute essential archiving;

In practice, a pragmatic approach to study acceptability may be necessary.

Table 26: Recommended pragmatic acceptance criteria for human exposure studies

Essential Requirements	Desirable Requirements	Rejection criteria
Aims of survey or study strategy[54]	Protocol for study	No stated objective
Identification of the process etc.	Full details of process, task, equipment, substance in use	No process or task description, substance unidentified
Number of subjects and samples	Number of unique subjects and samples	Many replicates (few subjects, many samples)
Work environment	Workplace information	No workplace information
Product used - form, packing, site delivery	Product form etc. and in-use assay	No product details
Duration of task / tasks	Full pattern of use data and work-rate	No data for use duration
Sampling methods	Sampling methods validation	No clearly stated sampling methods
Analytical outline and recovery data	Analytical method, validation, recovery, storage, detection limits	No recovery data (unless obvious)
Task sampled - task and sampling match	Sampling data linked to task data	Sampling time and task or duration mismatch,
In-use product	Bulk biocidal product samples taken	Missing bulk information

[54] GLP compliance of studies into exposure to biocidal products has at the moment no generic demand in the EU, as it has in the USA and Canada. Some Member States require GLP-compliant studies for pesticides.

M&L, application, or post-application information	M&L, application, or post-application sampling	No clear description of activity phase sampled
Controls, work clothing	Exposure controls and PPE used, laundry, etc	No data on work clothing or controls
Outline of disposal route	Detail of exposure route and recycling	No way of deducing disposal route
Data reported in full	Data reported in full	Data as summary (e.g. range and statistics)
Study date	Date	No indication

M&L= mixing and loading; PPE= personal protective equipment

Expert judgement will be required to evaluate whether certain aspects of a study do not fulfil some of the essential requirements.

Studies meeting any of the rejection criteria will still be evaluated to see if they contain any useful data on any aspect of exposure, such as the pattern of use or the environment in which the product was applied. The assessor must report on the acceptability of studies submitted.

In addition to the general desirable study characteristics set out above there are a number of specific contextual data items that should also be documented in a study report. These are shown in Table 27. Some of the data indicated in this table can be important for the evaluation of the adequacy of studies, for example, a study on inhalation exposure towards a volatile substance would probably be rejected if it provides no information on the location and the ventilation.

Table 27: Desirable contextual human exposure data

Data item	Desirable amount of detail to be recorded
Emission of biocides	Either: solid/liquid aerosol, vapour, mist; spray, splash or spill
Location of biocide use	Inside or outside a building; volume of room
General ventilation	Details of general ventilation, e.g. good mechanical ventilation, poor mechanical ventilation, natural ventilation; details of weather conditions if outside
Physical properties of biocidal product	Some indication of the dustiness of solids being handled or the volatility of liquids; qualitative details of the viscosity of liquid biocidal products
Mass of product used	The total mass of product used during the task or tasks
Biocide concentration	Record of the concentration of the active biocide, both in use and before any dilution
Proportion of the task exposed to biocide	Percentage time the person is exposed (by inhalation or dermal contact) to the biocide
Time near to the source	Proportion of the task where the person is close (within 1m) to the source of the biocide
Description of the handling of the biocide	Details of the process or activity; for example, handling contaminated objects, spraying, brushing, wiping, immersion etc.; details of the process, e.g. spray technology, spray pressure, nozzle diameter, etc.
Process temperature	Temperature of the biocide in use
Description of local	Presence of local ventilation for inhalation risks, ideally with some comment on

controls	its likely effectiveness; details of any other control measures applied at the source
Housekeeping	Description of the apparent cleanliness of the area; details of any accidental splashes, spills, etc.
Contaminated surfaces	Area of contaminated surfaces, concentration of biocide on surfaces, estimated personal contact rate (hands or body touches per hour) with surfaces.
Use of PPE	Type of respirator, gloves, clothing or other PPE worn while using biocide; brief description of training of people to use the equipment and administration of the PPE.
Physical activity involved with task	Categorised as: rest (e.g. sitting), light work (e.g. sitting or standing with moderate arm movements), moderate (walking with moderate lifting or pushing), heavy (e.g. intermittent heavy lifting with pushing or pulling), very heavy (e.g. shovelling wet sand).
Categorical (yes/no)	Inadvertent exposure of food through treatment/contamination

3.7. Requirements and evaluation of exposure studies

Measurements of application scenarios should be conducted in such a way that the process corresponds to the assessed task, covering realistic worst-case conditions in agreement with article 19 (2) of the BPR. Measurements shall be performed in accordance with relevant standards, e.g. EN ISO 482 for workplace air measurements. It is recommended to provide robust documentation (supported by videos or photos) to ensure the traceability of the results.

An important consideration is the number of measurements to be conducted. Each measurement must cover independent runs of the task being investigated, i.e. if several measurements are carried out in parallel for an individual task, these count together as one measurement. The number of measurements must be sufficient to demonstrate safe use and therefore depends on the results and their variability, in addition to the distance between the measured exposure levels and the reference values. Overall, a higher number of measurements is recommended.

However, for non-critical scenarios in clause 5.5.2 of EN ISO 689:2020 a screening test for 3 to 5 measurements is recommended which is also applicable for the assessment of biocides. Accordingly, it may be concluded safe use when

- All of 3 measurements are < 10% of the relevant reference value
- All of 4 measurements are < 15% of the relevant reference value
- All of 5 measurements are < 20% of the relevant reference value

The protective effect of PPE may be considered in this assessment.

If safe use cannot be concluded based in these criteria, more than 5 measurements are required. If more than 5 measurements are available, the assessment of biocides shall be conducted based on the indicative value which is to be selected as a percentile of the measured distribution as explained in section 3.8.

When carrying out measurements of similar activities, it may be possible to pool data depending on the results generated. This could have the advantage that data sets with more measurements are available, which may justify the selection of a more favourable percentile. This requires a robust justification for the existing similarity.

It should also be clearly explained why the investigated scenarios represent realistic worst-case situations. This is particularly necessary regarding the transferability of the exposure data to other applications/activities, possibly with different application quantities.

For further information, see OECD (2002)[55].

3.8. Selection of indicative exposure values

The following general rules should be used in selecting indicative exposure values from measured exposure data (see also Appendix 3-2). A minimum of six or more measurements has to be considered for this approach.

1. Moderate uncertainty. The dataset is sufficiently large and/or the variability sufficiently low that the exposure distribution can be characterised with a reasonable level of assurance. The 90% confidence intervals for the 75th percentile is typically less than a factor of 2[56]. For these datasets the 75th percentile can be used as an indicative exposure value.
2. Considerable uncertainty. The dataset is smaller, or the variability is greater than for datasets of moderate uncertainty. The degree of confidence in the characterisation of the exposure distribution is lower, with 90% confidence intervals for the 75th percentile typically greater than 2. For these datasets the 95th percentile can be used as an indicative exposure value.
3. High uncertainty. The dataset is small and/or the variability is great. The lognormal approximation to the exposure dataset may not be verifiable and confidence intervals based upon this assumption might be misleading. The exposure distribution is poorly characterised. The maximum exposure value can be used as an indicative value, or the numerical values can be disregarded.

For further information on how to deal with generic models and the corresponding choice of percentiles, please refer to *BHHEM*.

[55] *Guidance Document for the Conduct of Studies of Occupational Exposure to Pesticides During Agricultural Application*, OECD Series on Testing and Assessment, No. 9, OECD Publishing, Paris, https://doi.org/10.1787/9789264078079-en

[56] The factor between the confidence intervals can be calculated using MS Excel as follows (command names and punctuation differ in various language versions of MS Excel):

- "range" are the cells containing the measured values, e.g. "A1:A12"
- "size" is the number of measured values in the data set, e.g. "12"
- "alpha" is 10% (i.e., 100% - 90% confidence level)
- "standard_deviation" is calculated by [=stdevp(range)]
- "width" of the confidence intervals is calculated by [=Confidence(alpha,standard_deviation,size)]
- 75th "percentile" is calculated by [=percentile(range, 0.75)]

With these values, the factor between the confidence intervals is then calculated by [=(percentile+width)/(percentile-width)].

Appendix 3-1: Refinement of exposure estimates and hierarchy of controls

This appendix is aimed primarily at the applicant developing the risk assessment.

For occupational risk management, the general measures necessary for safety and health protection of workers (Article 6 of Directive 89/391/EC), the reduce-to-a-minimum principle (Article 6 of Council Directive 98/24/EC) and the hierarchy of RMM prescribed in the Chemical Agents Directive must be followed. This includes in particular:

- avoiding risks;
- evaluating the risks which cannot be avoided;
- combating the risks at source;
- giving collective protective measures priority over individual protective measures;
- replacing dangerous by non-dangerous or less dangerous;
- giving appropriate instructions to workers.

The recommended RMMs for the occupational setting should enable and support the employer to meet the goals of occupational safety and health protection. Manufacturers, importers and downstream users should therefore consider measures needed for controlling risk in the order of the following hierarchy of control:

- Eliminate risks by limiting the use of the substance in market or modification of process, by using intrinsically safe equipment or by automation;
- Reduce risk by limiting the concentration of a substance, and/or change form of physical state, and/or apply closed processes, and/or install effective local exhaust ventilation;
- General area ventilation and other workplace related measures (like segregation of dirty departments, safe storage, fire/explosion protection and prevention, eyebaths/showers);
- Other collective RMMs aimed at protecting the population of workers, e.g. organisational measures limiting the number of exposed workers or the duration of exposure;
- Personal protective equipment (respiration, skin, eyes) where exposure cannot be prevented by other means.

Apart from substance or process specific risk management measures, good occupational hygiene practice forms the basis to minimise exposure of workers during and after normal operations. Personal hygiene procedures (e.g. washing hands after handling of substances, changing contaminated clothes) and organisational settings (e.g. separation between exposure areas and non-exposure areas) should be supported by regular training/instruction of workers and consequent supervision. Application of PPE should be based on acceptance and a high level of comfort to achieve effective implementation.

Hierarchy of controls

Hierarchy of controls is a principle applied mostly for controlling exposure at the workplace. However, the principles should be considered relevant for use of biocidal products by the general public, where relevant.

For occupational risk management, the general measures necessary for safety and health protection of workers (Article 6 of Directive 89/391/EEC), the reduce-to-a-minimum principle

(Article 6 of Chemical Agents Directive 98/24/EC) and the hierarchy of RMM prescribed in the Chemical Agents Directive must be followed. This includes in particular:

- avoiding risks;
- evaluating the risks which cannot be avoided;
- combating the risks at source;
- giving collective protective measures priority over individual protective measures;
- develop a coherent risk prevention policy;
- replacing dangerous by non-dangerous or the less dangerous;
- giving appropriate instructions to workers.

The recommended RMMs for the occupational setting should enable and support the employer to meet the goals of occupational safety and health protection. Manufacturers, importers and downstream users should therefore consider measures needed for controlling risk in the order of the following hierarchy of the general workflow:

- Eliminate risks by limiting the use of the substance in market or modification of process, by using intrinsically safe equipment or by automatisation;
- Reduce risk by limiting the concentration of a substance, and/or change form of physical state, and/or apply closed processes, and/or install effective local exhaust ventilation;
- General area ventilation and other workplace related measures (like segregation of dirty departments, safe storage, fire/explosion protection and prevention, eyebaths/showers);
- Other collective RMMs aimed at protecting the population of workers, e.g., organisational measures limiting the number of exposed workers or the duration of exposure;
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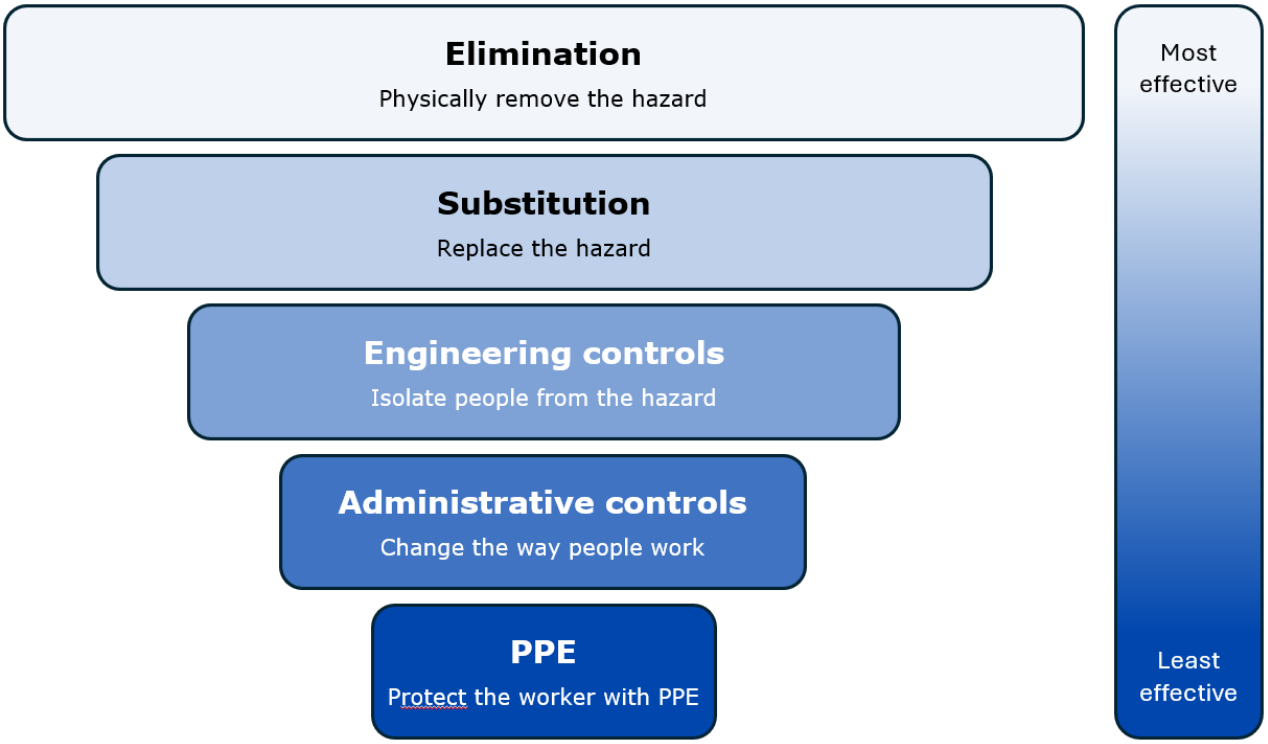
Further elaboration of technical measures/engineering controls that reduce dispersion, adapted from Hierarchy of controls applied to dangerous substances - OSHwiki | European Agency for Safety and Health at Work (europa.eu).

When measures at the source cannot sufficiently reduce the release of substances, technical measures that reduce further dispersion and consequently exposure of workers should additionally be considered. Local exhaust ventilation (LEV), which extracts the substances as close to the source as possible, should always be the first option to consider. Usually, it is much more effective than general (room) ventilation. However, daily checks of its proper functioning - by the worker, as well as periodic maintenance – to be organised by the employer – are crucial to the effectivity of these measures.

The STOP principle refers to Substitution, Technical measures, Organisational measures and Personal protection. Substitution (S) is normally not relevant in the context of active substance approval or biocidal product authorisation, except in the context of BPR Articles 10 (Active substances which are candidates for substitution) and 23 (Comparative assessment of biocidal

products). In accordance, the first steps for reducing the risk are to define technical (T) and organisational (O) measures, and only as the last resort PPE (P).

Figure 2: Hierarchy of controls



Control methods at the top of graphic are potentially more effective and protective than those at the bottom. Following this hierarchy normally leads to the implementation of inherently safer systems, where the risk of illness or injury has been substantially reduced.

When measures at the source cannot sufficiently reduce the release of substances, technical measures that reduce further dispersion and consequently exposure of workers should additionally be considered. The effectiveness of these measures depends on daily checks of their proper functioning by the worker, as well as periodic maintenance organised by the employer.

It is the responsibility of the applicants to provide sufficient information in their applications about the operational conditions set, the concerned worker groups and all measures undertaken, following the 'Hierarchy of control' considerations, to ensure the elimination or minimisation of risks for human health.

Local exhaust ventilation

Local exhaust ventilation (LEV) extracts the substances as close to the source as possible and should always be the first option to consider as it is much more effective than general (room) ventilation.

Designing effective LEV is a specialist activity. If the design, installation, maintenance or the operation of LEV is improper, its effectiveness will be reduced. It is advisable to consult a specialist supplier to ensure its effectiveness. Generally, well-designed and correctly operated LEV systems may be capable of reducing exposure by 80-99%. A general recommendation is to place the inlet of the system as close to the source as possible. For LEV hoods a maximum

distance equal to the diameter of the hood is often used as a rule of thumb. Other recommendations are to avoid long or bended ducts, and to take account of potentially turbulent air flows. Advantage should be taken of the direction and kinetic energy of the emitted substances. In many cases it will be necessary to (partially) enclose the process to increase the effectiveness of the LEV.

General ventilation

Although LEV generally is the preferred option, it is never 100% effective. Therefore, additional general ventilation is needed to prevent the uncaptured pollutants from building up to harmful concentrations. In scenarios where many small diffuse sources are present, general ventilation may even be the preferred option.

The design, installation and maintenance of general ventilation is a specialist task. Consideration is needed regarding the location of air inlets and outlets, to prevent short circuits where fresh air that is brought in is extracted again close to the inlet, without diluting the pollutants. In addition, the required air flow (in m^3/hour) or the number of air changes per hour (ACH) should be determined. The geometry of the room, any objects that might disturb airflows and interfering air flows should all be considered. The possibilities can be considered for recirculation, in relation to filtering options and energy demand for heating. In most cases, recirculation is not allowed when carcinogenic substances are present. It is advisable to consult a specialised supplier of ventilation systems to ensure its effectiveness.

Merely opening doors or windows is normally insufficient.

Technical measures for control of exposure to non-professionals

Bait boxes and child-resistant fastenings are good examples of technical measures to reduce possible exposure to non-professionals.

Organisational measures and administrative controls

Spatial measures aim at increasing the distance between the worker and the substances emitted, or ideally at full separation (segregation) of the worker from the source of the substances. Full separation may be achieved by access restrictions, e.g. to areas where biocidal products have recently been sprayed. This prevents exposure to vapours or mists by inhalation. Such restrictions can also be temporary. Access to work in confined spaces, e.g. to carry out maintenance in tanks, should be strictly limited to those who are properly instructed and protected.

A less efficient type of separation is the use of long-stemmed brushes, rollers, or mixing equipment. This type of equipment increases the distance from the source and may reduce both inhalation and dermal exposure.

Temporal measures such as task rotation may reduce the duration of the exposure for individual workers. Thoughtful work planning may reduce workers' exposure. For example, spraying of biocidal products could be carried out when other workers are not present.

Residential administrative control means the exclusion of residents from treated spaces until aerosols have dispersed and surfaces are dry. All subsequent exposure is secondary.

Workplace administrative control needs to consider proper supervision and training of workers, as well as procedural plans, event planning (such as accidental spill procedures) and permits to work.

'Safe systems of work', 'emergency procedures' and 'permits to work' mean that hazardous biocides can be used with minimal risk. For example, the risk is likely to be high in operations such as maintenance and when a 'permit to work' is needed. The permit sets out the steps to assure that situations are made safe before work starts, remains safe, and includes standby rescue and re-commissioning procedures.

PPE/RPE

PPE is used when residual exposure cannot be avoided after application of other means. Thus, exposure scenarios that rely on PPE as a primary risk management option should be avoided whenever possible. Selection and use of personal protective equipment will always need to be seen within the context of national occupational health and safety legislation where the full range of risks need to be considered. For example, it is necessary to consider the additional physiological burden introduced using PPE, such as heat stress, or impact on the hands due to long wearing of PPE, if appropriate breaks are not taken. It is the responsibility of the employer to ensure such risks are avoided, but the applicant should consider these in assessing the feasibility of the PPE. This may be particularly relevant to exposures for extended periods, for example when wearing of impermeable gloves and the national legislation requires that breaks are taken to avoid the effect of wet working. For example, the time for continuous wearing of the gloves may need to be limited to e.g. 2 or 4 hours.

For the risk characterisation the reduction factor is taken into account that is achieved by the use of the PPE. Justification should be provided when PPE is specified within exposure scenarios as the primary method to achieve acceptable exposures.

The use of RPE should usually be a temporary measure, during short time intervals, until other technical measures are provided to ensure safe use. RPE should be proposed for use well within its designed performance. This may mean an exposure assessment that indicates a performance of 90% but additional good practice advice may suggest equipment providing 95% or better performance is preferred to meet the requirement of other legislation, especially in cases where the exposures are close to the limit values.

PPE to protect against dermal exposure will often be needed due to the very variable and unpredictable nature of dermal exposure, and gloves are not sufficient when other parts of the body are exposed. The quantitative assessment should not be the only information used to propose suitable and adequate gloves and clothing.

It is an absolute requirement that the barrier properties of the glove material are known to be adequate to ensure the substance does not migrate through the material of the glove during the proposed use. Protective gloves may fail to protect the wearer from exposure due to:

- permeation – the process by which a chemical substance migrates through the protective glove at a molecular level;
- penetration – the bulk flow of a chemical substance through closures, porous materials, seams and pinholes or other imperfections in the protective glove;
- degradation – a damaging change in one or more physical properties of the protective glove as a result of exposure to a chemical substance.

The contaminant may also get inside the glove where it may reside against the skin for a longer period, which could result in higher exposure compared to not wearing gloves.

Gloves must be sufficiently described in the dossier so that there is assurance that suppliers of substances and formulations can effectively communicate (in section 8 of the Safety Data Sheet) the correct information to downstream users. Glove material type and breakthrough time need

to be stated by the Applicant. While glove manufacturers may provide indicative information, the best information derives from specific testing against the specific substance. Such information will also help producers of mixtures to select appropriate gloves for their products. Information such as "*suitable chemical resistant gloves tested according to EN ISO 374*" does not give sufficiently concrete information to ensure that the risk can be adequately controlled.

For coveralls, further details such as the type and category, material type, characteristics of penetration and permeation need to be stated by the Applicant.

Appendix 3-2: Re-entry scenarios for human exposure (inhalation)

Introduction

When applying a biocidal product with a volatile substance, a certain amount of these substances will evaporate into the air. During the application time and afterwards, the corresponding concentrations may increase until all biocidal product has evaporated. Depending on the application method, the amount of the biocidal product, the vapour pressure of the corresponding substances and solvents, the evaporation time might be similar or much longer than the application time. Due to ventilation (and in some cases chemical reaction) the concentration of the corresponding substances within the air will decrease over time.

The standard exposure scenario assumes that all workers will leave the area at the end of the exposure time. The related question is when persons may return to the contaminated area.

Depending on the predicted concentrations during exposure time and further estimates on the course of the concentrations of the relevant substances, considerations on when or how safe re-entry to the area is ensured must be performed. These considerations are the basis for the creation of a re-entry scenario. In the following, re-entry scenarios for substances with a given air concentration limit/reference value are discussed.

Re-entry with regard to systemic exposure or aspects of dermal exposure are not addressed here but may require additional considerations in specific cases.

A re-entry scenario shall be created, if the biocidal product contains one or more volatile active substances or substances of concern (i.e., the vapour pressure of the pure substance is > 0.01 Pa) and at least one of the following points is met:

1) The predicted concentration during exposure time is larger than the toxicological reference value (for example: the predicted mean event consideration is larger than the acceptable exposure concentration, AEC).
2) The predicted concentration during exposure time is smaller than the toxicological reference value, but at the end of the exposure time, the concentration is still increasing (based on, e.g., ConsExpo or 2-component model data).
3) Respiratory protection equipment (RPE) is mandatory during product application.

Waiving of a re-entry scenario may be possible with a proper justification showing that relevant exposure resulting from re-entry can be excluded.

Discussion of applicable models

Certain simulation tools can conduct re-entry scenarios. Those are mostly the deterministic (mass-balance based) models, such as ConsExpo (evaporation model) or the 2-component

model (HEAdhoc recommendation 16). They can predict time-dependent concentration profiles and thus to estimate when limit values are not exceeded.

Other simulation tools, e.g. the Advanced REACH Tool (ART) can produce an exposure estimate for the application scenario but cannot predict time dependent concentration courses that allow to calculate re-entry scenarios for themselves.

The choice between the two simulation approaches (e. g. ConsExpo or ART) should be based on the models used for the application scenario for which a re-entry scenario shall be examined. The choice of the model for the application scenario should be based on the existing harmonization (HEAdhoc recommendation 6).

Calculation of re-entry scenarios with ConsExpo / 2-component model

The re-entry scenario can be seen as an extended application scenario. The only difference is the increased exposure time, giving the possibility to monitor the concentration over time.

The following steps are recommended:

1) Enter the parameters into the evaporation model sheet of ConsExpo. All parameters remain the same (also the application time) as for the ConsExpo calculation for the application itself.
2) Extend the exposure time.
3) Monitor the result, based on the diagram of the air concentration or the corresponding table of individual numbers depending on the time. Check whether the concentration at the end of the exposure time is below the corresponding toxicological reference value and decreasing.
    - The concentration at the end of the exposure time is lower than the relevant toxicological reference value: Check the data to see at which point of time the concentration is below the reference value.
    - The concentration at the end of the exposure time is not lower than the relevant toxicological reference value: Conduct the calculation again with increased exposure time.

Calculation of re-entry scenarios with ART

ART is not capable of calculating re-entry scenarios as certain parameters (like treated surface, application time etc.) are not specified. Therefore, this approach should not be the method of choice.

For calculation of the re-entry time, the following formula can be used:

$$c(t) = c_0 * e^{(-\text{ventilation rate} * t)}$$

where c(t) is the concentration of the substance (time-dependant, in h), c_0 is the starting concentration (result/percentile of choice of the application scenario calculated in ART), ventilation rate (1/h) and t is the time (in h). As the time (t) increases, the concentration c(t) decreases. When c(t) falls below the toxicological reference value, the re-entry time is reached.

ART offers different options for ventilation, whereas not all options include obvious ventilation rates. The use of the ventilation rates in Table 28 are recommended in context with the formula above.

Table 28: Ventilation rates proposed for ART calculations

Air exchange rates proposals for calculations based on ART	Ventilation rate* (1/h)
No restriction on general ventilation characteristics	1
Only good natural ventilation	2
Mechanical ventilation giving at least 1 ACH	4
Specialised room ventilation with more than 10 ACH	20
0.3 ACH	0.3
1 ACH	1
3 ACH	3
10 ACH	10
30 ACH	30

** Ventilation rate is considered synonymous to ACH*

For this approach it must be ascertained that no additional substance is released into the room air, e.g., after wiping activities all surfaces must have dried, dipping baths must be closed or emptied, etc.

Interpretation of re-entry times

The re-entry time is the time a user must wait until they may enter an area again, where they worked before using a volatile biocidal product.

The models used for the determination of re-entry times have certain limitations. The conditions at the user´s place might be different and difficult to foresee for applicant and regulator.

Therefore, instead of specifying an exact time, the following statement is recommended in the SPC:

> *Re-entry is only permitted once the air concentration has dropped below X ppm (Y mg/m³) or relevant lower national reference value.*
>
> (X = reference value of the corresponding substance in ppm; Y = corresponding national reference value in mg/m³)

In this case, the user may conduct representative measurements on their own or rely on read-across from literature.

If the applicant or the evaluating competent authority want to insist on their own measurements each time at the user's site, the following statement may be used:

> *Re-entry is only permitted once the air concentration has dropped below X ppm (Y mg/m³) or relevant lower national reference value.*
>
> *Use a calibrated sensor to confirm the exposure is ≤X ppm (Y mg/m³) or the corresponding national reference value prior to re-entry.*

When considering whether re-entry times are realistic to ensure safe use, the following aspects must be assessed case-by-case:

1) Is it possible that the various applications of the biocidal product can wait for such a long time (respecting the re-entry time)?
2) Is it possible that the overall process of work opens space for an area not to be entered for such a long time?

Workplace measurements may be used to refine the exposure scenario and allow checking if actual re-entry times are shorter than previously predicted and therefore feasible.

Appendix 3-3: Reverse reference scenario example

This example reflects primary exposure of professional and non-professional remedial treatment of timber using wood preservative containing 0.5% active substance pastes by brush, trowel, caulking gun and gloved hand. This task is performed for approximately 30 minutes per day.

Assumptions for the example:

- Task duration: 30 minutes per day
- $AEL_{long\text{-}term}$: 0.25 mg/kg/d
- Dermal absorption: 10%

There are no generic exposure data for application of pastes. In the absence of generic data or a suitable mathematical model, an option is to assess the maximum acceptable exposure to the active substance and then assess the likelihood that exposures will exceed this level.

The maximum exposure to the active substance allowable is given by $AEL_{long\text{-}term}$. For a non-volatile paste it is assumed that inhalation exposure is negligible.

To exceed the $AEL_{long\text{-}term}$, active substance contamination to the skin would need to exceed:

0.25 mg/kg/d × 10 = 2.5 mg/kg/d

If the operator weighs 60 kg then active substance contamination would need to exceed:

2.5 mg/kg/d × 60 kg = 150 mg/d

As the maximum concentration of active substance in the ready-for-use paste formulation is 0.5% w/w, the weight of paste product containing 150 mg active substance will be:

150 / 0.005 = 30 000 mg = 30 g

Assuming that dermal exposure will be predominantly to the hands and that gloves are worn, the rate of actual dermal exposure to the hands inside gloves would need to exceed:

30 g / 30 min = 1 g/min

The worked examples database for professional users contains approximately 400 measurements of actual hand exposure inside gloves across a wide range of tasks. The maximum exposure to an in-use formulation is 360 mg/min with a 95^{th} percentile of 23 mg/min.

In conclusion, for chronic exposure the reverse reference scenario indicates a high margin of safety. This calculation is presented in the standard format in Table 29.

Table 29: Presentation of reverse reference scenario exposure assessment in standard format

Application of curative pastes	
Product	
active substance % w/w	0.50%
Potential body exposure	
Indicative value mg/min	0
Duration min	30
Potential dermal deposit mg	0
Clothing type	Cotton coveralls, 20% penetration
Clothing penetration %	20%
Actual dermal deposit [*product*] mg	0
Hand exposure	
Indicative value mg/min (actual)	1000
Duration min	30
Potential hand deposit mg	30 000
Mitigation by gloves	None
Actual hand deposit [*product*] mg	30 000
Total dermal exposure	
Total dermal deposit [*product]* mg	30 000
Active substance mg	150
Dermal absorption %	10%
Systemic exposure via dermal route mg	15
Exposure by inhalation	
Indicative value m^3/min	0
Duration	30
Inhalation rate m^3/h	1.25
Mitigation by RPE	None
Inhaled [*product*] mg	0
Systemic exposure via inhalation route mg	0
Systemic exposure	
Total systemic exposure a.i. mg	15
Body weight kg	60
Systemic exposure mg kg^{-1} day^{-1}	0.25

Appendix 3-4: Deterministic and probabilistic approaches

When performing estimation of exposure, two approaches can be followed.

Deterministic approach provides an estimate based on a single value for each model input and a corresponding individual value for a model output, without quantification of the cumulative probability or, in some cases, plausibility of the estimate with respect to the real-world system being modelled. This term is also used to refer to a model for which the output is uniquely specified based on selected single values for each of its inputs.

In **probabilistic analysis** distributions are assigned to represent variability or uncertainty in quantities. The output of a probabilistic analysis is a distribution.

Mathematical models are calculation routines that are based on the physico-chemical properties of a substance and the environment into which these substances are released. Although the basis for the calculation algorithm is scientific, these models can be gross approximations as the full range of real variables cannot be accounted for and are therefore assigned very conservative defaults. Although mathematical models are usually meant to be conservative, this does not hold true for all models or assessed scenarios: some model outcomes may underestimate exposure substantially. Few of the models have been validated against real situations.

Generally, exposure models fall into one of three types:

1) **mathematical mechanistic models** predict exposure levels from a mechanistic description of a process;
2) **empirical/knowledge-based models** predict exposure levels based on an empirical database;
3) **statistical mathematical models** predict exposure levels based on statistical relations.

Some models are further described within the *BHHEM*.

The use of exposure models requires the selection of various input parameters. Insufficiently detailed information on exposure scenarios or lack of sufficient data may require the use of default values. Input data or default values used for the calculations must be clearly documented. Computer programs have been developed to implement mathematical predictive models and empirical models. Statistical models have been developed using available data and appropriate statistical methods. Model choice should be justified by showing that the model uses the appropriate exposure scenario (e.g. as judged from the underlying assumptions of the model). Expert judgement may be required to check the realism of the exposure value derived from a model, particularly if default or realistic worst-case values have been used. Modelling of exposure can be performed either by taking discrete values (point estimate) or distributions for the model variables (probabilistic modelling).

Mathematical mechanistic models

Mathematical mechanistic models are generally based on mass balance equations and are often used for assessing inhalation exposure to volatile compounds.

These can incorporate the physical and chemical properties of the substance, together with patterns of use. They are used to characterise the rate of release of the product into a space, and its subsequent behaviour. Mathematical models should cover all relevant processes or tasks contributing to exposure in a scenario. For many tasks, a number of models could be appropriate. The underlying assumptions for each model, and the processes it represents, help the assessor

in model selection. More than one model can be run to assure consistency. The advantages of mechanistic models are:

- the mechanisms and main processes are clearly stated;
- the inputs and outputs are clearly stated;
- they are well documented and can be validated;
- they can be improved using real life data.

However, if the underlying assumptions do not apply to the task, they can be poor approximations of the real world. Importantly, they make a number of simplifying assumptions, for example instantaneous complete mixing of the substance in air, and they account only for the main variables that affect exposure.

Care must be taken not to rely completely on point prediction.

Empirical models

Empirical models can be described as models based on exposure measurements obtained from real situations. This type of model can be used to predict the likely exposure in other comparable situations, i.e. the informed use of generic data. If sufficient and high-quality data are used in empirical models they are likely to account for the many variables that influence exposure.

The main advantage of empirical models is their amalgamation of multiple studies into a large data set, which reflects the distribution of results better than a small exposure study. The disadvantages include:

- uncertainties about the quality of the information fed into the model;
- uncertainties about input default settings;
- important factors that influenced the recorded exposure level may become hidden;
- the output from the model may be misapplied or misinterpreted;
- outputs may be imprecise.

Statistical mathematical models

Statistical mathematical models use empirical relationships to predict exposures from statistical indicative distributions together with historical data. They reflect a combination of empirical and mechanistic models together with consideration of the distribution of the input parameters. One of the most important steps in the procedure is represented by the implementation of the probabilistic approach, which allows the use of distributions in the calculation.

Probabilistic techniques use distributions instead of point values for variables in model estimations. Distributions reflect the variability and uncertainty of a variable, enabling the assessor to introduce an additional approach to describe data quality. Probabilistic analysis may reveal the factors that really drive the exposure. It may also help to differentiate sub-populations with respect to exposure, and thus to identify groups of people at risk. Knowledge of the range and distribution of exposures allows the assessor to select from appropriate points in the distribution to inform the decision-making process and to perform an appropriate sensitivity analysis.

A large amount of exposure data is needed to establish a distribution and allow the application of statistical methods. Probabilistic analysis therefore requires input data of sufficient number and quality. Otherwise, misinterpretations of the probability distribution that represents the

variables, for example, underestimating the variance, can seriously hinder and prevent the interpretation of the outcome. In cases where the assessor has little data of low quality, a realistic worst-case estimate of exposure in combination with expert judgment is preferred.

In summary, probabilistic assessments integrate distributions of exposure factors to produce an estimate of exposure. They increase insight in the uncertainty of the assessment (via uncertainty analysis) and the contribution of each exposure factor in the final result (via sensitivity analysis). If data quality is adequate, a probabilistic analysis is preferred, at least to underpin a deterministic presentation of the results.

4 Risk characterisation

4.1 Introduction

According to Annex VI of the BPR, risk characterisation is defined as follows:

> *the estimation of the incidence and severity of the adverse effects likely to occur in a human population, animals or environmental compartments due to actual or predicted exposure to any active substance or substance of concern in a biocidal product. This may include "risk estimation", i.e. the quantification of that likelihood.*

In addition, according to Article 19(1)(b)(iii), an assessment is required also with regard to residues, as it has to be established that:

> *the biocidal product has no immediate or delayed unacceptable effects itself, or as a result of its residues, on the health of humans, including that of vulnerable groups, or animals, directly or through drinking water, food, feed, air, or through other indirect effects*

Therefore, risk characterisation is performed to assess the risk associated with the exposure to the active substance or a substance of concern, or to residues arising from the use of the biocidal products. Residues include parent compound metabolites, breakdown or reaction products occurring in foodstuffs and diverse environmental matrices including drinking water.

Risk characterisation can be either quantitative or qualitative or a combination of the two, depending on the nature of the effects and the information available.

The methodology for risk assessment of the active substance can be defined as the combined processes of (a) hazard identification, (b) hazard characterisation, (c) exposure assessment and (d) risk characterisation. Hazard characterisation, i.e. identification of the dose-response relationship, is performed for the active substance during the evaluation of the biocidal active substance, and the agreed reference values will then be used in the biocidal product evaluations. Risk assessment must also address exposure via treated articles where relevant.

During the approval of an active substance, the realistic combination of some uses or scenarios should also be addressed. Combined exposure to multiple chemicals (from one or multiple uses/releases) needs to be assessed considering in particular cumulative and synergistic effects (see section 4.3.2).

In the interest of harmonising the assessments under different regulatory frameworks, the conclusions under other regulatory frameworks should be taken into consideration to support the assessment at all stages, i.e. by the applicant before submitting the application, by the eCA during evaluation, and by all actors during the opinion forming stage.

4.1.1 Considerations on formulations

An active substance may be formulated in a number of diverse matrices for the specific uses to maximise the performance of the product for its end use while reducing toxicity of the product to the end-user or the environment, improving durability, extending shelf life and reducing wastage. Achieving all these objectives may not be possible at the same time, as improvement in some areas may cause sacrifices in others.

The effect of how the active substance is formulated into a product will also depend on the properties of the active substance, including its inherent physico-chemical characteristics.

Formulation may have the potential to increase or decrease both the hazard and exposure as compared to the active substance.

The following aspects will (among others) contribute to increasing or reducing exposure to the active substance:

- Physical state (liquid, aerosol, vapour, powder, pellets);
- Particle size;
- Encapsulation, soluble bags etc.;
- Chemical and physical interaction inside the product (partitioning, adsorption).

The following aspects contribute to increasing or reducing systemic exposure to the active substance:

- Changes in absorption through dermal layers;
- Changes in passage through protective clothing and PPE;
- Disposition changes caused by increased droplet size or reduced surface tension

In addition, additive, synergistic and antagonistic effects need to be considered.

All the above factors will need to be considered when assessing the risk of a formulated product. Remaining uncertainties due to e.g. missing information on the effect of a particular factor will need to be assessed on a case-by-case basis.

Product specific information may be available according to BPR Annex III, but such studies will not cover all endpoints and in some cases it may not be possible to perform the studies in a way that would provide the most informative results. As an example, it is difficult to estimate dermal absorption from a product that will form a dry layer soon after use, such as paints and antifouling products[57] (see also Section 1.3.3.3 where further specific dermal absorption guidance is referred to, including that for rodenticides, as well as further guidance for rodenticide formulations[58, 59]). The available information will therefore need to be considered as a whole, taking into account all information sources including physical-chemical properties and *in vitro* information.

4.1.2 Local or systemic risk characterisation

Whether local or systemic effects are more critical depends on several factors including the inherent properties of the active substance (e.g. highly reactive at the site of contact), the concentration of the active substance in the product and the intended use of the product. Theoretically, administration of high doses of substances at low concentration may be more critical for systemic effects, whereas for local effects lower doses administered at higher

[57] See also *Dermal absorption of PT 21 active substances* (https://webgate.ec.europa.eu/s-circabc/d/a/workspace/SpacesStore/c9893d6f-92c6-48ab-9bdb-ccc443e90a50/Dermal absorption_PT 21.pdf)
[58] Dermal absorption values for anticoagulant rodenticides: https://webgate.ec.europa.eu/s-circabc/d/a/workspace/SpacesStore/f98f9676-4716-448a-80d6-50fa671f7860/Dermal_abs_anticoagulant_rodenticides.docx
[59] Dermal absorption values for anticoagulant rodenticides: Alternative approach for the occupational setting: https://webgate.ec.europa.eu/s-circabc/d/a/workspace/SpacesStore/c59f1958-3032-418c-a10b-0fa7ba93ffe8/Dermal_abs_anticoagulant_rodenticides_alternative.docx

concentrations may be critical. Local toxicity is also influenced by the potential of co-formulants and solvents to induce local effects, as well as the pH of the product.

For substances and products having local toxicity, the observed systemic effects could be true primary effects or secondary to the local toxicity of the substance. Therefore, a hazard assessment and hazard characterisation for systemic effects should be performed in addition, unless there are no systemic effects or it can be concluded that all effects are secondary to local toxicity.

If a biocidal product is used exclusively together with a non-biocidal product that is classified for local or systemic effects, the non-biocidal product may need to be included in the risk assessment.

4.1.3 Refinement of risk characterisation

If a safe use is not identified in the initial assessment (first tier), or in a borderline situation, the risk characterisation should be refined in a second tier, considering hazard and exposure. This may address both quantitative and qualitative risk characterisation approaches or one of them, as necessary. Identifying a 'safe use' is synonymous to concluding that the risk is acceptable in a specific case.

An uncertainty analysis can provide more accurate estimates for hazard or exposure side, or information on the uncertainties; see *REACH Guidance R.19* and *EFSA Guidance on Uncertainty Analysis*.

In this second tier a refined exposure estimate is established by introducing:

- Risk management measures that were not yet included in the first tier;
- Options for exposure reduction;
- Exposure databased on surveys or studies with the actual product or with a surrogate.

A refinement in the hazard assessment is generally not possible apart from active substance approval/renewal: the toxicological reference values will be established at this stage and cannot be adjusted for the purpose of assessing a product. In exceptional cases it might however be possible to take into account e.g. considerations on the sensitivity of the relevant subpopulation if only a specific sub-population will be exposed due to restrictions on the approval. See section 2.3.6 for consideration on the possibility of exceptionally deriving different reference values for professionals and non-professionals.

If the second tier still shows risk, further risk management measures may be required.

For non-professionals, risk reduction by personal protection equipment usually cannot be assumed, as no assumptions can be made on the protective effect of risk management measures that require a minimum level of knowledge, skill and concerted action. For non-professionals, the assessment will thus not consider PPE unless specifically agreed otherwise, e.g. wearing gloves when using antifouling products. Risk management measures applicable for non-professionals would normally consist of measures ensuring that the biocidal product is provided in a form that reduces or excludes exposure without the need of specific action by the user, such as technical measures like bait boxes for rodenticides and insecticides, safety locks on bait stations.

Professional users come into contact with active substances in the biocidal products in their professional life. In most circumstances the professional user is subject to worker protection

legislation (Directive 89/391/EC and Council directive 98/24/EC). As a general rule, the hierarchy of control should be employed according to the STOP principle (Substitution, Technical measures, Organisational measures, Personal protection). This principle ranks exposure-mitigating measures in order of priority, first priority being substitution and last one personal protective equipment.

The type of professional users also needs to be considered, as some will be trained professionals having expert knowledge and skills in handling hazardous biocidal products. For such users, the variability in exposure for a certain task can be assumed lower than for non-specialised users.

On the other hand, some workers (e.g. self-employed, farmers) may have limited knowledge and skills to handle biocidal products, particularly if the use of the biocidal product is not routinely required in their workplace. The exposure of these users might be similar to non-professional users.

As a general rule, risk reduction measures for professionals are aimed to mitigate either single (peak) exposure or (e.g. daily) average values. The AEL/AEC selected should reflect this aim, considering also the time-dependency of toxicity in deciding on the most appropriate risk management strategy.

4.2 Quantitative risk characterisation

4.2.1 Introduction

Where a critical effect is **threshold-based**, quantitative risk assessment should be carried out for each exposed population, product-type, and method of application relevant for the respective biocidal products based on exposure assessment. The acute, medium-term and long-term AELs are used as general health-based reference values for the human population as a whole (in exceptional cases different values have been derived for professional and non-professional users; see also section 2.3.6).

Where quantitative hazard characterisation is possible (see Section 2), reference values are derived for use in quantitative risk characterisation together with the outcome of exposure assessment, as shown in Figure 3.

In quantitative risk characterisation, exposure estimates are compared to the corresponding AEL for each use and relevant time frame. A tiered approach has to be followed according to the same principles as described in section 3.2.4. Where the exposure/AEL ratio is ≤1, the risk is considered acceptable. Ratios >1 are considered unacceptable and further refinement is needed in exposure assessment if possible, including risk management measures.

In general, in the **first tier,** systemic AELs derived for acute, medium-term, and long-term exposure are compared with the total internal body burden expressed as mg/kg bw/day, based on potential exposure without PPE. If the estimated exposure is lower than the reference value, there is no cause for concern and no further refinement is necessary. If RMMs (including PPE) are required due to qualitative risk characterisation (e.g. for local effects), these RMMs should be taken into account also in the quantitative risk characterisation. Similarly, technical specifications and operational conditions may reduce exposure and should in this case be included in the assessment at this stage. It is however generally not necessary to revise calculations that already show safe use if further RMMs are required at a later stage due to local effects.

If the first tier results in an unacceptable level of risk for any of the scenarios, a refinement is needed in the **second tier**. The refinement can be a revision of the exposure assessment and/or using more specific absorption rates, giving special attention to route specific contributions of exposure and protection measures as well as to uncertainty analysis underlying both hazard and exposure components of risk characterisation. The refinement is in practice iterative: additional refinements are included until safe use can be identified or until no further refinements are possible. See section 4.1.3 for further guidance on the refinement possibilities.

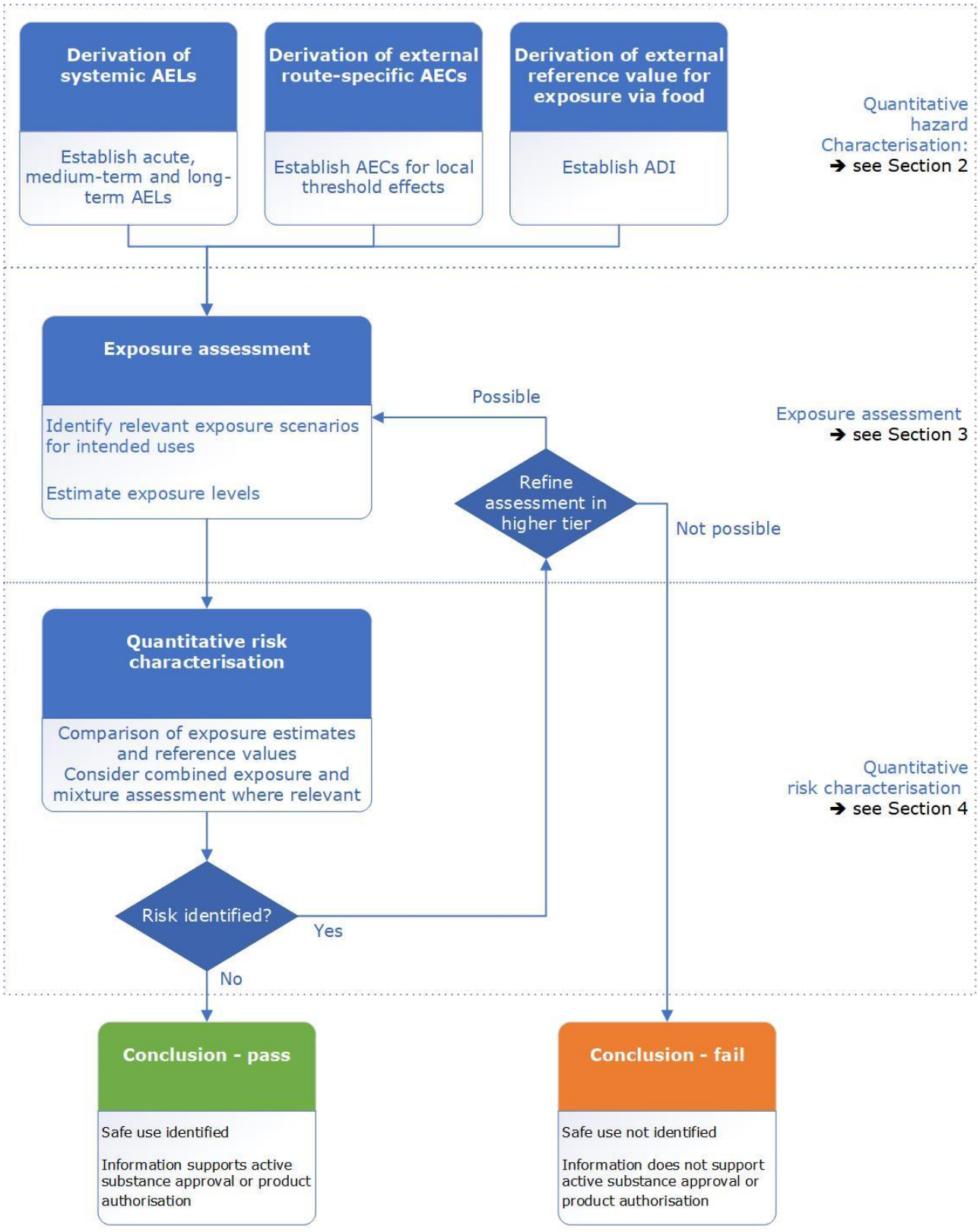
4.2.2 Exposure via food

For exposure via food, please refer to *ECHA Guidance Vol III Part D*. Further guidance is also available on the ECHA Website[60].

If exposure via food is possible, derivation of ADI and ARfD is necessary (see Section 2.3.8).

[60] https://echa.europa.eu/about-us/who-we-are/biocidal-products-committee/working-groups/assessment-of-residue-transfer-to-food

Figure 3: Concluding on safe uses in quantitative hazard and risk characterisation



4.2.3 Corrosive formulations/dilutions

For corrosive formulations/dilutions, qualitative assessment is performed, resulting in the requirement for personal protective equipment and risk management measures. This is independent of whether the corrosivity is due to the biocidal active substance or a co-formulant.

It can however not be assumed that PPE and RMMs ensure in all situations no direct contact with corrosive formulations/dilutions, which may penetrate, permeate or by-pass the PPE. Therefore, systemic risk characterisation is needed also when corrosive products are used, unless 1) the product has only local effects and no primary systemic effects, or 2) it is possible to justify that exposure is negligible due to a thorough description of technical RMMs (see Table 41 for examples of possible RMMs). For example, if a corrosive product contains an active substance having systemic toxicity, a quantitative risk characterisation might need to consider exposure of the professional user by applying the protection factors of gloves[61].

The systemic risk characterisation should cover all routes of exposure and is performed in addition to the qualitative assessment.

4.2.4 Threshold of toxicological concern (TTC)

The TTC approach is a screening and prioritisation tool for the risk assessment of chemicals when hazard data are incomplete and human exposure can be estimated. For biocides, the TTC could be used in assessing the toxicity of e.g.:

- impurities;
- metabolites (including groundwater metabolites);
- transformation products formed from water treatment processes on residues of the AS or its metabolites in surface water and/or groundwater abstracted for the production of drinking water.

The TTC concept may be of use as a risk management tool when negligible exposure and potential for waiving specific data requirements is under consideration. Therefore, the use of TTC concept requires a high level of confidence in the exposure data or estimates. Generally, exposure below the relevant TTC would be considered negligible (or at least acceptable).

TTC values indicate generic human chronic exposure thresholds that have been established by grouping experimental toxicity data from animal bioassays by the oral route. TTC values are derived by applying a probabilistic methodology such that the chance of adverse effects is low at human exposure levels below these values. The TTC values are provided in Table 30.

The TTC concept has been incorporated in the risk assessment by regulatory bodies, such as FDA, EFSA, EMA and JECFA (the Joint Expert Committee on Food Additives of the U.N. Food and Agriculture Organization and the World Health Organization).

The *EFSA TTC Guidance* provides step-by-step instructions for using the TTC approach. It defines inclusion and exclusion criteria and explains the TTC decision tree. The EFSA guidance should be used as the reference guidance for the TTC assessment under BPR.

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[61] See also the BPC Opinion on *Questions on the risks of exposure of workers to corrosive particles during the use of biocidal products by coarse spraying*; available at https://echa.europa.eu/regulations/biocidal-products-regulation/approval-of-active-substances/opinions-on-article-75-1-g

This section presents an introduction to the EFSA TTC approach, its limitations, criteria for use and the field of use under BPR.

The approach can be used when:

- the chemical structure of the substance is known;
- there are limited chemical-specific toxicity data; and
- the exposure can be estimated.

The TTC approach should not be used:

- for substances for which EU legislation requires the submission of toxicity data,
- when sufficient data are available for a risk assessment,
- if the substance under consideration falls into one of the exclusion categories.

Table 30: TTC values for the different substance categories

Substance category	TTC value (µg/person /day)	TTC value (µg/kg bw/d)	Basis of TTC value
DNA-reactive mutagens and/or carcinogens	0.15	0.0025	Analysis of EFSA Scientific Committee 2012
Organophosphates or carbamates	18	0.3	Analysis of EFSA Scientific Committee 2012
Cramer Class III	90	1.5	Database of 613 chemicals with 2941 NOAELs (Munro *et al.*, 1996)
Cramer Class II	540	9	Database of 613 chemicals with 2941 NOAELs (Munro *et al.*, 1996)
Cramer Class I	1800	30	Database of 613 chemicals with 2941 NOAELs (Munro *et al.*, 1996)

If the estimated exposure to a substance is higher than the relevant TTC value, a non-TTC approach is required to reach a conclusion on potential adverse health effects.

The Cramer classification scheme is presented below. More details on the development and implementation of the Cramer classification are included in the *EFSA TTC guidance*.

The structural classes for chemicals in the Cramer scheme are as follows:

- **Class I:** Substances with simple chemical structures and for which efficient modes of metabolism exist, suggesting a low order of oral toxicity. This class would include normal constituents of the body (excluding hormones); simply-branched, acyclic aliphatic hydrocarbons; common carbohydrates; common terpenes; substances that are sulfonate or sulfamate salts, without any free primary amines.
- **Class II:** Substances which possess structures that are less innocuous than Class I substances, but do not contain structural features suggestive of toxicity like those substances in Class III. This class would include common components of food; substances containing no functional groups other than alcohol, aldehyde, side-chain ketone, acid,

ester, or sodium, potassium or calcium sulfonate or sulfamate, or acyclic acetal or ketal and are either a monocycloalkanone or a bicyclic substance with or without a ring ketone.

- **Class III:** Substances with chemical structures that permit no strong initial presumption of safety or may even suggest significant toxicity or have reactive functional groups. This class would include structures that contain elements other than carbon, hydrogen, oxygen, nitrogen or divalent sulfur; certain benzene derivatives; certain heterocyclic substances; aliphatic substances containing more than three types of functional groups.

The TTC concept should not be applied for substances that are not represented in the database or are outside the domain of applicability of the TTC concept:

- Inorganic substances;
- Proteins;
- Nanomaterials;
- Radioactive substances;
- Organosilicon substances;
- Metals in elemental, ionic or organic form.

However, in the case of organic salts, where the counter ion is an essential metal (e.g. sodium), the EFSA Scientific Committee recommended that the TTC approach could be applied to the organic ion.

The TTC concept should not be applied to substances with the following properties:

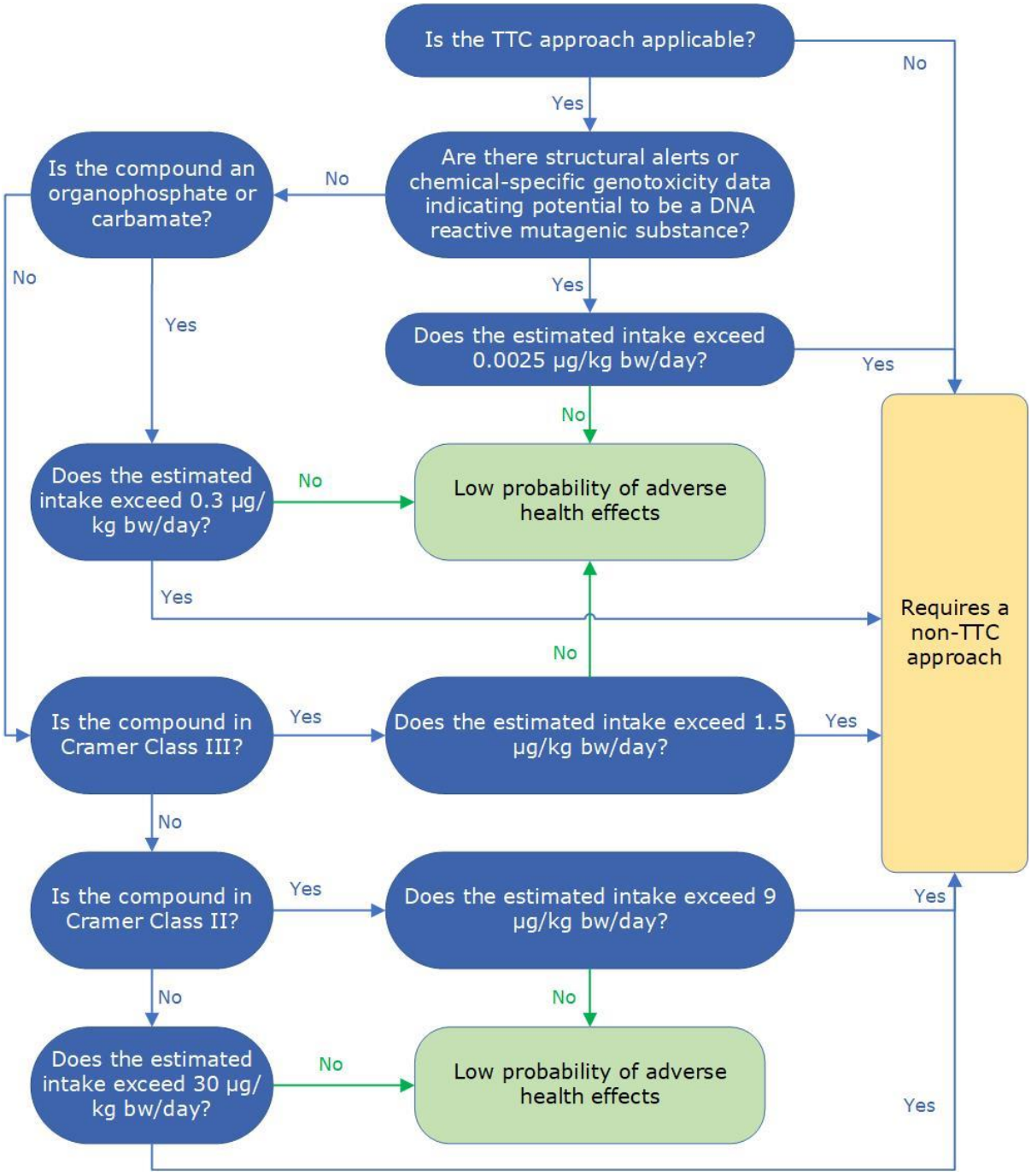
- High potency carcinogens: aflatoxin-like, azoxy- or N-nitroso substances and benzidines;
- Steroids;
- Substances with a potential for bioaccumulation according to the analysis of EFSA 2012, including substances like polyhalogenated-dibenzodioxins, -dibenzofurans and -biphenyls.

The use of TTC concept is limited to systemic effects and exposure by the oral route, because the TTC values are derived on databases covering primarily systemic effects from oral exposure. This is especially important to note where inhalation or dermal exposure is the main route of contact.

Local effects such as irritation and sensitisation are not covered by the TTC values.

Figure 4 provides a decision tree for applying the TTC concept. Before using this decision tree, 1) an exposure assessment should be made for the appropriate duration, and 2) a literature search should be performed for the substance (or a structural analogue) to decide whether there are sufficient data for risk assessment, including any read-across considerations. If the substance is a member of a group that has well-established toxicity data, the TTC approach is not applicable.

Figure 4: TTC decision tree



4.3 Systemic risk characterisation for combined exposures

In the evaluation of dossiers for biocidal products, the possibility of cumulative or synergistic effects shall be taken into account (Article 19(2)(d-e) and Annex VI of BPR). Furthermore, BPR Article 8(3) refers to the necessity to consider cumulative effects from the use of biocidal products containing the same or different active substances. For cumulative, aggregate and combined exposure, see also Section 3.5.

4.3.1 Terminology

There are no internationally harmonised definitions for cumulative, aggregate and combined exposure. The following definitions are applied in the current guidance:

- **Cumulative exposure**: combined exposure to multiple substances from any source or use.

  In this context, 'multiple substances' would normally share a common toxicological profile and/or have the same mode of action and/or have the same target organ. The substances may be from any source, including non-biocidal uses. The need to assess cumulative exposure could arise in a situation where health concerns may be expected due to exposure to more than one substances having the same mode of action or the same target organ.

  Cumulative exposure would normally be assessed only in specific cases on the basis of an identified or presumed health concern.

  Where an assessment is necessary, the approach described in Section 4.3 may be followed and adapted as necessary. Experience from other regulatory frameworks should also be considered, in particular where the same substances or the same mode of action has been assessed for example by EFSA or US EPA.

- **Aggregate exposure**: exposure to a single substance from any source or use and by any route of exposure.

  The assessment of aggregate exposure concerns the situation when the biocidal active substance is also used for other purposes or is e.g. a naturally occurring substance, and exposure to it can take place for example by using different chemical products or via the environment or in food. The need to assess aggregate exposure could arise in a situation where health concerns may be expected due to aggregate exposure via several of these sources and routes, where the biocidal use being assessed is only one contributing factor.

  Aggregate exposure would normally be assessed only in specific cases on the basis of an identified or presumed health concern. It can also be performed in a specific situation where several biocidal products are used e.g. during a workday and an assessment of 'combined exposure' (see below) is not needed because there is only one biocidal active substance and no substances of concern. Exposure may also take place to a range of treated articles for which the leaching rates should be considered.

- **Combined exposure to multiple substances**: exposure to two or more substances in a specified context.

  Assessing combined exposure may be performed to all substances in a product (simultaneous exposure), or to several products that one person is using during a workday. The difference to cumulative exposure is that for combined exposure to multiple substances, the substances can be very different while for cumulative exposure the mode of action and/or the target organ are the same.

Combined exposure to multiple substances would normally need to be assessed for a biocidal product that contains more than one active substance and/or substances of concern. However, since assessment of combined exposure to multiple substances can also be needed in other situations, it is always necessary to specify what is covered in the assessment (single product, several products, specified time or use situation etc.).

'Mixture' should be used as defined in the CLP Regulation:

- **Mixture**: a mixture consists of at least two substances that are intentionally or unintentionally mixed. Therefore, biocidal products can be considered as mixtures in most cases, and the principles for classification and labelling of mixtures apply as described in the *CLP Guidance*.

  The terms mixture toxicity and mixture assessment refer to the hazard assessment and hazard characterisation of multiple chemicals/mixtures.

4.3.2 Systemic risk characterisation for combined exposure to multiple substances

Note: please refer to the Glossary of terms in the beginning of this Guidance, as well as Section 4.3.1 Terminology.

4.3.2.1 Introduction to combined exposure to multiple substances

Among the first steps in hazard assessment of mixtures is the identification of whether the chemicals present in the mixture interact and produce an increased or decreased overall response compared to the sum of each chemical acting independently of each other.

The combined actions of components of mixtures can be due to non-interaction or due to interaction. In both cases similar or dissimilar mode of action can take place.

For **non-interaction**, there are in principle two possible approaches:

- Independent action: for chemicals with differing effects on the body, the combined effect equals the separate effect of each one alone. The risk for the mixture/product is considered acceptable if the risk for each substance in the mixture is acceptable. The risk for the mixture is not acceptable if there is an unacceptable risk for any of the substances.
- Dose addition: when two or more chemicals have the same effect on the body, differing only on potency, the combined effect can be estimated from the total dose of all chemicals together, adjusting for their relative potencies. This approach assumes that all substances in a biocidal product act as if they were dilutions or concentrations of each other. Substances are considered to act similarly if they have similar effect(s) on the same target organ or tissue. In most cases similar action cannot be ensured with certainty, but the conservative approach of concentration (dose) addition can be used as a first tier.

For **interaction**, one must assume one of the two possibilities:

- Synergism: the combined effect of two chemicals is greater than dose addition.
- Antagonism: the combined effect of two chemicals is less than dose addition or independent action.

For synergism or antagonism, there are no established methodologies that should be applied in assessing biocides; see however the approach presented in Section 4.3.2.3. In addition, PBK

modelling can be considered in elucidating possible toxicokinetic interactions of chemicals in mixtures.

A tiered approach is provided for risk assessment of biocidal products containing at least two substances for which a quantitative assessment is required for systemic effects. These substances can be active substances or SoCs. No assessment is required for co-formulants that are not SoCs.

There is normally very limited test data available for biocidal products, and the hazard assessment mostly relies on data on individual ingredients of the product. In addition to active substances, products may contain substances of concern (SoC) for which a systemic quantitative assessment may be needed[62].

The same assessment principles can be used in other situations where combined exposure to multiple substances needs to be considered, including e.g. dilutions of products and exposure to multiple products. To cover all these situations, the term 'mixture(s)' is used below.

In the various steps, the following terms are used:

- **Hazard quotient** (HQ) for each substance is the ratio of internal exposure and AEL (internal exposure divided by AEL).
- **Hazard index** (HI) is the sum of HQs calculated for each substance separately.
- **Surrogate AEL** is not derived according to the principles described in Section 2.3.6 but is used similarly as an AEL. Such value would normally rely on another value derived in other regulatory frameworks (e.g. OEL, DNEL).

Before the tiered assessment, two preliminary steps are performed to (Step a) verify acceptability of each substance in the mixture(s) one by one, and (Step b) whether there are synergistic effects.

Tier 1 is a worst-case assessment of combined exposure to the substances in the mixture(s), applying simple additivity (dose addition). If Tier 1 shows risk, a more complex but more realistic assessment is performed by identifying common target organs in Tier 2 and, where needed, setting adjusted AELs in Tier 3. Tier 4 (analysis of mechanism of action) is provided as an option for completeness while most often information would not be sufficient to follow it.

Noting that lower Tiers are more conservative and higher tiers become more realistic, one may proceed to higher tiers to verify if the requirement of PPE and RMMs identified in lower Tiers is necessary. Proceeding to higher tiers would be recommended if achieving safe use in lower tiers requires PPE or RMMs that are additional with respect to those required for individual substances of the mixture(s) and are considered to limit the use of the product(s). In using reference values, the time frame needs to be considered, e.g. acute, medium-term or long-term (surrogate) AEL.

Where (surrogate) AELs are derived from e.g. OEL or DNEL, the information that was used in setting these values need to be considered. OELs and DNELs are often route specific, most commonly derived for inhalation, and may correspond to external reference values rather than systemic ones. Correction for bioavailability/absorption should be considered where possible.

[62] See CA-Nov14-Doc.5.11 (https://circabc.europa.eu/ui/group/e947a950-8032-4df9-a3f0-f61eefd3d81b/library/e8b77b92-0867-4c7a-9dde-8de0c2031c29/details).

Figure 5 presents an overview of the methodology, and an example of applying the methodology is provided in Section 4.3.2.8.

A fully quantitative risk assessment is required for SoCs with classifications as Carc (1A, 1B, 2), Repr (1A, 1B, 2), Lact (H362), Muta (1A, 1B, 2), STOT RE (1, 2)[63]. Substances with these classifications need to be included in a RC for combined exposure.

Considerations on feasibility for SoCs:

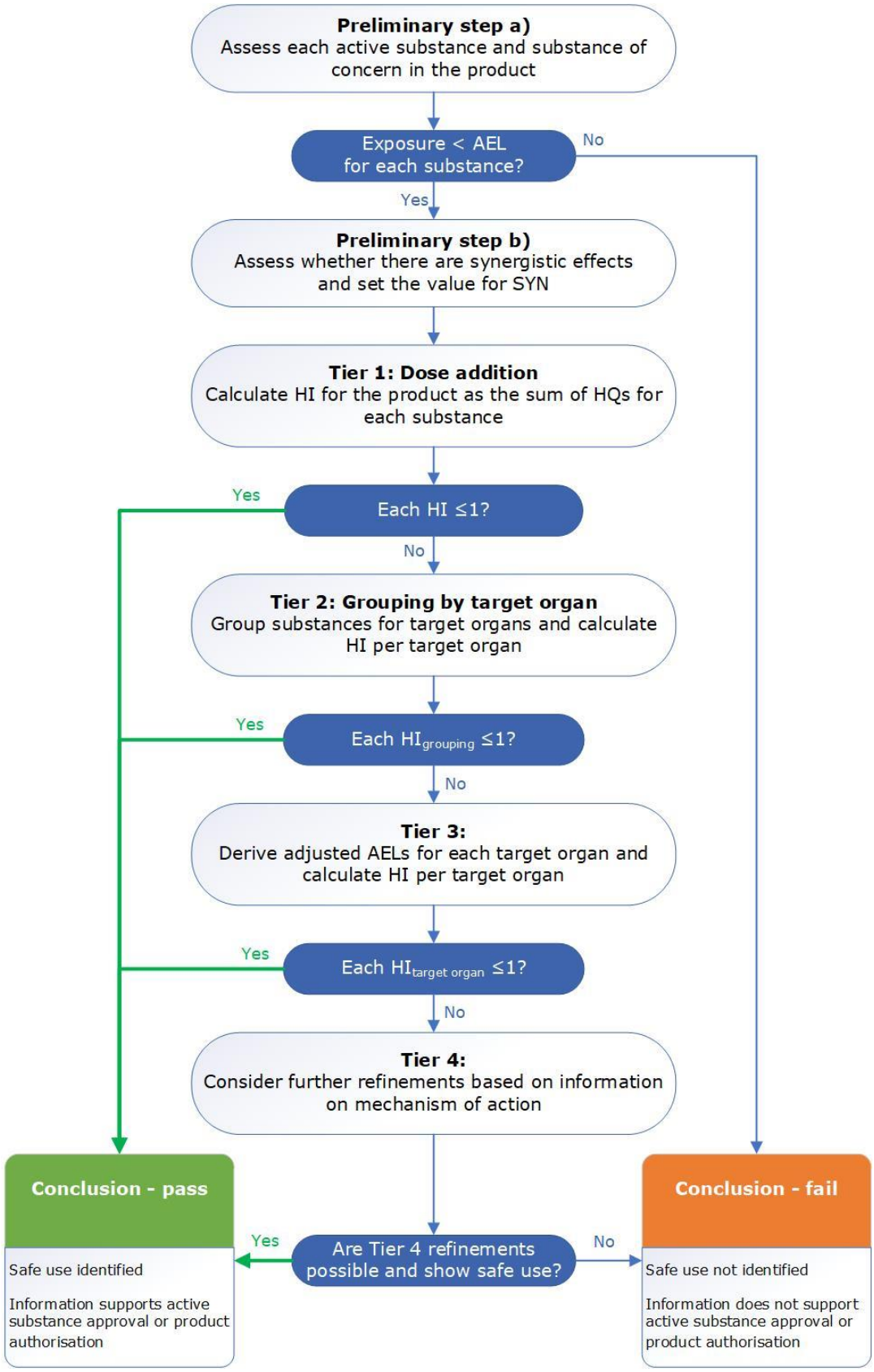
- A prerequisite for including a substance in a RC for combined exposure assessment is that an AEL is available or a surrogate AEL can be derived from an existing value such as DNEL, AOEL or OEL. These values should be used only if they are set by a European authority (e.g. ECHA or EFSA).
- If an AEL is not available and a surrogate value cannot be derived, it is not possible to include the substance in a RC for combined exposure to multiple substances. The lack of a reference value could be because no suitable values and data are available, or because the substance has non-threshold properties or only local toxicity. Where a full quantitative risk assessment is required but cannot be performed due to insufficient information, it is in principle not possible to conclude that the use is safe. According to CA-Nov14-Doc.5.11[64], it is therefore proposed that the use of such co-formulants/SoCs in biocidal products should be discouraged.

DNELs from safety data sheets and REACH Registration dossiers should normally not be considered, as generally they are not subject to evaluation by the Member States or ECHA. For substances that have been subject to Substance Evaluation (SEV), the SEV conclusion documents can provide valuable support regarding acceptability of a DNEL where the evaluating Member State has derived a DNEL based on their own assessment or stated their agreement with the DNEL proposed by the Registrant. However, SEV conclusion documents are not peer reviewed and only represent a snapshot in time. Further hazard data from e.g. higher tier mammalian studies may become available after conclusion documents have been published. This new information may result in the need for a lower DNEL.

[63] CA-Nov14-Doc.5.11 (https://circabc.europa.eu/ui/group/e947a950-8032-4df9-a3f0-f61eefd3d81b/library/e8b77b92-0867-4c7a-9dde-8de0c2031c29/details). Note that substances considered as endocrine disruptors are not included in this list. For ED substances, specific considerations are needed as quantitative RC for ED substances is currently not supported. See also Section 1.11.6 *Concluding on suitability for risk assessment* (under 1.11 *Endocrine disruption*).

[64] https://circabc.europa.eu/ui/group/e947a950-8032-4df9-a3f0-f61eefd3d81b/library/e8b77b92-0867-4c7a-9dde-8de0c2031c29/details

Figure 5: Overview of RC for combined exposure to multiple substances



RAC sets reference DNELs that are used as a basis for characterising risk in Applications for Authorisation or restriction proposals (for SVHC substances with for example CMR, ED, PBT, vPvB, PMT or vPvM properties). Reference DNELs do not have a formal legal basis but are used by Applicants on a voluntary basis. Reference DNELs benefit both applicants and RAC by ensuring the risk characterisation is always based on the same hazard conclusions. These reference DNELs are developed by a member of RAC or ECHA secretariat and are peer reviewed and agreed upon by members of RAC. The reference DNELs that have been developed and published are available at the ECHA website[65].

RAC (and formerly SCOEL) provides opinions on occupational exposure limits under the Carcinogens, Mutagens or Reprotoxic substances Directive (2004/37/EC) and the Chemical Agents Directive (98/24/EC) on worker protection from risks related to exposure to substances found in the workplace. Information on the activities planned, ongoing or completed by ECHA in relation to occupational exposure limits is available at the ECHA website[66].

Noting the shortcomings in the approach presented for combined exposure to multiple substances, case-by-case assessment is required using expert judgment to avoid disproportionate conclusions.

4.3.2.2 Preliminary step a) Assessment of each substance in the mixture(s)

This step is performed to verify that a safe use can be identified for each substance individually; if this is not the case, a RC for combined exposure to multiple substances is not necessary.

Each active substance and SoC in the mixture(s) is assessed individually in terms of systemic risks to primary and secondary exposure following all the scenarios relevant to the uses, considering the required level of PPE.

- ➔ If the estimated level of exposure to each substance in each scenario is lower than the relevant (surrogate) AEL (acute, medium-term, long-term), proceed to preliminary step b).
- ➔ If the estimated level of exposure in any of the scenarios is above the relevant (surrogate) AEL and no refinement is possible, the use is not safe and there is no need to proceed with the RC for combined exposure to multiple substances for the use.

4.3.2.3 Preliminary step b) Synergistic effects

This step is performed to confirm whether synergistic effects are identified or there is convincing evidence that justifies assuming synergism for a specific substance combination. The modes of action of the substances should be reviewed, taking into account all available information, including literature, to identify potential mixture effects and synergism. Where information is limited, there is higher uncertainty in the risk assessment for combined exposure to multiple substances.

[65] https://echa.europa.eu/applying-for-authorisation/evaluating-applications; see the row 'Reference DNELs'.
[66] https://echa.europa.eu/oels-activity-list

If no synergism is demonstrated, a safety factor for synergism (SYN) is set to 1. If the conclusion of the assessment is that synergism is demonstrated for a specific substance combination, SYN is applied on the basis of all available information.

- When synergism is shown, SYN should normally be 4[67].
- The value should normally be set to 2 if the available information justifies a reduction or there are indications of synergism but without conclusive evidence.

Other values should be used only where specific data justifies this.

4.3.2.4 Tier 1: Assessment of mixture by dose addition

As a pragmatic and conservative first tier assessment, dose additivity is considered for the effects used to establish the (surrogate) AELs for each substance in the mixture(s).

The assessment is performed with the same parameters as in the preliminary step a). The HQ for each substance is used to calculate HI for the mixture(s) as the sum of the HQs for each substance, multiplied by SYN. The default value for SYN is 1, and deviations from this should be made only if synergistic effects were identified in preliminary step b).

The HI is calculated as follows (see an example of applying the methodology in Section 4.3.2.8):

HI = (SYN) × Σ (internal exposure / AEL)

➔ If HI ≤ 1 the risk is acceptable and no further assessment is needed.

➔ If HI > 1 the risk is not acceptable:

- Include realistic RMMs (and PPE for professionals) in a stepwise manner, taking into account the hierarchy of control, until a safe use is identified (HI ≤ 1).
- If the risk remains unacceptable (HI > 1) and no further RMMs/PPE can be included, proceed to Tier 2.

4.3.2.5 Tier 2: Grouping by target organ

Target organs for each substance are listed and the substances are grouped according to their common target organs. Subgrouping may be needed if there are several modes of action affecting one target organ. For each group, HI per target organ ($HI_{target\ organ}$) is calculated.

Where synergism was seen and the value of SYN was set above 1, it needs to be considered if the synergistic effects are not relevant for some of the target organs and it would be justified to set SYN as 1.

The HIs per target organ are calculated as follows (see an example of applying the methodology in Section 4.3.2.8):

$HI_{grouping}$ = (SYN) × Σ (internal exposure / AEL)

[67] There is overall lack of scientific information to establish default values for synergism, but Boobis et al. (2010) showed that the magnitude of synergism at low doses did not exceed the levels predicted by additive models more than by a factor of 4.

➔ If each $HI_{grouping}$ ≤1 the risk is acceptable and no further assessment is needed.

➔ If one or more $HI_{grouping}$ > 1 the risk is not acceptable:

- Include realistic RMMs (and PPE for professionals) in a stepwise manner, taking into account the hierarchy of control, until a safe use is identified (HI ≤ 1).
- If the risk remains unacceptable (HI > 1) and no further RMMs/PPE can be included, proceed to Tier 3.

Note: If there is no target organ or mode of action in common, dose addition is not confirmed, and the risks are already covered by the preliminary step a).

4.3.2.6 Tier 3: Adjusted AELs

In each group (established in Tier 2) for which risk is not acceptable, adjusted AELs ($AEL_{target\ organ}$) are derived for each identified target organ/mode of action and each substance, if possible. This is done based on all available information, considering also the time frame (acute, medium-term, long-term). Adjusted AELs are derived using the same principles and safety factors described in Section 2.3, where applicable.

Based on the exposure estimates calculated in preliminary step a), HI per target organ ($HI_{target\ organ}$) is calculated for each group.

Where synergism was seen and the value of SYN was set above 1, it needs to be considered if the synergistic effects are not relevant for some of the target organs and it would be justified to set SYN as 1.

The HIs per target organ are calculated as follows (see an example of applying the methodology in Section 4.3.2.8):

$$\mathbf{HI_{target\ organ} = (SYN) \times \sum (internal\ exposure\ /\ AEL_{target\ organ})}$$

➔ If each $HI_{target\ organ}$ ≤ 1 the risk is acceptable and no further assessment is needed.

➔ If one or more $HI_{target\ organ}$ > 1 the risk is not acceptable:

- Include realistic RMMs (and PPE for professionals) in a stepwise manner, taking into account the hierarchy of control, until a safe use is identified (HI ≤ 1).
- If the risk remains unacceptable (HI > 1) and no further RMMs/PPE can be included, proceed to Tier 4.

4.3.2.7 Tier 4: Mechanism of action

If available, information on the mechanism of action can be used to further refine the assessment. If one or more HI is above 1 even when including realistic RMMs (and PPE for professionals), the risk is not acceptable and no further refinement is possible.

Note that mode of action (MoA) and mechanism of action (MOA) concern information of different levels: mode of action describes the functional/anatomical effects and is considered in Tier 2, while mechanism of action concerns the molecular level and is considered in Tier 4.

The likelihood of having sufficient information to perform a Tier 4 assessment seems low. If a Tier 4 assessment is possible in a specific case, the mechanistic information will be handled on a case-by-case basis.

4.3.2.8 Example of risk characterisation for combined exposure to multiple substances

An example of performing a RC for combined exposure to an active substance (AS) and two co-formulants (SoC1, SoC2) in a biocidal product is described. The same approach can be used for any number of active substances or SoCs.

The assessment concerns both professional and non-professional users. The exposure assessment is the same for both user groups, with the difference that PPE can be required for professionals.

Preliminary step a) Assessment of each substance in the product

The relevant (surrogate) AELs and results of the exposure assessment and the resulting HQs are provided in Table 31. The exposure values are relevant for both acute and long-term exposure. HQs are calculated by dividing the systemic exposure by the relevant AEL, considering the required level of RMM and/or PPE (RMM/PPE).

Table 31: RC for combined exposure to multiple substances – preliminary step a)

		AS	SoC1	SoC2	Conclusion
Systemic exposure (mg/kg bw/day)	No RMM or PPE	0.0125	0.0075	0.01	
	Exposure reduced with RMMs or PPE	0.005	0.0025	0.003	
Acute	AEL (mg/kg bw/day)	0.1	0.2	0.5	
	HQ, no RMM/PPE	0.125	0.0375	0.02	All acceptable
	HQ with RMM/PPE	0.05	0.0125	0.006	All acceptable
Long-term	AEL (mg/kg bw/day)	0.05	0.01	0.02	
	HQ, no RMM/PPE	0.25	0.75	0.5	All acceptable
	HQ with RMM/PPE	0.1	0.25	0.15	All acceptable

As shown in the table above, all HQs are below 1 as the estimated level of exposure to each substance is below the relevant AEL values for both acute and long-term exposure. The risk for each substance individually is acceptable and a risk assessment is necessary for combined exposure to multiple substances.

Preliminary step b) Synergistic effects

No indications of synergism were seen in a literature search or in acute studies performed with the product. The value for SYN is therefore 1.

Tier 1: Assessment of mixture by dose addition

The exposure information, AEL values and HQs shown in Table 31 is applicable. The results of the assessment by dose addition are shown in Table 32.

Table 32: Assessment based on dose addition – Tier 1

		AS	SoC1	SoC2	HI	Conclusion
Acute	HQ, no RMM/PPE	0.125	0.0375	0.02	0.185	Acceptable
	HQ with RMM/PPE	0.05	0.0125	0.006	0.0685	Acceptable
Long-term	HQ, no RMM/PPE	0.25	0.75	0.5	1.5	Not acceptable
	HQ with RMM/PPE	0.1	0.25	0.15	0.5	Acceptable

In Tier 1, acute exposure is acceptable without PPE while long-term exposure is acceptable only with PPE. This would support acceptable long-term use for professionals only.

Tier 2 assessment will be performed to assess the acceptability for non-professionals. This will also confirm whether PPE should be required for professionals.

Tier 2: Grouping by target organ and mode of action

To perform a Tier 2 assessment, more information on the substances is necessary. For many SoCs, the available information may not be sufficient, and the assessment would stop at Tier 1. The effects seen in target organs are listed in Table 33 and the HI for each target organ or mode of action is calculated in Table 34.

Only long-term effects and long-term exposure will be considered because acute exposure is safe in Tier 1.

Table 33: Target organs for each substance – Tier 2

Target organ (mode of action)	AS	SoC1	SoC2
Liver	✓	✓	✓
Thyroid	✓	✓	
Kidney	✓		✓
Eye (cataract)	✓		
Fertility		✓	

Table 34: Calculation of HI for each target organ or mode of action – Tier 2

Target organ / Mode of Action	RMM/PPE	HQ			HI
		AS	SoC1	SoC2	
Liver	None	0.25	0.75	0.5	1.5
	RMM/PPE	0.1	0.25	0.15	0.5
Thyroid	None	0.25	0.75		1
	RMM/PPE	0.1	0.25		0.35
Kidney	None	0.25		0.5	0.75
	RMM/PPE	0.1		0.15	0.25
Eye (cataract)	None	0.25			0.25
	RMM/PPE	0.1			0.1
Fertility	None		0.75		0.75
	RMM/PPE		0.25		0.25

The results of Tier 2 assessment show that long-term exposure is not acceptable without RMM/PPE due to liver effects. For thyroid effects, HI is 1 which is formally safe but as a borderline case this would likely require some further considerations in the assessment.

The risk for liver effects can be refined in Tier 3.

Tier 3: Adjusted AELs

An AEL adjusted for liver effects is derived risk was identified in Tier 2. Using the relevant NOAEL values for liver, the liver adjusted AEL is derived by applying a default AF 100. The AF can also be different from this default value; the principles described in section 2.3.4 apply.

Table 35: Deriving liver adjusted AEL values – Tier 3

	AS	SoC1	SoC2
NOAEL liver (chronic) (mg/kg bw/day)	5	2	2
AEL liver (chronic) (mg/kg bw/day)	0.05	0.02	0.02

The liver adjusted AEL values are used to recalculate the HI for liver effects.

Table 36: Calculation of HI for long-term liver effects – Tier 3

		AS	SoC1	SoC2	HI	Conclusion
Systemic exposure (mg/kg bw/day)	No RMM/PPE	0.0125	0.0075	0.01		
	With RMM/PPE	0.005	0.0025	0.003		
AEL liver (mg/kg bw/day)		0.05	0.02	0.02		
HQ	No RMM/PPE	0.25	0.375	0.5	1.125	Not acceptable
	With RMM/PPE	0.1	0.125	0.15	0.375	Acceptable

The results of Tier 3 assessment show that the risk without RMM/PPE is still not acceptable. The risk is acceptable when the RMM/PPE are applied.

Tier 4: Mechanism of action

This example does not demonstrate the use of Tier 4 as the guidance also provides no principles for this and the likelihood of having sufficient information is low.

Conclusion for the example

Conclusion for combined exposure to multiple substances:

- Professional users: The risk is acceptable with RMM/PPE.
- Non-professional users: The risk is acceptable if the RMMs are applicable for non-professionals and are sufficient without PPE.

4.4 Semi-quantitative and qualitative risk characterisation

Semi-quantitative and qualitative risk characterisation may be required for effects that are not covered by reference values, or where reference values cannot be derived. This may be the case for effects such as irritation/corrosion, eye damage, sensitisation, mutagenicity, carcinogenicity and endocrine disruption.

In cases where both quantitative and qualitative approaches need to be followed (e.g. systemic and local effects), these should complement each other in terms of risk management measures, and both must demonstrate adequate control of risks.

The purpose of the qualitative risk characterisation is to assess the likelihood that effects are avoided when implementing the technical, organisational and operational conditions and risk management measures that define each scenario.

A qualitative risk characterisation approach has to be followed when there is no basis for setting an acceptable exposure level for a certain human health endpoint, i.e. when the available data for this effect do not provide quantitative dose-response information, but there is toxicity data of a qualitative nature. The endpoints where the available data may trigger a qualitative risk characterisation are normally irritation/corrosion, eye damage, sensitisation, carcinogenicity, mutagenicity and endocrine disruption.

4.4.1 Non threshold mutagens and carcinogens

Genotoxicity

It is generally accepted that a threshold for genotoxicity may be established only for non-DNA reactive substances that are not clastogenic nor causing gene mutations (see section 1.8.5). Apart from this exception, it is usually assumed that a threshold does not exist for genotoxicity, and genotoxicity studies cannot provide any quantitative input to the risk characterisation. However, a conclusion on potential for genotoxic activity is a fundamental qualitative input to risk characterisation.

According to BPR, active substances classified as mutagens category 1A or 1B shall not be approved (exclusion criteria in BPR Article 5(1)) unless the derogation conditions are fulfilled (BPR Article 5(2)). However, if a risk assessment needs to be conducted for a mutagen (e.g. following derogation), a qualitative approach should be followed. Non-professional use and secondary exposure of the general public to these substances would normally be unacceptable.

Category 2 mutagens are substances or products for which there are indications of possible genotoxic effects in somatic cells but there is insufficient evidence to place the substance in category 1B. The risk from a category 2 mutagenic substance in a biocidal product should be also considered qualitatively on a case-by-case basis, taking into account exposure conditions. A thorough assessment of possible groups entering treated areas or handling treated goods is essential. The possibility of exposure and the available measures to control and limit exposure would also influence whether the risk was so low as to be acceptable.

Carcinogenicity

According to BPR, active substances classified as carcinogens Cat 1A or 1B shall not be approved (exclusion criteria in BPR Article 5(1)) unless the derogation conditions are fulfilled (BPR Article 5(2)). However, if derogation is granted, risk evaluation still needs to be performed.

The acceptability of the risk from active substances contained in biocidal products for which there is carcinogenic potential will depend on the category of carcinogenicity classification, the likely mechanism of carcinogenicity and the extent of exposure.

Products classified as Carc. Category 1A or 1B shall not be authorised for non-professional uses. The approval of active substances meeting the criteria for category 1B classification will be strongly dependent on the mechanism and levels of exposure.

If the known or most likely mechanism has a threshold, then a quantitative threshold risk assessment approach can be taken. However, an additional AF to cover for the severity of effect might be used (e.g. if the PoD is based on increased incidence of tumours).

If more data on the mechanism is awaited (one of the criteria for category 2) or if it is believed that a genotoxic non-threshold effect may be responsible for the carcinogenic potential, then a threshold approach to risk assessment is not possible and the acceptability of the risk must be carefully considered qualitatively and/or in a semi-quantitative approach which provides a means to assess the efficiency of RMMs ensuring negligible exposure.

To perform a semi-quantitative approach for non-threshold carcinogens, see section 2.4.1. The DMEL methodology, the 'linearised' approach or the 'Large Assessment Factor' approach should be used to judge the remaining/residual likelihood of risks after RMMs and operational conditions are implemented. The derived reference dose (e.g. DMEL corrected for absorption) is then compared to the exposure estimate to conclude whether the risk is as low as reasonably practicable.

4.4.2 Local effects – qualitative and semi-quantitative risk characterisation

4.4.2.1 General considerations

Risk characterisation for local effects concerns irritation, corrosion and sensitisation.

The purpose of risk characterisation for local effects is to assess the likelihood that effects are avoided when implementing the technical, organisational and operational conditions that define each scenario. These are constituted by risk management measures (RMMs) and personal protective equipment (PPE). In this context, the hierarchy of controls and the STOP principle need to be considered (See Appendix 3-1), the first steps being to define technical and organisational measures, and only as the last resort PPE.

The qualitative RC for local effects focuses on the product, rather than the active substance only. For active substance approval, the assessment is performed for the representative product. Where unacceptable risk is identified for the product due to co-formulants, authorisation of the product would not be granted but approval of the active substance is still possible.

Using this guidance requires expert judgment and flexibility to avoid disproportionate conclusions, always considering reliability of the information, in particular any quantitative information, the WoE and any realistic exposure scenarios. While such considerations are relevant for all risk assessment methodologies, the importance is specifically highlighted for local RC.

In addition to this guidance, any documents agreed at the Biocides CA meeting[68] need to be considered, informing e.g. upon the possibility to require PPE for non-professional users.

4.4.2.1.1 Definitions for risk characterisation for local effects

In **quantitative local RC**, the hazard, exposure and risk parts of the RC are quantitative. An AEC or other reference value such as EU-OEL, is compared with quantitative exposure estimates.

In **qualitative local RC**, the hazard, exposure and risk parts of the RC are qualitative. For hazard characterisation, it is only considered whether classification criteria are met of the product itself and any in-use dilutions to which exposure may occur. For the exposure part, only qualitative information is used, i.e. who is exposed (industrial, professional, general public, children, infants), description of the exposure scenario, potential exposure routes, use frequency, duration of exposure, potential degree of exposure (amount and concentration of substance used) and relevant RMMs. Acceptability or non-acceptability of the risk is concluded on the basis of qualitative arguments.

In **semi-quantitative local RC**, the RC for local effects is not strictly defined and may be a combination of quantitative and qualitative approaches, depending on the information available. Such an approach will provide a description of the nature and severity of effects that may result from exposure. The assessment may include a comparison of a substance specific reference value (AEC, NOAEC) with the concentration of that substance in a biocidal product or a dilution.

[68] Documents finalised at the CA meetings are available at: https://circabc.europa.eu/ui/group/e947a950-8032-4df9-a3f0-f61eefd3d81b/library/386abfea-55ce-4764-8a31-f9d4f6ceaf0a?p=1&n=10&sort=modified_DESC

4.4.2.1.2 Decision logic for performing (semi-)quantitative or qualitative local RC

Table 37 provides the decision logic for performing (semi-)quantitative or qualitative RC for local effects considering the different routes of exposure, provided that the route is relevant for human exposure.

Table 37: Decision logic for local RC

Route of exposure	Effect	Qualitative RC	(Semi-)quantitative RC
Inhalation	Irritation Corrosion	Performed only if classification is triggered as one or more of the following: • STOT SE or STOT RE (respiratory tract) • H317 - *May cause an allergic skin reaction* • H335 - *May cause respiratory irritation* • H370 - *Causes damage to organs* (respiratory tract) • H371 - *May cause damage to organs* (respiratory tract) • H372 - *Causes damage to organs* (respiratory tract) *through prolonged or repeated exposure* • H373 - *May cause damage to organs (*respiratory tract*) through prolonged or repeated exposure* • EUH071 - *Corrosive to the respiratory tract*	Quantitative RC should be performed whenever possible, i.e. whenever an inhalation AEC (or another reference value such as an EU-OEL) is available[69]. If a relevant reference value (e.g. AEC, EU-OEL) is available for a substance of concern, a low concentration may justify not performing a quantitative assessment at all if it is clear that the exposure concentration will not reach the reference value. Where information is not sufficient or sufficiently reliable to derive a reference value, a semi-quantitative RC should be attempted.
	Respiratory sensitisation	Performed only if classification is triggered as: • H334 – *May cause allergy or asthma symptoms or breathing difficulties if inhaled*	Not applicable
Oral	Irritation Corrosion	Performed only if classification is triggered (with relevance to gastrointestinal tract) as one or more of the following: • H370 - *Causes damage to organs* (gastrointestinal tract*)*	The possibility of performing a semi-quantitative local RC should be considered on a case-by-case basis. A quantitative assessment would normally not be most relevant because the effects will depend on a number of parameters such as concentration, dosing system, exposure time and the

[69] For concluding on SoCs, see CG-45_e-c Harmonized appr._SoC and workplace exp. limits_vf available at https://webgate.ec.europa.eu/s-circabc/w/browse/89efe476-1017-46af-8a31-6ad845f79d04

		• H371 - *May cause damage to organs* (gastrointestinal tract) • H372 - *Causes damage to organs* (gastrointestinal tract) *through prolonged or repeated exposure* • H373 - May cause damage to organs (gastrointestinal tract) *through prolonged or repeated exposure* • H314 – *Causes severe skin burns and eye damage* • H315 – *Causes skin irritation*	frequency of exposure. Furthermore, the experimental design may not be corresponding to human oral exposure (e.g. testing by gavage). If systemic effects are present and are the most serious effects observed, the local gastrointestinal tract effects will be covered by the systemic risk assessment using AEL values derived from oral studies. For occupational settings, the oral route is normally not considered relevant due to occupational hygiene.
Dermal	Irritation Corrosion	Performed only if classification is triggered as one of the following: • H314 – *Causes severe skin burns and eye damage* • H315 – *Causes skin irritation* • H370 - *Causes damage to organs* (skin) • H371 - *May cause damage to organs* (skin) • H372 - *Causes damage to organs* (skin) *through prolonged or repeated exposure* • H373 - May cause damage to organs (skin) *through prolonged or repeated exposure* • EUH066 – *Repeated exposure may cause skin dryness or cracking* See also Figure 6.	Semi-quantitative local RC should be performed if classification for H314 or H315 is <u>not</u> triggered and: - a NOAEC is available and is relevant for the product, and - the NOAEC is based on prolonged or repeated exposure, and - the use concentration is above the NOAEC. If performed, this should include information regarding NOAEC/LOAEC for local effects and the expected dermal effects in the exposure situations, taking into account the amount and concentration to which exposure takes place, as well as the frequency and duration. The nature of the expected effects should be considered together with exposure considerations in deciding whether PPE or RMMs are required to limit the effects. When the dermal NOAEC is based on prolonged or repeated exposure, a semi-quantitative assessment is necessary because exposure may result in substantial local effects. When the dermal NOAEC is based on acute effects and classification is not triggered, semi-quantitative assessment is not needed because it would not impact the conclusion on acceptability. See also Figure 6.
	Skin sensitisation	Performed only if H317 classification is triggered as: • H317 – *May cause an allergic skin reaction*	Normally not applicable*

Eye	Irritation Corrosion	Performed only if classification is triggered as one of the following: • H318 - *Causes serious eye damage* • H319 - *Causes serious eye irritation* • H370 - *Causes damage to organs* (eyes) • H371 - *May cause damage to organs* (eyes) • H372 - *Causes damage to organs* (eyes) *through prolonged or repeated exposure* • H373 - May cause damage to organs (eyes) *through prolonged or repeated exposure*	Not applicable

** See also the subheading 'Sensitisation' below, in this same section.*

Where classification (of the product or the in-use dilution) is a trigger for local RC in the principles described in Table 37 and Figure 6, harmonised classification of co-formulants in that product is not required for triggering the local RC. In deciding on the classification of the product[70], the available information is prioritised as follows:

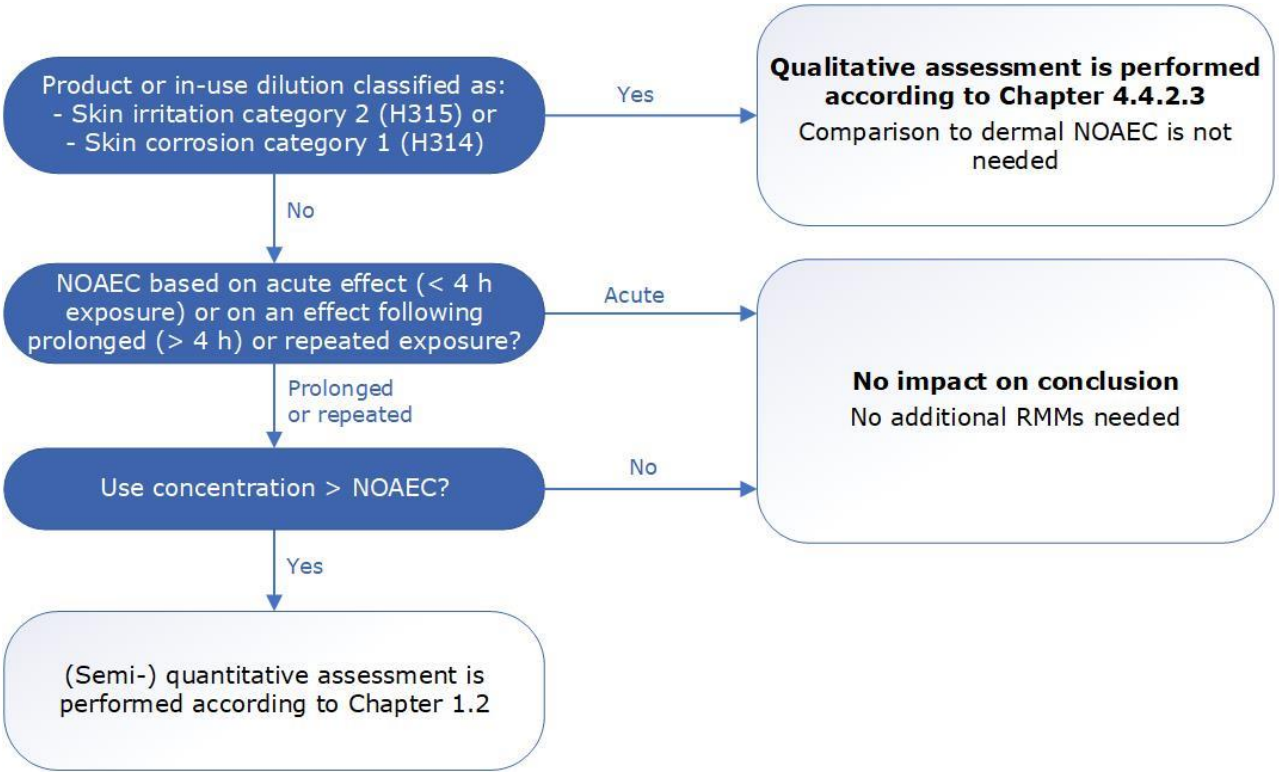
1. Harmonised classification (existing entry in Annex VI to CLP or a RAC opinion) is always used for both the active substance and co-formulants.
2. For substances and hazard classes where harmonised classification (CLH) is not available, the classifications proposed in a submitted dossier for CLH are used for both the active substance and co-formulants.
3. For substances and hazard classes where a dossier for CLH has not been submitted:
    - For a co-formulant, the self-classification recorded in the SDS submitted by the Applicant is normally taken as the basis for classifying the product, noting however that the eCA can deviate from it if there is information justifying this.
    - For an active substance, the classification included in the BPC opinion is applied, and where the BPC opinion is not yet available, the classification proposed by the active substance eCA is applied.

The above decision logic is followed for each constituent of the product.

According to *Introductory guidance on the CLP Regulation*, self-classification is the decision on a particular hazard classification and labelling of a substance or mixture taken by the manufacturer, importer or downstream user of that substance or mixture, or, where applicable, by those producers of articles who have the obligation to classify. Further information is available at the ECHA website[71].

[70] See also CA-Nov15-Doc.4.1-Final available at https://circabc.europa.eu/ui/group/e947a950-8032-4df9-a3f0-f61eefd3d81b/library/386abfea-55ce-4764-8a31-f9d4f6ceaf0a (direct link: https://circabc.europa.eu/ui/group/e947a950-8032-4df9-a3f0-f61eefd3d81b/library/d9b30c99-7d7b-41d0-b7f6-31eba3fac7c3/details)
[71] https://echa.europa.eu/regulations/clp/classification

Figure 6. Decision tree for performing local RC for dermal irritation/corrosion effects



For (semi-) quantitative RC, the AEC or NOAEC should have been peer reviewed and agreed under the BPR and must be relevant for the product in question (considering formulation). Expert judgment is required in concluding on the relevance of such values for each product, considering among other things the compositions of the product and of the test substance, the role of pH in local effects, and the frequency, duration and route of exposure in the study used to derive these values.

In selecting the most relevant study results for setting the NOAEC/LOAEC for local dermal effects, dosing should optimally resemble the expected human exposure in terms of amount, concentration, frequency and duration. The differences in the test formulation and the product formulation must be considered: for example, results from a test with the active substance in a vehicle would normally not be valid for a complex formulation with the same active substance concentration but may nevertheless provide useful information in decision making. Before setting a NOAEC/LOAEC in a given study, consideration should be given to the relevance of the information in assessing human exposure situations. The semi-quantitative information could be omitted from the assessment if the effects are only seen in conditions that are not relevant for human exposure.

Sensitisation

For respiratory sensitisation and dermal sensitisation, only a qualitative RC should be carried out. In some cases, if there are adequate and good quality human data available, including detailed information on exposure, quantitative RC may be possible. In this context, information on elicitation and a possible threshold for it will be crucial. By using *in chemico*, *in vitro*, and/or *in vivo* data, it is possible to estimate the potency of a sensitiser, but the proposed quantitative methodologies for dermal sensitisation are currently neither harmonised nor considered sufficiently protective and need further scientific clarification. Currently this is a topic of scientific

debate. New approaches should be considered when they receive regulatory acceptance e.g. within OECD.

The approach under REACH, including potency estimation, is provided in *REACH Guidance R8*, Appendix R. 8-10.

Performing both a qualitative and (semi-) quantitative assessment:

Where both a qualitative and (semi-) quantitative assessment are triggered, both of these are performed, and both need to be acceptable to conclude on a safe use. Applying the principles above, this situation may arise mainly 1) for the inhalation route, and 2) for the dermal route when the product or in-use dilution is both sensitising and has irritant/corrosive properties.

- ➔ If a reliable quantitative assessment (covering all relevant routes of exposure) shows no risk, it may be possible to exceptionally make a case-by-case decision whether the qualitative assessment could be disregarded. This exception is expected to be possible only for the inhalation route when the product is a pure active substance or only active substance in water solution, and the AEC value is based on the same effect as the classification.

If the available information does not allow performing a reliable (semi-) quantitative assessment, it is necessary to consider which specific additional information has to be requested (such as exposure data) that would enable performing this assessment. If such information cannot be specified or requested, the qualitative assessment must be relied on.

4.4.2.1.3 Uncertainties to be considered for risk characterisation for local effects

Uncertainties for all exposure routes

Data that are potentially useful for a quantitative RC for local effects usually contain several types of additional uncertainties compared to information on systemic effects.

AFs are used to address uncertainties due to LOAEC to NOAEC extrapolation, exposure time extrapolation and intraspecies/interspecies differences. However, Databased probabilistic information on extrapolation uncertainties are only available for systemic effects, while AFs used for local effects are substantially more uncertain as they are not based on probabilistic databases (see e.g. *REACH Guidance R.19*).

A key difference in the RC for local effects, compared to RC for systemic effects, is the need to consider the pH of the product, as well as the presence of co-formulants that may strongly influence the potential of the active substance to induce local toxicity, either increasing or decreasing the likelihood and severity of adverse effects. A local NOAEC/AEC established for the active substance may not be appropriate for the RC because of the product composition, noting that the local RC always concerns the product and not individual substances.

Additional considerations for the dermal route

Co-exposure to additional dermal stressors is particularly important in relation to local effects. Aggravation of the effects by mechanical and physical stress on the skin needs to be considered at the workplace in selecting the appropriate PPE, e.g. reducing contact with water at wet work places.

Endpoint uncertainty needs to be considered: skin irritation or sensitisation may be quantified by various methods and parameters (heat, redness, swelling and dysfunction) of different sensitivity. The relevance of semi-occlusive conditions and amount of substance per treated skin

area in the animal test need to be considered in comparison to the actual human exposure situation.

Exposure models or measurements usually provide highly uncertain dermal exposure values that are intended for assessing systemic effects and may not be suitable for assessing local effects. The values tend to be averaged over time and skin surface, while local effects are mainly driven by peak and localised substance/product concentrations on skin, e.g. in wrinkles.

Secondary exposure may need to be considered in situations where the product is applied at concentrations not triggering classification, but where drying may cause the concentration to increase in the applied product. In such situations, normally qualitative or semi-quantitative risk characterisation would be performed.

Additional considerations for the respiratory route

Airway anatomy, respiratory rate, deposition patterns and local and total clearance rates differ between animal models and humans. Since rats are obligate nose breathers and have a higher respiratory rate and higher filtering efficiency of inhaled particles and gases, the effects in the rat could result in overestimating the effects in human upper airways and underestimating the effects deeper in the respiratory tract. According to *REACH Guidance R.8*, when there is no data to inform on these uncertainties, "*it is prudent to assume that humans would be more sensitive than animals to effects on the respiratory tract. In such a situation, a chemical-specific remaining uncertainties factor or the default factor of 2.5 should be applied, as would be the case for systemic effects*."

The effects may differ due to the physical form of the active substance and products. For example, exposure to gas or aerosol may lead to different effects due to distributions in the respiratory tract.

With aerosol exposure, effects may differ due to different active substance concentrations in the aerosol, different aerosol mass per air volume, and different aerosol droplet size distribution.

Additional considerations for the oral route

The relevance of the rat forestomach irritation is questionable for human risk assessment. The epithelia of the rodent forestomach are not identical to the epithelia of the human oesophagus or stomach. The rodent forestomach is a cornified stratified squamous epithelium without glands, while the human oesophagus is a non-keratinizing stratified squamous epithelium with submucosal glands (providing some protection of the epithelium by mucus secretions) and the human stomach is lined by columnar epithelial cells with diverse glands. The rodent forestomach pH is 4.5 to 6, human oesophagus pH is 7 and human stomach pH is 1 to 2 (fasting). In humans, the contact time between the oesophagus epithelium and ingested material is negligible when compared to the rodents' forestomach that functions as a storage organ. The contact time in the human stomach and intestine may be significant, as is the contact time in the rodent glandular stomach and intestine.

Overall, NOAELs or concentrations for irritant effects are more relevant in those parts of the animal gastrointestinal tract having a counterpart in humans, such as oral cavity, pharynx, oesophagus, glandular stomach, and intestine.

Additional considerations for sensitising effects

Skin sensitisation tests simulate the induction and/or challenge phase of allergic contact dermatitis in humans/type IV hypersensitivity reactions in animals (= sensitisation and elicitation). Where induction has not been prevented and people are already sensitised,

approaches focusing on induction will not be sufficiently protective because of the lower doses required for elicitation.

For respiratory sensitisation, no internationally accepted tests are available. Models are being developed that can indicate respiratory sensitisation potential.

4.4.2.2 (Semi-) quantitative RC for local respiratory and skin effects

Respiratory effects: quantitative approach

The most reliable and relevant non-irritating concentration in animal or human studies (respiratory NOAEC) should be used to calculate the $AEC_{inhalation}$. With the interest of harmonisation between regulatory fields, the AFs in *REACH Guidance R.19* are applied with the exception that the same intraspecies AF is applied for professionals and non-professionals (referred to as "workers" and "general population" in REACH).

AFs for respiratory exposure:
Interspecies AF = 2.5 (default)
Intraspecies AF = 10

Deviation from the default AF proposed in REACH should be considered on a case-by-case basis, and the scientific reasoning/justifications should always be given.

This AEC is compared with the external inhalation exposures, normally expressed in mg/m^3.

Skin irritation: semi-quantitative approach

For dermal irritation effects, a full quantitative RC (using AFs) is of limited value because of uncertainty in dermal exposure models and measurements in terms of dermal dose per surface area. Furthermore, the usefulness of the information available from animal studies may be limited because the study setup would not necessarily reflect the human exposure situation.

Skin irritation mostly depends on peak exposure while exposure measurements are usually integrated or averaged over time. Peak exposure also cannot be estimated, for example in wrinkles. There is also considerable uncertainty in the dose per body surface area when personal protection is worn.

The NOAEC or LOAEC identified from the available animal or human data should normally be expressed as a percentage concentration (%) and compared directly with the in-use concentration (%) of the active substance in the representative product in each scenario without applying AFs. The aim of this comparison is to provide only an approximation of the magnitude of the effects that can be expected rather than a precise, quantitative measure of the risks involved.

A dermal AEC should not normally be derived, as it is preferable not to set a defined limit for acceptable exposure due to local dermal effects. An AEC would express a concentration above which the use would become unacceptable, and setting this level below a NOAEC would be questionable. However, where reliable and appropriate information is available regarding cumulative dermal effects and this information is considered relevant for humans, an AEC could be derived.

Addressing uncertainties of quantitative or semi-quantitative risk assessment for local effects

An uncertainty analysis should be considered. In line with *REACH Guidance R.19*, the uncertainties in the hazard and exposure assessment may be evaluated in addition to the quantitative or semi-quantitative risk assessment. A general checklist in this REACH guidance can be tailored to case-specific needs to indicate which uncertainties were addressed by AFs and which remaining uncertainties tend to over- or underestimate the risk estimate or influence it in either direction.

4.4.2.3 Qualitative RC for local effects

If a qualitative RC for local effects is necessary, all available information on potential local effects and possible exposure should be considered.

With the interest of harmonisation between regulatory fields, the principles described for the qualitative RC within *REACH Guidance Part E* should be considered.

The steps in the assessment are described in sections 4.4.2.3.1 and 4.4.2.3.2.

The examples in Section 4.4.2.3.3 may be used as templates to describe the hazard, exposure, risk and related uncertainties.

4.4.2.3.1 Identification of exposure scenarios - indicators and arguments

The following qualitative information on each exposure scenario should be provided:

- Who is exposed: general public (adults, children, infants), professionals or industrial workers, animals
- Tasks, uses and processes: see examples in Section 4.4.2.3.3
- Potential exposure route: skin, eye, respiratory tract, gastrointestinal tract

The following information should be provided for each exposure scenario:

1. Frequency and duration of potential exposure

   A realistic worst-case estimate should be provided. The likelihood of exposure increases with the frequency and duration of the task/use/process, while the duration of potential exposure might be significantly higher or lower than the duration of task/use/process and may be different for different exposure routes.

2. Potential degree of exposure

   If the degree of exposure can be estimated in terms of exposure estimates in ml/m^3 air or ml/cm^2 skin or mg/person, these can be considered together with all the other information in concluding on the acceptability of exposure.

3. Operational conditions and other RMMs already in use or additionally required.

4. PPE required

   Operational conditions in terms of technical and organisational provision and other RMM (including e.g. special formulations with microencapsulation, or special packaging, see 4.1.1) as well as PPE should be considered. Potentially relevant RMMs are listed in Table 41, and PPE in Tables 39 and 40.

In considering the acceptability of a particular exposure scenario, Table 2 provides examples of qualitative arguments that can be used to support acceptability or non-acceptability of the risk. Note that some arguments in the table may not be valid in all cases, and they need to be considered on a case-by-case basis. As examples, 'high viscosity' could increase the actual exposure time if dermal exposure takes place and the product remains on the skin, and "used with low frequency" would not be a valid argument for a sensitiser. The terms such as 'high' and 'low' are intentionally vague as they need to be considered case-by-case considering all of the available information.

For transparency, the assessment should report arguments supporting both acceptability of the risk and non-acceptability of the risk.

Table 38: Examples of qualitative arguments that can be used to support acceptability or non-acceptability

Arguments generally supporting lower risk	Arguments generally supporting higher risk
Reversible effect	Irreversible and/or severe effect[72] (e.g. Cat. 1 effect)
Adverse effect expected only after repeated, prolonged exposure (e.g. STOT RE and EUH066)	Adverse effect occurring after a brief exposure
Used with low frequency	Used with high frequency
Used for short duration	Used for long duration
Low likelihood for exposure of critical initial sites of contact: skin, eye, RT, GI(T)	High likelihood for exposure of critical initial sites of contact: skin, eye, RT, GI(T)
Low exposure (approximate information):	High exposure (approximate information):
Low amount used per event	High amount used per event
Low vapour pressure	High vapour pressure
Low aerosol formation (liquid or solid)	High aerosol formation (liquid or solid)
High viscosity of product (less aerosol formation and potential for splashes)	Low viscosity of product
High ventilation expected, e.g. due to outdoor use or a use for which high ventilation is standard	Low ventilation expected (e.g. non-professional indoor use)
No direct contact with skin, eye, GT expected	Direct contact with skin, eye, GT expected
Low exposure level compared to adverse effect concentration (LOAEC) or no adverse effect concentration (NOAEC) if available	High exposure level compared to adverse effect concentration (LOAEC) or NOAEC, if available
High degree of operational RMMs already in use or recommended and compliance expected	Operational RMMs cannot be applied or compliance not expected

[72] Severity of the effect can be assessed if any relevant information is available. Scores from specific *in vitro* tests may also be used as an information source.

High level of containment	
Easy maintenance	
Minimization of manual phases	
Local exhaust ventilation	
High degree of organisational RMMs already in use or recommended and compliance expected	Necessary organisational RMM not applicable
Permit to work procedures	
Trained workers	
Intensive supervision of workers regarding correct use of RMM	
Professionals using appropriate PPE	General public cannot be expected to use PPE
Package design eliminating exposure	
Child-proof closure	Potential children and infant exposure
Appropriate instructions for use	
Special formulation effects (such as encapsulation, coating, partitioning or adsorption of substances within the product, exposure reduction by particle size or aerosol/droplet size control, pellet formation and antagonistic co-formulant effects, see section 4.1.1) reduce or eliminate exposure and/or expression of the hazard	Special formulation effects increase exposure and/or expression of the hazard

4.4.2.3.2 Concluding qualitatively on the acceptability of risk

A qualitative assessment aims at reducing or avoiding contact with potentially hazardous products and any in-use dilutions. Implementation of RMMs, including engineering controls, need to be proportional to the degree of concern for the health hazard. For example, a stricter control strategy should normally be applied to strong sensitisers than to irritants, and life-threatening consequences require the most stringent measures.

Tables 39 and 40 provide indicative guidance for the acceptable frequency, duration and degree of potential exposure for each effect, and recommend PPE for products and in-use dilutions. Table 41 provides possible RMMs.

The degree of potential exposure under best practice conditions is described qualitatively in terms of tasks and expected exposures. Some of the descriptions for the different exposure indicators are intentionally vague to allow flexible application of the guidance.

It must be stressed that acceptability of a scenario is affected by the combination of the pattern and situation of use, all risk management measures taken and any possible PPE. Since all these need to be considered in conjunction, it is not possible to establish definite rules or values for a certain parameter. The same acceptability criteria should not be valid for exposure time in

situations that may be the same regarding containment RMMs and appropriate PPE, but when in one case automation is also included: automation should enable longer theoretical exposure time if this leads to significantly reduced extent of exposure.

➔ As a specific example, for spraying application one needs to consider the type of spraying equipment (knapsack, trigger etc.), the pressure applied, droplet size distribution, direction of spraying, distance of the operator from source of spray, ventilation, including local exhaust ventilation, closed conditions or not, indoor or outdoor use, automation, PPE etc. Since all these variables affect the exposure in a manner that is in principle independent of the other variables, it would not be appropriate to set for example minimum requirements for one of these without considering all the others.

The assessment should balance the indicative duration and degree of exposure with the effectiveness of the RMMs and PPE for each exposure scenario. For example, longer exposure time could be acceptable when the degree of exposure can be minimised by RMMs and PPE.

Expert judgment is necessary when evaluating (a) if the RMMs and PPE are feasible in each exposure scenario and (b) if deviations may be acceptable from the indicative frequency, duration and potential degree of exposure as well as from the proposed RMMs and PPE (including e.g. missing RMMs/PPE, substitution by other means). The arguments in Table 38 supporting either acceptable or non-acceptable risk provide further support in this decision making.

The proposed measures to be applied in terms of acceptable exposure, RMMs and PPE depend on the nature, severity and potency of the effects expected, listing from most stringent to least stringent:

- Most stringent measures are necessary for strong respiratory sensitisers to which exposure should be strictly contained because current methodologies do not allow an adequate assessment of the risks associated with their use. The same applies to extreme skin sensitisers and corrosives because they can cause serious, potentially irreversible effects even at low concentrations.
- Less stringent measures are required for strong and moderate skin sensitisers, moderate respiratory sensitisers, corrosives to the respiratory tract and products/in-use dilutions causing serious eye damage. These can cause serious, irreversible effects.
- The least stringent measures concern moderate irritants and products/in-use dilutions which cause skin dryness. These are moderate, reversible effects.

The potency evaluation and hazard categorization could potentially result in two products with very different concentrations of active substance requiring the same measures. In deciding the measures, careful scientific consideration is therefore necessary using all relevant information, including tests on substances and products, concentration of the substance, physical form, physico-chemical interactions and any possible formulation effects (see 4.1.2).

Table 39: Guidance for concluding qualitatively on the acceptability of the risk for non-professionals. The second and third columns advise when the risk can be considered to be under control: these columns should be considered together, as for example a very low degree of exposure would allow a higher frequency. To conclude on acceptability, the information also needs to be considered together with qualitative arguments such as the examples provided in Table 38 and the possible RMMs provided in Table 41.

Effects	Acceptable frequency and duration of exposure[73]	Acceptable degree of exposure
Skin Sens 1A or Skin Sens 1 (H317) and potency evaluated as "extreme" according to *CLP guidance* Resp Sens 1A (H334) or Resp Sens 1 and potency evaluated as "strong" according to *CLP guidance* Skin corr. 1A (H314) STOT SE 1 (H370) (local effects skin, gastrointestinal tract, respiratory tract, eyes), H370 STOT RE 1 (local effects skin, gastrointestinal tract, respiratory tract, eyes), H372	Not acceptable	Not acceptable Products are normally not to be sold to general public Exceptions are possible when: 1) exposure is so low that unacceptable risk to human health is not expected (negligible exposure), or 2) the hazard is not relevant due to the route of exposure, or 3) there is a clear benefit to public health such that withdrawal of the product may result in more serious health concerns
Skin Sens 1A or Skin Sens 1 (H317) and potency evaluated as "strong" according to *CLP guidance* Resp Sens 1B (H334) or Resp Sens 1 and potency evaluated as "moderate" according to *CLP guidance* Skin corr 1, 1B, 1C (H314) Eye dam 1 (H318) Corrosive to the respiratory tract, EUH 071 Skin sens. 1B, H317, or Skin Sens 1 (H317) and potency evaluated as "moderate" according to *CLP guidance*	Equal to or less than once per week and few minutes per day	Practically no exposure Example: use of toilet cleaner For corrosive substances and products, the probability of exposure may be best linked to the duration of the task.
Skin irrit 2, H315 EUH066 - Repeated exposure may cause skin dryness or cracking Eye irrit 2, H319 STOT SE 3, H335 (may cause respiratory irritation) STOT SE 2 (local effects skin, gastrointestinal tract, respiratory tract, eyes), H371 STOT RE 2 (local effects skin, gastrointestinal tract, respiratory tract, eyes), H373	Equal to or less than one hour per day, considering the relevant exposure route	Examples: - Use of dish cleaning product - Low volume outdoor spray application - Downward spray application in areas with good ventilation

[73] Duration of potential exposure can be shorter than task/use/process

Table 40: Guidance for concluding qualitatively on the acceptability of the risk for professionals. The second and third columns advise when the risk can be considered to be under control: these columns should be considered together, as for example a very low degree of exposure would allow a higher frequency. The fourth column provides a non-exhaustive list of possible PPE that can be used to achieve acceptable exposure levels. To conclude on acceptability, the information in this table needs to be considered together with qualitative arguments such as the examples provided in Table 38. In concluding on acceptability, one should consider also what is an acceptable degree of exposure for non-professionals (Table 39).

Effects	Acceptable frequency and duration of exposure[a]	Acceptable degree of exposure[b]	Possible PPE
Skin Sens 1A, or Skin Sens 1 (H317) and potency evaluated as "extreme" or "strong" according to *CLP guidance*	Few minutes per day or less	Very high level of containment, practically no exposure Example: connecting tubes with technical RMM and PPE	All skin and mucous membranes with potential exposure protected with appropriate PPE
Resp Sens 1A or 1B (H334), or Resp Sens 1 and potency evaluated as "strong" or "moderate" according to *CLP guidance*	Few minutes per day or less		Appropriate respirator mandatory unless complete containment is verified for all phases of the operation
Skin corr. 1A (H314)	Few minutes per day or less		Face shield Substance/task appropriate chemical resistant gloves, coveralls, chemical protective footwear, chemical protective aprons Substance/task appropriate respirator Substance/task appropriate shoes, e.g. acid/base resistant
Skin corr 1, 1B, 1C (H314)	Few minutes per day or less	High level of containment, practically no exposure; no splashes, no hand to eye transfer, no (liquid or solid) aerosol formation Example: brief contact with technical RMM and PPE (touching contaminated surfaces)	Face shield Substance/task appropriate gloves Skin coverage with appropriate barrier material based on potential for contact with the chemicals (examples: coveralls, chemical protective footwear, chemical protective aprons) Substance/task appropriate respirator Substance/task appropriate shoes, e.g. acid/base resistant

Eye dam 1 (H318)	Few minutes per day or less		Face shield
Corrosive to the respiratory tract, EUH 071	Few minutes per day or less	High level of containment, practically no exposure; no splashes, no (liquid or solid) aerosol formation Example: brief contact with technical RMM and PPE	Substance/task appropriate respirator
Skin sens. 1B, H317, or Skin Sens 1 (H317) and potency evaluated as "moderate" according to *CLP guidance*	Few minutes per day or less	High level of containment, practically no exposure Example: brief contact with technical RMM and PPE (touching contaminated surfaces)	Substance/task appropriate gloves Skin coverage with appropriate barrier material based on potential for contact with the chemicals (examples: coveralls, chemical protective footwear, chemical protective aprons, face shield) Substance/task appropriate respirator Face shield
STOT RE 1 (local effects skin, gastrointestinal tract, respiratory tract, eyes)	Few minutes per day or less		Substance/task appropriate protection (select from box above)
Skin irrit 2, H315	More than few minutes but equal to or less than few hours per day For exposure duration of less than few minutes per day, no RMM or PPE are normally necessary	Controlled exposure Examples: spray application with high ventilation or technical RMM and PPE cleaning and maintenance work with high ventilation or technical RMM and PPE	Face shield Eye protection (chemical goggles, safety glasses)[c] Substance/task appropriate gloves Protective coverall
EUH066 - Repeated exposure may cause skin dryness or cracking			
Eye irrit 2, H319			Face shield Eye protection (chemical goggles, safety glasses)
STOT SE 3, H335 (may cause respiratory irritation)			Substance/task appropriate respirator
STOT RE 2 (local effects, RT, eyes, skin)			Substance/task appropriate protection (select from boxes above)

[a] *Duration of potential exposure can be shorter than task/use/process. RMMs may reduce the duration of exposure for example by ensuring the user not being present all the time.*
[b] *The degree of exposure can be reduced by PPE, or by RMMs such as requiring LEV.*
[c] *Eye protection in the form of goggles/safety glasses is only necessary in cases where the specific application could lead to exposure of the eyes.*

Where PPE or RPE is required, these need to be specified with regard to any relevant standards and materials, including further details such as breakthrough times where necessary. The

feasibility of the PPE should be ensured. If a standard is not stated, the PPE has to be clearly specified by indicating e.g. the material, breakthrough time and protection factor.

Table 41 provides a non-exhaustive list of possible RMMs that may be used where relevant. RMMs for child-resistant fastening are triggered by classification in specific hazard classes and categories according to CLP Regulation EC 1272/2008 for a substance or mixture supplied to the general public. However, the CLP does not require child-resistant fastening for products classified for e.g. sensitisation, skin or eye irritation or serious eye damage, while such RMMs could be considered necessary based on a qualitative risk assessment. Overall, the RMMs must be carefully considered to ensure compliance with both the BPR and the CLP Regulation.

Table 41: Possible risk management measures

	Risk management measures
Technical measures Normally not considered for non-professionals	Very high level of containment required, except for short term exposures e.g. taking samples
	Closed system (less exposure, easier maintenance)
	Automation
	Equipment under negative pressure
	Regular cleaning of equipment and work area
	Containment as appropriate
	Segregation of the emitting process
	Effective contaminant extraction
	Good standard of general ventilation
	Minimisation of manual phases
	Avoidance of contact with contaminated tools and objects
	Minimisation of splashes and spills
	Minimisation of exposure to aerosols (e.g. ventilation, local exhaust ventilation, no spraying upwards, increasing distance from aerosol source)
	Sensors to detect safe/excessive concentrations in air with a corresponding alarm system
Organisation Normally not considered for non-professionals	Control staff entry to work area
	Control of re-entry times after biocide application
	Recording of any 'near miss' situations
	Permit for maintenance work
	Management/supervision to check that the RMMs are used correctly and operational conditions followed
	Training for staff on good practice
	Procedures and training for emergency decontamination and disposal
	Good standard of personal hygiene
	Sensitisers: pre-employment screening and appropriate health surveillance

	Minimise number of staff exposed Ensure all equipment well maintained
Product and packaging For professionals and non-professionals	Labelling, pictograms, instructions for use Child proof closure (not relevant for professional use) Packaging eliminating exposure and/or facilitating safe handling (e.g. handles, ensuring good grip, limiting package size) Formulation reducing exposure, e.g. viscosity Application methods that reduce exposure, e.g. avoiding spraying or avoiding/reducing aerosol formation

4.4.2.3.3 Examples on risk characterisation for local effects including sensitisation

Example 1: Qualitative risk assessment for local effects

A) Primary exposure: use of product

Hazard		Exposure							Risk	
Hazard category	Additional relevant hazard information	PT	Who is exposed?	Tasks, uses, processes	Potential exposure route	Frequency and duration of potential exposure	Relevant RMM & PPE	Potential degree of exposure	Conclusion on risk	Uncertainties attached to conclusion may increase (↑) or decrease (↓) risk or both (↑↓)
Eye irrit. Cat 2, H319	-	2	General public: adults	Dilute product by pouring 100 ml to 10 L water (=1%)	Skin Eye (splashes, hand to eye transfer)	2/year; Few minutes or less per day	Labelling as eye irritant Child proof closure Instructions for use packaging reducing risk for eye exposure by splashes Washing of hands after use	Low	Acceptable: - Reversible effect - Low frequency	Frequency of use may be higher than recommended (↑) Instructions for use and packaging as well as adherence to it, including washing of hands may vary (↑↓)

B) Primary exposure: use of application solutions

The application solution containing 1% of the product is poured into the garden pond resulting in a concentration of 0.01% of the product in garden pond water. Children and pets may accidently play or drink the garden pond water. However, these dilutions are below the classification limit, therefore the risk for local effects is considered as acceptable.

Example 2: Qualitative risk assessment for local effects

A) Primary exposure: use of product

Hazard		Exposure							Risk	
Hazard category	Additional relevant hazard information	PT	Who is exposed?	Tasks, uses, processes	Potential exposure route	Frequency and duration of potential exposure	Relevant RMM & PPE	Potential degree of exposure	Conclusion on risk	Uncertainties attached to conclusion may increase (↑) or decrease (↓) risk or both (↑↓)
Eye irrit. Cat 2, H319	-	10	General public: adults	Loading product into spraying device and mixing/ diluting it for final application (17%)	Skin Eye (splashes, hand to eye transfer)	2-3/year Few minutes or less per day	Labelling as eye irritant Child proof closure Instructions for use Packaging reducing risk for eye exposure by splashes Washing of hands after use	Low	Acceptable: - Reversible effect - Low frequency	Frequency of use may be higher than recommended (↑) Instructions for use and packaging as well as adherence to it, including washing of hands may vary (↑↓)
Eye irrit. Cat 2, H319	-	10	Professionals	As above	As above	Not daily, but ≥ 1/week Few minutes or less per day	Labelling as eye irritant Instructions for use minimizing exposure Packaging reducing risk for eye exposure by splashes Washing of hands after use	Low	Acceptable: - Reversible effect - Professionals following instructions for use - Experience expected	Instructions for use and packaging as well as adherence to it, including washing of hands may vary (↑↓)

B) Primary exposure: use of application solutions

Hazard		Exposure							Risk	
Hazard category	Additional relevant hazard information	PT	Who is exposed?	Tasks, uses, processes	Potential exposure route	Frequency and duration of potential exposure	Relevant RMM & PPE	Potential degree of exposure	Conclusion on risk	Uncertainties attached to conclusion may increase (↑) or decrease (↓) risk or both (↑↓)
Eye irrit. Cat 2, H319	No clinical signs or macroscopic pathological effects with 5000 mg/m3 (~5 ml/m3) after 4 hours RT exposure of rats[1]	10	General public: adults	Spraying on masonry, outdoor with 17% solution	Skin Eye (splashes, hand to eye transfer) RT	2-3/year ~ 60 min/ day	Labelling as eye irritant Child proof closure Instructions for use Only spray downward Washing of hands after use Washing of face/eye after accidental exposure	Low	Acceptable: - Reversible effect - Low frequency - Low intensity: outdoor use, low intensity compared to additional hazard information[1]	Ventilation in outdoor situations may vary (↑↓)
As above			Professionals	As above		Not daily, but ≥ 1/ week ~ 60 min/ day	Labelling as eye irritant Instructions for use Only spray downward Washing of hands after use Washing of face/eye after accidental exposure Instructions for use minimizing exposure for professionals	Low	Acceptable: - Reversible effect - Low intensity: outdoor use, low intensity compared to additional hazard information - Professionals following instructions for use - Experience expected	

[1] With eye irritation also respiratory tract irritation is expected but no threshold is available, therefore acute product test data are used as additional information for semi-quantitative RC.

Example 3: Qualitative risk assessment for local effects

A) Primary exposure: use of product

Hazard		**Exposure**							**Risk**
Hazard category	Additional relevant hazard information	PT	Who is exposed?	Tasks, uses, processes	Potential exposure route	Frequency and duration of potential exposure	Relevant RMM & PPE	Potential degree of exposure	Conclusion on risk
Eye dam. Cat 1, H318	-	19	General public: adults, children, infants	Poured into hands and spread over skin of arms and legs	Skin Eye (splashes, hand to eye transfer)	Up to more than 1/day for weeks	Labelling for eye damage, Child proof closure Instructions for use Packaging reducing risk for eye exposure by splashes Washing of hands after use	Considerable	Not acceptable: - Irreversible or severe effect - Frequent use - High amount per event - High probability for eye exposure - Children and infant exposure

Example 4: Qualitative risk assessment for local effects

A) Primary exposure: use of product

Hazard		Exposure							Risk	
Hazard category	Additional relevant hazard information	PT	Who is exposed?	Tasks, uses, processes	Potential exposure route	Frequency and duration of potential exposure	Relevant RMM & PPE	Potential degree of exposure	Conclusion on risk	Uncertainties attached to conclusion may increase (↑) or decrease (↓) risk or both (↑↓)
Skin corr. Cat 1A, H314	-	4	Industrial	IBC containers containing the product are connected to CIP via installed pipes	Skin Eye	Few minutes per day or less	Technical and organisational RMM adequate for the very high hazard category are achievable Transfer in closed systems and industrial RMM excluding risk for skin and eye exposure Use of appropriate gloves and mask	Low	Acceptable No exposure expected since: - Technical and organisational RMM adequate for the very high hazard category are achievable	Frequency of use may be higher than recommended (↑) Industrial users (↓)

IBC: intermediate bulk container; CIP: cleaning in place

B) Primary exposure: use of application solutions

Hazard		Exposure						Risk		
Hazard category	Additional relevant hazard information	PT	Who is exposed?	Tasks, uses, processes	Potential exposure route	Frequency and duration of potential exposure	Relevant RMM & PPE	Potential degree of exposure	Conclusion on risk	Uncertainties attached to conclusion may increase (↑) or decrease (↓) risk or both (↑↓)
Skin irrit. Cat 2, H315 Eye irrit. Cat2, H19	-	4	Industrial	Exceptional maintenance work with 0.3% to 2% dilution	Skin Eye	Very low frequency More than few minutes but equal to or less than few hours per day	Technical and organisational RMM adequate for the low hazard category are achievable Use of appropriate gloves, eye protection, filter mask	Low	Acceptable: - Reversible effects - Installed RMM at place - Trained workers - Use of appropriate PPE	Frequency of use may be higher than recommended (↑) Correct use of the PPEs (↓) Industrial users (↓)

Example 5: Qualitative risk assessment for local effects

A) Primary exposure: use of application solutions

Hazard		Exposure						Risk		
Hazard category	Additional relevant hazard information	PT	Who is exposed?	Tasks, uses, processes	Potential exposure route	Frequency and duration of potential exposure	Relevant RMM & PPE	Potential degree of exposure*	Conclusion on risk	Uncertainties attached to conclusion may increase (↑) or decrease (↓) risk or both (↑↓)
Skin corr. Cat	Based on extreme pH and the acid alkaline	2, 3	Professionals (not trained for the task)	Low pressure coarse	Skin Eye	Not daily, but ≥ 1/month	PPE:	Considerable	Not acceptable	Irreversible and/or severe effect (↑)

1, H314	reserve the mixture may be corrosive			spraying application Disinfection of surfaces and animal housing for target organisms that require concentrated products	Respiratory tract (non-volatile active substance and solvents)	Exposure duration 120 minutes	- Substance/task appropriate chemical resistant gloves, coveralls (including hood) - Chemical protective footwear, appropriate shoes, e.g. acid/base resistant The following PPE may not be worn appropriately by professionals without specific training for the task: - Face shield - Substance/task appropriate respirator - Chemical resistant tape for sealing PPE RMMs: - Regular cleaning of equipment and work area - Containment as appropriate - Segregation of the emitting process (lance) The following RMMs may not be respected by professionals without specific training for the task: - Minimisation of splashes and spills - Minimisation of exposure to aerosols (e.g. ventilation, local exhaust - Ventilation, no spraying upwards, increasing distance from aerosol source)		The risks could not be adequately managed without ensuring appropriate training	Used for long duration (↑) Uncertainty on proper use of the RMMs (↑) Direct contact with skin, eye, respiratory tract expected (↑) (that cannot be ruled out as PPE might not be used adequately by this user category) Low vapour pressure (↓)

							- Sensors to detect safe/excessive concentrations in air with a corresponding alarm system - Control entry to area			
Skin corr. Cat 1, H314	Based on extreme pH and the acid alkaline reserve indicate that the mixture may be corrosive	2, 3, 4	Professionals (trained for the task)	Low pressure coarse spraying application (non-volatile active substance and solvents) Disinfection of surfaces and animal housing for specific target organism requiring more concentrated products (e.g. oocysts)	Skin Eye Respiratory tract	Potential daily workday, ≥ 1/ week Exposure duration 120 minutes	PPE: - Face shield - Substance/task appropriate chemical resistant gloves, coveralls (including hood) - Chemical protective footwear, appropriate shoes, e.g. acid/base resistant - Chemical resistant tape for sealing PPE - Substance/task appropriate respirator RMMs: - Regular cleaning of equipment and work area - Automation - Containment as appropriate - Segregation of the emitting process (lance) - Minimisation of splashes and spills - Minimisation of exposure to aerosols (e.g. ventilation, local exhaust - Ventilation, no spraying upwards, increasing	Low	Acceptable The risks could be managed adequately	Irreversible and/or severe effect (↑) Used for long duration (↑) No direct contact with skin, eye, respiratory tract expected (↓) - but could be ruled out as PPE might be used adequately (cover whole body, contamination prevented) by workers trained for the task Workers trained for the task(↓) Intensive supervision of workers regarding correct use of RMM (↓) Low vapour pressure (↓)

							distance from aerosol source) - Sensors to detect safe/excessive concentrations in air with a corresponding alarm system - Control entry to area - Management/supervision to check that the RMMs are used correctly and operational conditions followed - Ensure all equipment well maintained - Professional training: Training must be provided for the users to ensure appropriate handling of the corrosive product and the PPE required.			

* Additional product specific information may be considered, such as viscosity and ventilation expected (indoor/outdoor uses).

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